

Asthma phenotypes and lung function up to 16 years of age - the BAMSE cohort

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Conflict of interest

JH, PT, ESS, ASM, MB, FC, IK, PMG and EM have stated there is no conflict of interest.

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Abstract

Background

Asthma is a disease affecting many locations throughout the airway. Most studies have used spirometry as the primary assessment of airway obstruction, a method that may be less sensitive in regard to peripheral airway obstruction. The aim of the current study was to elucidate the associations between asthma phenotypes based on age of onset and duration of symptoms and; (1) spirometry, and (2) small airway involvement measured by impulse oscillometry (IOS) in adolescence.

Methods

Children and adolescents taking part in BAMSE, a prospective birth cohort study, performed spirometry at 8 and 16, and IOS at 16 years of age. Based on data collected in questionnaires, children were categorized to the following groups; “never asthma”, “early transient asthma”, “early persistent asthma”, and “late onset asthma”.

Results

Compared with the never asthma group, all asthma groups were associated with lower FEV₁ at 16 years of age (early transient -119 ml, 95% Confidence Interval -204 to -34; early persistent -410 ml, 95%CI -533;-287 and late onset -148 ml, 95%CI -237;-58). Between 8 and 16 years, significantly less increase of FEV₁ was observed in the early persistent and late onset groups. The small airway index “R₅₋₂₀” was significantly associated with active asthma at 16 years, but not transient asthma.

Conclusions

All asthma phenotypes studied were negatively associated with FEV₁ in adolescence. IOS measurements showed that active asthma is associated with small airway impairments. These results provide new insights into the physiology underlying wheezing phenotypes based on age of onset and duration of disease.

Introduction

In order to understand the physiological mechanisms underlying asthma disease and symptom expression, differentiation of the asthma phenotypes based on age at onset and duration of disease has been explored by studying lung function over time. The onset of asthma in early life has been associated with impairments in lung function that track throughout life, not only in those with persisting disease, but also after early transient symptoms (1-4).

Most studies have used spirometry as the primary assessment of airway obstruction, a method that may be less sensitive with regard to peripheral airway obstruction as it reflects mostly total airway resistance, and peripheral airway resistance to a lesser degree (5, 6). In addition, unevenly distributed airway obstruction, which is thought to be a common phenomenon in asthma (7, 8) can be masked by increased flow via non flow limited airways (9).

Nevertheless, asthma is an inflammatory disease affecting large parts of the airway system, including the small airways (10, 11). Impulse oscillometry (IOS) has been described as a simple and reliable method that provides information about the proximal peripheral airway function (12). Recently, it has been suggested that the degree of small airway involvement as measured by IOS is associated with severity of disease (13), asthma control (14) and the presence or absence of respiratory symptoms in individuals with asthma(15). To our knowledge, the relationship between age at onset of asthma, asthma duration, and degree of

small airway involvement has been only partly described in one study of high-risk children that showed associations only between early onset rhinovirus-induced wheezing and parameters of small airway disease (16).

We have previously reported a negative association between early onset asthma and lung function as measured by spirometry in the 8-year follow-up of the BAMSE cohort (17). Here we report lung function at 16 years of age, with the aim to further elucidate the associations between age at onset, duration and presence of concurrent symptoms with (1) lung function as measured by spirometry at 8 and 16 years of age, and (2) small airway involvement as measured by impulse oscillometry at 16 years of age.

Material and methods

Study subjects

The BAMSE (Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology) study is a longitudinal, population-based prospective birth cohort including 4089 children born between 1994 and 1996 in Stockholm (18). The cohort was designed to study risk factors for asthma and other allergic diseases in childhood, and to study factors of importance for disease prognosis. Questionnaires on respiratory symptoms and medication were answered at age of 1, 2, 4, 8 and 16 years(19). Response rates ranged from 96-82% at each occasion. At the 8 and 16 year follow-up, spirometry was performed and at the latter, impulse oscillometry was also measured.

Measurements of lung function

Lung function testing was performed at 8 years of age using the 2200 Pulmonary Function Laboratory (Sensormedics, Anaheim, CA, USA) (20) and at 16 years with the Jaeger

MasterScreen-IOS system (Carefusion Technologies, San Diego, California). See the online supplement for details.

Briefly, for IOS, pressure impulses were sent from a loudspeaker through the respiratory system. The subjects were encouraged to perform tidal breathing with their lips tightly sealed around the mouthpiece while supporting the cheeks with their hands to avoid impulse pressure loss due to upper airway shunt. After quality inspection, the mean value of resistance at 5 and 20 Hertz (R_5 , R_{20}), frequency dependence of resistance (R_{5-20}) and the square root of the area of reactance ($AX^{0.5}$) were used for analysis.

Maximal expiratory flow volume (MEFV) measurements were performed following IOS. All subjects performed several MEFV measurements. The highest values of forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV_1), and maximum expiratory flow when 50% of the FVC remains to be exhaled (FEF_{50}) were extracted and used from analysis, provided that the subject's effort was accepted as being maximal by the test leader, the MEFV curve passed visual quality inspection, and that the two highest FEV_1 and FVC readings were reproducible according to ATS/ERS criteria (21). FEV_1 /FVC ratios were expressed as percentages.

Definition of asthma groups

Based on answers provided in the questionnaires, participants were grouped according to whether or not they had asthma and/or wheeze at the age of 1, 2, 4, 8, and 16 years, see the online supplement for detailed criteria descriptions. Children were categorized in the following groups based on time of onset and duration of symptoms; “never asthma” (reference group, did not fulfilled any asthma criteria from birth to 16 years of age), “early transient asthma” (asthma between age 0-4 years, no wheeze after) “early persistent” (asthma

in the first 4 years and asthma at 16 years), "late onset asthma" (no asthma at age 1, 2 or 4, but asthma at age 16).

Statistical analysis

See the online supplement for detailed descriptions. The associations between asthma groups and spirometry variables were assessed using linear regression. To make full use of the repeated data sampling, a longitudinal analysis with mixed models was used. Time-dependent covariates included in the model were height, weight and age. Asthma group and sex were time-invariant covariates. To assess potential variations in the effect of asthma on lung function over time, an interaction term between the time and asthma group was included in the model.

The distribution of IOS was skewed to the right. We utilized quantile regression to estimate associations between asthma and IOS variables at the 50th and 90th percentiles, while adjusting for sex, height, weight and age. For completeness, we also performed linear regression on the mean for IOS data. P-values of < 0.05 were considered statistically significant. Analyses were performed using the Stata 12.1 software package (StataCorp LP, College Station, TX, USA).

The BAMSE study was approved by the Ethical Committee of Karolinska Institute, Stockholm, and all parents provided consent for their child to participate in the study.

Results

Study Sample

Of the 4,089 children in the original BAMSE cohort, 2620 (64%) and 2605 (64%) participated in the 8 and 16 year follow-up, respectively. The characteristics of participating

and non-participating children are presented in E-Table 1 in the online supplement. The numbers of successful lung function tests performed at the 8 year follow-up was 1832 (spirometry only), and at the 16 year follow-up, 2056 and 2453 (spirometry and IOS, respectively). Of those participating in the 16 year follow-up, 2355 (90%) had sufficient data on wheeze/asthma in order to be classified according to pre-defined asthma groups. The never asthma group consisted of 1928 individuals (82% of the eligible population). The early transient asthma group included 139 individuals (6%), early persistent asthma 61 individuals (3%) and late onset asthma 109 individuals (5%). Four percent of the available children did not belong to any of the specified groups. The characteristics of the children in the different groups can be found in Table 1.

Asthma and spirometry

Spirometry data obtained at the 8 year follow-up can be found in E-table 2. Early persistent asthma was associated with lower values for all spirometric parameters except FVC, when compared with the never asthma group. The group with early transient asthma presented with a significantly lower FEV₁/FVC ratio and FEF₅₀, while lung function values in the late onset asthma group were no different from the reference group. At 16 years of age, all asthma groups were associated with lower FEV₁, FEV₁/FVC and FEF₅₀ values in comparison with the reference group (Table 2). The lowest values were seen for the early persistent group with FEV₁ and FEF₅₀ reductions of 410 ml (or 10%) and 1141 ml (or 23%), respectively, compared with the never asthmatics. In addition, a significantly lower FVC was seen only in the persistent asthma group.

Asthma and lung function change between 8 and 16 years

Using a longitudinal mixed model incorporating spirometry data from both follow-up examinations, we observed significantly less increase in FEV₁ and FEF₅₀ between the ages of

8 and 16 years for the early persistent (FEV_1 -262 ml [95% CI -377;-147] and FEF_{50} -668 ml [95% CI -934;-401]) and late onset groups (FEV_1 -124 ml [95% CI -213;-36] and FEF_{50} -350 ml [95% CI -557;-142]) as compared with the reference group. The early transient asthma group showed less increase in FEF_{50} , compared with the reference group (FEF_{50} -221 ml [95% CI -415;-27]), but no difference in FEV_1 increase (Table 3).

Asthma and impulse oscillometry (IOS) at 16 years

Differences between each asthma group and the reference group are shown in Table 4 and Table E3. All of the estimated means, and all but one of the estimated percentiles, for R_5 and R_{20} were significantly smaller in the referent than in any other group. In particular, the 90th percentiles for R_5 and R_{20} in the persistent asthma group were 92 Pa/L/s (or 4.8%) and 48.5 Pa/L/s (or 3.6%), respectively, greater than in the never asthma group. $AX^{0.5}$ showed a similar pattern.

Figure 1 show all percentiles for R_{5-20} , an index which is thought to indicate heterogeneity in resistance over the airway system. The distribution of R_{5-20} values was similar in never asthma and in the transient asthma groups. As shown in Table 4, the late-onset group differed from the never asthmatic group in all percentiles above the median (90th percentile difference from never asthmatics = 24.6 Pa/L/s, or 3.7% larger). The persistent asthma group showed a heterogeneous pattern including a wide range of R_{5-20} values and a skewed distribution with extremely large R_{5-20} values (90th percentile difference from never asthmatics = 61.1 Pa/L/s, or 9.1% larger).

Discussion

Principal findings

Using a longitudinal prospective birth cohort study, we present data indicating impaired airway function in adolescents with current and/or past asthma symptoms. The lung function deficits associated with asthma did not improve between the ages of 8 and 16 years, instead impairments were further pronounced in the group with early persistent symptoms. Such impairments were also observed in those with transient symptoms, although to a lesser extent. Finally, we describe the association between IOS measures and asthma, showing that small airway involvement is a feature of ongoing asthma in adolescence.

In our cohort, only a small proportion of children with asthma symptoms during the first four years of life continued to have asthma at age 16 years. This benign outcome of early wheeze has previously been shown in a number of cohorts (1, 2, 22-25). Even so, the current study also showed that early transient asthma was associated with impaired spirometric values at both examinations, indicating remaining airway obstruction from the age of 8 up to 16 years. The long-lasting effects of early respiratory symptoms on lung function throughout childhood have also been described, although the above mentioned studies aimed to investigate the natural course of asthma symptoms and did not specifically stratify findings according to the presence or absence of symptoms after age 6-7 years (1, 2). The Norwegian ECA study did stratify according to current symptoms at age 16, and in concordance with the results of the present study, they found both transient and persistent early wheezing to be associated with lower lung function. It was further reported that although the children in the transient asthma group no longer fulfilled the criteria for asthma at 16 years, up to a third of the group had symptoms still present or access to asthma treatment(3). In the current study, the negative effects on lung function remained although wheeze was reported to be completely quiescent at

the time of lung function assessment, confirming and extending the findings of the ECA study. However, 14.8% of children with transient asthma in our study answered that they had used some kind of asthma medication at in the last 12 months at 16 years. Although the transient asthma children were symptom free during the same period, we cannot completely rule out that medication use in a minority of the cases could reflect a very mild ongoing disease.

The longitudinal analysis of spirometry data showed that less increase in FEF_{50} was observed in both the transient, late onset and persistent groups, compared with reference group, while the negative effect on FEV_1 was only apparent for children with late onset or persisting asthma. Although a number of studies with follow-up spans of up to 50 years have described lung function as being remarkably stable after children start school (1, 26, 27), others show similar results to ours regarding early persistent wheezers (28, 29). To assess the importance of their findings, Lodge et al. used data on normal lung function decline in adulthood from a population in Tucson, Arizona (29, 30), to show that their reported reduction in FEV_1 growth for asthmatics represented between 4.9 and 12.9 years of adult lung capacity. In our study, the same estimation would suggest that the less than expected increase in FEV_1 between the ages of 8 and 16 years seen for the early persistent asthma group could be estimated to that of the natural decline in FEV_1 over a period of 10 and 13 years in adulthood, depending on sex. Providing a more clinical perspective, the CAMP study showed that lung function in asthmatics seem to worsen over time in that an increasing number of children with abnormal FEV_1/FVC ratio were observed throughout childhood(4).

Finally, we found that airway resistance measured by impulse oscillometry showed overall higher values in those with asthma at 16 years compared to the never asthma group, thereby

confirming the negative association between asthma and spirometry indices. A frequency dependency of resistance was observed only in the group with active asthma - a higher resistance value at 5Hz than at 20 Hz indicating inhomogeneity of airway resistance, and thus the presence of peripheral airway disease. In particular the early persistent group showed extremely large values at the highest percentiles. The relationship between onset of disease and later IOS is sparsely described in the literature and direct comparisons are difficult. Children up to the age of 4 years were described by Oostveen et al. (31) to have increased resistance at 4 Hz, A_x and frequency dependency of resistance if they had had suffered from asthma in their first years of life compared with never asthmatics. Guilbert et al. (16) examined children at 8 years of age and found impairments in spirometry measures and the IOS parameter R_5 in children with previous rhinovirus-induced wheezing, but not other wheezing subgroups. In summary, by measuring lung function using impulse oscillometry, we have presented further evidence of the heterogeneity of the asthma phenotypes during childhood and adolescence, suggesting that early and late onset asthma is associated with an overall increase in airway resistance, while differences in symptom expression and level of lung function could, in part, be attributed to the location of the disease within the airways.

Strengths and limitations

A strength of the BAMSE study is its long follow-up span with relatively limited loss to follow-up. Nevertheless, the individuals who participated in the clinical testing were more often female, had more parental atopy and less maternal smoking during pregnancy than those who did not participate, which might to some extent limit the generalizability of the results to the larger population. The use of age and duration to classify asthma and wheeze has been shown to be relevant not only to long-term outcomes (32), but also when methods such as

unsupervised clustering are used (2). In the current study, our aim was to combine symptom history in early childhood with ongoing disease at 16 years in order to draw further conclusions regarding the impact of transient or persistent symptoms on lung function in adolescence. Our choice of phenotype definitions may limit the comparability between studies, but also adds to the understanding of the relationship between symptoms and lung function over time. The use of quantile regression over multiple percentiles in the analysis of IOS data has provided important information regarding the characterization of the associations between small airway disease and asthma phenotypes. One of the major limitations in our study is that it would have been desirable to have post bronchodilator measurements in order to explore whether the observed impairments are due to static changes after remodeling of the airways, or reversible airway obstruction.

Interpretations/conclusions

All asthma phenotypes studied in this paper were negatively associated with lung function at 16 years of age, although in different ways. Early onset transient asthma was associated with an obstructive pattern at both 8 and 16 years. The level of FEV₁ did not change over time in comparison with the reference group, indicating that the timing of lung function impairment occurred between conception and school age. Early onset persistent asthma showed lung function impairments at 8 years of age, with further reductions up to age 16, supporting the two-hit hypothesis with an initial early lung function impairment and ongoing pathological progress with global reductions in spirometric lung function thereafter. Taken together, these results provide further insight into the physiology underlying different wheeze phenotypes up to adolescence.

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Figure 1. Distribution of R_{5-20} values for the never asthma group (A) and difference between each asthma group and the “never asthma” group at all percentiles (B-D). Estimates at each percentile are shown as solid black lines with 95 per cent confidence bands (shaded gray areas). The mean value (solid) and 95 per cent confidence intervals (dashed) are shown as parallel black lines.

Legend:

A. Distribution of R_{5-20} values in the never asthma group

B. Difference in R_{5-20} between early transient asthma and never asthma

C. Difference in R_{5-20} between early persistent asthma and never asthma

D. Difference in R_{5-20} between late onset asthma and never asthma

Table 1. Characteristics of children with asthma data 0–16 yrs who participated in the clinical examination at 16 years (N=2355).

	Never asthma	Early transient asthma	Early persistent asthma	Late onset asthma
No. of children (% of tot.)	1,928(82)	139(6)	61(3)	109(5)
Male (%)	919(47.7)	86(61.9)**	31(50.8)	44(40.4)
Sensitization at 16 y n(%)	738(39.1)	63(46.0)	43(74.1)***	80(74.8)***
Parental atopy in Q0 n(%)	554(28.9)	53(38.7)*	35(57.4)***	44(40.7)**

Maternal smoking during pregnancy (%)	188(9.8)	29(20.9)***	13(21.3)**	9(8.3)
Member of the household smoking at age 16	321(17.6)	37(28.5)**	15(25.4)	20(18.7)
Personal smoking at age 16	235(12.3)	15(11.0)	2(3.3)*	10(9.3)
Premature birth	94(4.9)	13(9.4)*	6(9.8)	7(6.4)
Any asthma medication at 8 yrs	91(4.7)	16(11.5)***	44(75.9)***	34(32.4)***
Any asthma medication at 16 yrs	118(6.1)	20(14.4)**	55(90.2)***	90(82.6)***

*Q0 = questionnaire at age 0 yrs, premature birth = being born in gestation week 36 or earlier, any asthma medication = regular or periodic use of short-acting beta-agonists, inhaled corticosteroids, combination therapies and/or leukotriene antagonists in the last 12 months, * = $p < 0.05$ compared with never asthma group, ** = $p < 0.01$ compared with never asthma group, *** = $p < 0.001$ compared with never asthma group*

Table 2. Difference in adjusted mean spirometry values and 95% CI at 16 yrs between asthma groups and never asthma group (linear regression adjusting for height, age and weight at examination).

	FVC (ml)			FEV ₁ (ml)			FEV ₁ /FVC (%)			FEF ₅₀ (ml/sec)		
	Dif f.	(95% CI)	P- val ue	Diff .	(95% CI)	P- valu e	Diff .	(95% CI)	P- valu e	Di ff.	(95% CI)	P- valu e
Never	46			396			85.			47		
asthma*	46			1			7			79		
Early	-		0.8	-	-204;-	0.00	-3.7;-		<0.0	-	-646;-	<0.0
transient	-9	1034;8	45	119	34	6	-2.4	1.2	01	43	224	01

	5								5			
Early	-	-									-	
persistent	16	3034;-	0.0	-	-533;-	<0.0	-7.4;-	<0.0	11	-1446;-	<0.0	
	8	32	16	410	287	01	-5.7	3.9	01	41	837	01
Late onset		-									-	
		1712;2	0.1	-	-237;-	0.00	-3.2;-	0.00	40	-6234;-	<0.0	
	-72	7	55	148	58	1	-1.9	0.6	4	1	179	01

*Mean adjusted value for the reference group.

Table 3. Difference in mean adjusted lung function increase from 8 to 16 years of age between asthma groups and the never asthma group; using a longitudinal effects model of adjusted lung function with an interaction term between asthma groups and time.

	FVC (ml)			FEV ₁ (ml)			FEV ₁ /FVC (%)			FEF ₅₀ (ml/sec)		
	Di	(95%	P-	Di	(95%	P-	Dif	(95%	P-	Dif	(95%	P-
	ff.	CI)	valu	ff.	CI)	value	f.	CI)	val	f.	CI)	valu
			e						ue			e
Never	ref			ref			ref			ref		
asthma												
Early										-		
transient	-	0.3	-	-	-	-	-	-	0.1	22	-415;-	0.02
	46	47;138	35	44	126;39	0.303	0.8	1.9;0.3	56	1	27	6
Early				-						-		
persistent	-	-	0.2	26	-377;-	<0.00	-	-	0.0	66	-934;-	<0.0
	83	211;45	05	2	147	1	1.4	2.9;0.1	68	8	401	01

Late onset	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	0.0	12	-213;-	-	-	0.1	35	-557;-	0.00	
	84	183;15	97	4	36	0.006	0.8	2.0;0.4	66	0	142	1

* Time-dependent covariates included in the model were height, weight and age. Asthma phenotype and sex were included as fixed covariates. For the model, the number of observations was 3241, the number of groups was 2164 and the average number of observations per group was 1.5 (minimum. 1; maximum. 2).

Table 4. Estimated difference and 95% confidence interval in the 10th, 50th, and 90th percentile, and mean of IOS values between each asthma group and the “never asthma” group; estimated using linear regression adjusted for height, age, and weight at examination.

	50 th percentile			90 th percentile			Mean		
	Diff.	(95% CI)	P-value	Diff.	(95% CI)	P-value	Diff.	(95% CI)	P-value
R₅₋₂₀									
Never asthma*	390.9		-	669.3		-	21.5		-
Early transient	0.1	-7.8;8.0	0.980	0.0	-19.1;19.1	0.999	2.0	-5.5;9.6	0.594
Early persistent	19.1	7.2;30.9	0.002	61.1	32.5;89.6	1	33.9	22.7;45.2	<0.001
Late onset							12.		
	14.5	5.6;23.4	0.001	24.6	3.2;46.0	0.024	6	4.2; 21.0	0.003
AX^{0.5}									

Never	80.2		-	109.0		-	15.		-
asthma*							2		
Early									
transient	0.6	-0.2;1.4	0.124	1.5	-0.9;3.9	0.212	0.6	-0.3;1.4	0.180
Early			<0.00			<0.00			<0.0
persistent	2.6	1.4;3.7	1	9.2	5.7;12.8	1	4.6	3.3;5.8	01
Late onset									<0.0
	0.9	0.1;1.8	0.031	4.2	1.5;6.9	0.002	2.1	1.2;3.1	01

*Adjusted value for the reference group.

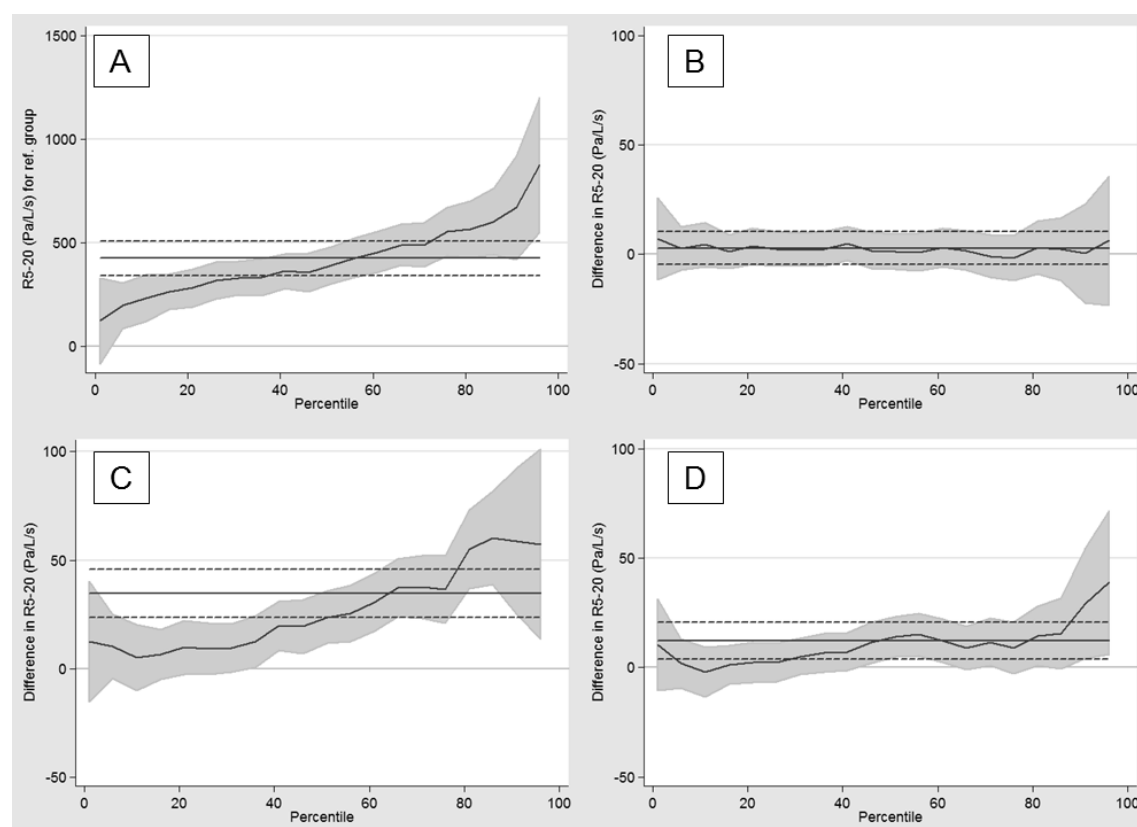


Table E1. Characteristics of children included in the BAMSE study who did or did not contribute with lung function data.

	Full BAMSE cohort	Took part in examination at 16 years of age		Acceptable spirometry at 16 years		Acceptable IOS at 16 years	
		No	Yes	No	Yes	No	Yes
No. of individuals	4089	1484	2605	2035	2054	1637	2452
Male n/N(%)	2065/4089 (50.5)	806/1484 (54.3)	1259/2605 (48.3)***	1132/2035 (55.6)	933/1121 (45.4)***	874/1637 (53.4)	1191/2452 (48.6)**
Parental atopy in Q0 n/N(%)	1200/4041 (29.7)	390/1460 (26.7)	810/2581 (31.4)**	537/2604 (26.8)	663/2035 (32.6)***	437/1613 (27.1)	763/2428 (31.4)**
Maternal smoking during pregnancy n/N(%)	527/4088 (12.9)	231/1484 (15.6)	296/2604 (11.4)***	304/2035 (14.9)	223/2053 (10.1)***	252/1637 (15.4)	275/2451 (11.2)***

Q0 = questionnaire at age 0 yrs. * $p < 0.05$ comparing “yes-group” with “no- group”, ** $p < 0.01$ comparing “yes-group” with “no- group”,

*** $p < 0.001$ comparing “yes-group” with “no- group”.

Table E2. Difference in adjusted mean spirometry values and 95% CI at 8 yrs between asthma groups and never asthma group (linear regression adjusting for height, age and weight at examination).

	FVC (ml)			FEV ₁ (ml)			FEV ₁ /FVC (%)			FEF50 (ml/sec)		
	Diff.	(95% CI)	P-value	Diff.	(95% CI)	P-value	Diff.	(95% CI)	P-value	Diff.	(95% CI)	P-value
Never asthma	ref			ref			ref			ref		
Early transient	10	-32;52	0.650	-21	-58;17	0.279	-1.3	-2.5;-0.2	0.023	-157	-265;-49	0.004
Early persistent												<0.00
	-15	-75;46	0.635	-86	-139;-32	0.002	-3.5	-5.2;-1.9	0.000	-375	-529;-222	1
Late onset	-9	-57;39	0.727	-24	-66;19	0.275	-0.7	-5.2;-1.9	0.271	-29	-152;93	0.639

Table E3. Estimated difference and 95% confidence interval in the 10th, 50th, and 90th percentile, and mean of IOS values between each asthma group and the “never asthma” group; estimated using linear regression adjusted for height, age, and weight at examination.

	50 th percentile			90 th percentile			Mean		
	Diff.	(95% CI)	P-value	Diff.	(95% CI)	P-value	Diff.	(95% CI)	P-value
R₅									
Never asthma*	1524.4		-	1914.5		-	365.5		-
Early transient	18.0	2.2;33.9	0.026	5.3	-23.2;33.8	0.716	13.9	1.1; 26.7	0.034
Early persistent	45.3	21.6;69.0	<0.001	92.0	49.5;134.5	<0.001	62.3	43.2;81.4	<0.001
Late onset	15.0	-2.8;32.7	0.098	31.0	-0.7;62.8	0.056	29.4	15.1;43.7	<0.001
R₂₀									
Never asthma*	1115.0		-	1363.2		-	344.0		-
Early transient	12.8	-0.1;25.7	0.052	-1.6	-26.2;22.9	0.897	11.8	1.1;22.6	0.031
Early persistent	20.0	0.8;39.3	0.042	48.5	11.9;85.1	0.009	28.3	12.3;44.4	0.001
Late onset	17.0	2.6;31.4	0.021	9.6	-17.8;37.0	0.494	16.8	4.8;28.8	0.006

1 ONLINE DATA SUPPLEMENT

2

3 **Asthma phenotypes and lung function up to age 16 years of age - the BAMSE cohort**

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6

7

1 **Methods**

3 **Measurements of lung function**

4 Forced expiratory spirometry with recordings of maximum expiratory flow volume curves
5 (MEFV) were performed at age 8 years using the 2200 Pulmonary Function Laboratory
6 (Sensormedics, Anaheim, Ca, USA). At age 16, lung function was assessed by spirometry and
7 a variant of the FOT called impulse oscillometry (IOS) using the Jaeger Masterscreen system
8 (Care Fusion, San Diego, Ca, USA). The same spirometry test protocols were used on both
9 occasions. Test subject performed at least three MEFV (Maximum Expiratory Flow Volume)
10 recordings in the sitting position, wearing a nose clip. The highest values of forced vital
11 capacity (FVC), forced expiratory volume in 1 sec (FEV₁), forced expiratory volume in 0.5
12 sec (FEV_{0.5}) and maximum expiratory flow when 50% of the FVC remains to be exhaled
13 (MEF₅₀) were extracted and used from analysis, provided that the subject's effort was coded
14 as being maximal by the test leader, the MEFV curve passed visual quality inspection, and
15 that the two highest FEV₁ and FVC readings were reproducible according to ATS/ERS (1).
16 IOS measures respiratory system impedance during tidal breathing by superimposing small air
17 pressure waves generated from a loudspeaker. Resistance (R), representing the energy
18 required to propagate the pressure wave through the airways, and reactance (X), representing
19 the viscoelastic properties of the respiratory system, were assessed for frequencies between 5
20 and 20 Hz (2). Measurement was performed during tidal breathing with the lips tightly sealed
21 around the mouthpiece and supporting cheeks with their hands. Signals free from artefacts
22 that lasted for at least 20 seconds were saved for analysis. At least two recordings were
23 performed per subject. Quality control was performed at the time for examination by visual
24 inspection of the waveforms, and given that coherence, which is a measure of testing
25 reliability, was >0,80 at 10 Hz, the mean value of R₅, R₂₀, R₅₋₂₀ and AX was used for analysis.

The IOS software extracts resistance (R) and reactance (X) in the range of 3-35Hz from the impedance. Resistance at a low frequency (e.g. R_5) is thought to represent properties of both proximal and distal airways, while resistance at higher frequency (e.g. R_{20}) represents proximal airway resistance. Frequency dependence of resistance i.e. R_{5-20} , may thus represent distal (small) airway resistance.(3) The portion of respiratory system impedance that is out of phase is called reactance (X). Reactance has two components with opposite numerical values to the resistive force to acceleration of the air column (inertance; I), and the other reflecting the elastic properties of the respiratory system expressed as capacitance (C). The frequency where these two opposing properties of the respiratory system are balanced is called the resonant frequency (f_{res}). In the frequency range of 5Hz to f_{res} , X represents capacitance mainly. In the presence of increasing small airway dysfunction X in the range 5Hz to f_{res} will be increasingly negative and f_{res} will increase. Goldman proposed to combine these responses to small airway obstruction in one index called AX (area of reactance), which is the area under the curve (AUC) of X with respect to frequency in the range of 5Hz to f_{res} , popularly called the “Goldman triangle”. While AX seems to be a more robust measure than X or f_{res} on their own, AX may boost the assessed response to small airway obstruction inappropriately compared to the FDR response because it multiplies two partly independent reactions of the airways. We therefore “linearize” AX by reporting its square root also (“ $AX^{0.5}$ ”). The IOS test was performed before spirometry according to clinical standards. The IOS system was calibrated each day using a 3l precision syringe and a reference resistance (0.20 kPa/l/s).

Definition of asthma groups

Based on their answers on the questionnaires, participants were grouped based on whether or not they had asthma and/or wheeze at the age of 1, 2, 4, 8, and 16 years. Wheeze was defined as one or more episodes of wheezing in the last 12 months. Asthma at age 1 and 2 years was

defined as at least 3 episodes of wheezing in the last 12 months, combined either with inhaled steroids or signs of hyper-reactivity (wheezing or severe coughing at exaltation and cold weather, or disturbing cough at night) without ongoing cold. At 4, 8 and 16 years of age, the definition of asthma was at least 3 episodes of wheezing in the last 12 months, or 1 episode if the child had been given inhaled steroids(4, 5).

Based on time of onset and duration of symptoms, children were categorized in groups as follows “never asthma” (reference group, never fulfilled any asthma criteria from birth to 16 years of age), “early transient asthma” (asthma between age 0-4 years, no wheeze after) “early persistent” (asthma in the first 4 years and asthma at 16 years), "late onset asthma” (no asthma at age 1, 2 or 4, but asthma at age 16).

Other definitions

Measurements of specific IgE antibodies

Blood samples were taken at the 16-year follow-up. Allergen-specific IgE antibodies were measured against a mix of common inhalant allergens (birch pollen, timothy grass pollen, mugwort pollen, house-dust mite, mould, and cat, dog, and horse dander) using the Pharmacia CAP System™ (Phadia AB, Uppsala, Sweden) in a certified laboratory (the Unit of Clinical Immunology and Allergy, Department of Medicine, Karolinska University Hospital). An IgE value greater than 0.35 kU/L was regarded as positive.

Parental atopy

Mother and/or father with doctor’s diagnosis of asthma and asthma medication and/or doctor’s diagnosis of hay fever in combination with furred pets- and/or pollen allergy at the time of questionnaire 0.

Any asthma medication

Regular or periodic use of short-acting beta-agonists, inhaled corticosteroids, combination therapies and/or leukotriene antagonists in the last 12 months.

Statistical analysis

Mean height and weight were compared between the reference group and asthma groups by the t-test. Comparisons for sensitization status, parental heredity, the mother's smoking habits during pregnancy, household smoking at 16 years of age, personal smoking and premature birth (defined as being born in gestation week 36 or earlier) were performed by the chi-square test.

The associations between asthma groups and spirometry variables were assessed with linear regression. Lung function variables were adjusted for age, height, weight and sex. The residuals were plotted and visually inspected to evaluate goodness of fit and possible presence of outliers and leverage points. To make full use of the repeated data sampling, a longitudinal analysis using mixed models was used. Time-dependent covariates included in the model were height, weight and age. Asthma group and sex were time-invariant covariates. To assess potential variations of the effect of asthma on lung function over time, an interaction term between time and asthma group was included in the model.

The distribution of the values of IOS was skewed to the right. We utilized quantile regression to estimate associations between asthma and IOS variables at the 10th, 50th and 90th percentiles, while adjusting by sex, height, weight and age. Quantile regression allowed us to estimate multiple points of the distribution rather, not only at the mean. This regression method is more robust to outliers or skewness of the distribution of the dependent variable than ordinary mean

1 regression. The interpretation of the quartile regression coefficients is similar to that of those
2 of linear regression, except that the parameter describes the difference in lung function
3 percentiles, rather than its mean. For completeness, we also performed linear regression on
4 the mean for IOS data. P-values of < 0.05 were considered statistically significant. Analyses
5 were performed with the Stata 12.1 software package (StataCorp LP, College Station, TX,
6 USA). The BAMSE study was approved by the Ethical Committee of Karolinska Institute,
7 Stockholm, and all parents provided consent for their child to participate in the study.

Results

Characteristics of participating and non-participating children are presented in E-Table 1 in the online supplement. In brief, those participating in the 16 year follow-up were more likely than non-participating children to be female, have a parent with atopic disease and have a mother that smoked during pregnancy, compared with non-participants.

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