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Characteristics of asthma and airway hyper-responsiveness after premature birth

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Asthma-like symptoms and airway hyper-responsiveness (AHR) are frequently reported in children subsequent to premature birth and bronchopulmonary dysplasia (BPD). There is limited knowledge on the mechanisms underlying these respiratory manifestations. Generally, childhood asthma and AHR is described within a context of inheritance, allergy and eosinophilic airway inflammation, and often in relation to cigarette exposures. We investigated these factors in relation to current asthma and AHR in a population-based cohort of 81 young people, born with gestational age ≤ 28 wk or birth weight ≤ 1000 g, and in a matched term-born control population. In the pre-term population, asthma and AHR were additionally studied in relation to neonatal respiratory morbidity. At follow up, more pre-term than control subjects had asthma. Forced expiratory volume in first second (FEV_1) was reduced, AHR was substantially increased, and the level of the urinary leukotriene metabolite E4 ($U\text{-}LTE_4$) was increased in the pre-term population compared to the term-born. In control subjects, asthma and AHR was associated with a pattern consistent with inheritance, allergy, airway inflammation, and cigarette exposures. In the pre-terms, asthma and AHR was either unrelated or *less* related to these factors. Instead, AHR was strongly related to a neonatal history of BPD and prolonged requirement for oxygen treatment. In conclusion, asthma and AHR subsequent to extremely premature birth differed from typical childhood asthma with respect to important features, and AHR was best explained by neonatal variables. These respiratory manifestations thus seem to represent a separate clinical entity.

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Asthma is a complex syndrome with many phenotypes in both children and adults (1). The disease is characterized by a set of symptoms, variable airflow obstruction, airway hyper-responsiveness (AHR), and a characteristic airway inflammation (2). Asthma may begin in infancy, and genetic as well as environmental factors contribute to its start and progression (1–3). The most important cause of chronic lung disease in infancy is premature birth with subsequent development of bronchopulmonary dysplasia (BPD) (4, 5). Premature birth and BPD is furthermore associated with asthma-like symptoms, airflow obstruction and AHR in later childhood and early adult life (6–13). The pathogenesis of BPD is multifactorial and not well established. The initial phase is characterized by an inflammatory process (4, 14), which is not fully understood. Beyond infancy, the pathophysiology of BPD is poorly described. While an eosinophil-driven inflammatory process is central in asthma (2), we know little about the nature and relevance of airway inflammation in long-term BPD survivors. Recently, Baraldi et al. (15) described low levels of exhaled nitric oxide (eNO) in school aged BPD survivors compared to FEV_1-matched asthmatic children. This suggests different aetiologies for these two types of pediatric obstructive lung disease. However, due to a similar clinical presentation, asthma

medication is frequently prescribed (5–7), despite few studies documenting effect (16, 17).

Typically, pediatric asthma and AHR are associated with inheritance, allergy, eosinophilic airway inflammation, and exposures to cigarette smoking. The purpose of this population-based, long-term follow-up study was to investigate the relevance and importance of these variables in relation to asthma and AHR occurring in young people after extremely premature birth and BPD. In the pre-term population, asthma and AHR were also studied in relation to neonatal variables.

Methods

Subjects and study design

Pre-terms consisted of two population-based cohorts of subjects with gestational age ≤ 28 wk or birth weight ≤ 1000 g, born consecutively within the study region in western Norway in the years 1982–1985 (first birth-cohort) and 1991–1992 (second birth-cohort). Medical care was provided by a senior medical staff that was largely the same in the two periods. Pre-terms were considered enrolled when admitted to the neonatal department. Neonatal data were accessed from hospital charts. Gestational age was assessed according to department policy, being identical in the inclusion periods (18). Neonatal respiratory morbidity was classified as suggested by Jobe and Bancalari (4), i.e., mild or moderate/severe BPD if requiring supplemental oxygen at a post-natal age ≥ 28 days or a post-menstrual age (PMA) ≥ 36 wk, respectively. For each pre-term, the temporally nearest term-born subject of the same gender with birth weight between 3.0 and 4.0 kg (Norwegian 10th and 90th centiles) was recruited as control. If this subject refused to participate, the next-born subject was approached, and so on, until one term-born and willing control had been recruited for each enrolled pre-term. All subjects were seen twice within 2 wk in 2001/2002 at the pediatric Cardio-Respiratory Laboratory at Haukeland University Hospital. On the first visit, the questionnaire from the *International Study of Asthma and Allergy in Childhood* (ISAAC) (19) was filled in by all subjects and their guardians. Subsequently, a full medical examination was done by one of two consultant pediatricians (TH or BTS). A methacholine provocation test was done on the first visit, while tests for exercise induced asthma (EIA) and reversibility to salbutamol was done on the second. Spirometry with flow volume loops was done on both visits. No subjects were examined within 2 wk of a respiratory tract infection or an asthma event. To obtain adequate and comparable conditions for bronchial provocation and reversibility testing, subjects using asthma medication were instructed to stop prior to testing as appropriate, i.e., inhaled corticosteroids and short acting β_2 agonists from the day before, and long acting β_2 agonists from 2 days before. The Regional Ethics Committee approved the study. Informed written consent was obtained from all participating subjects and their parents.

Lung function measurements and definitions

Spirometries were performed according to standard quality criteria (20) with a Vmax 22 spirometer (SensorMedics Inc., Anaheim, USA). Forced expiratory volume in the first second (FEV_1) was expressed as percentage of the predicted values (FEV_1%) (21). Mean values from the two test days were used for statistical analysis. Methacholine provocation test (PD_{20}) was performed (22) with an inhalation-synchronised, dosimetric nebulizer, Spira Elektra 2® (Respiratory Care Centre, Hameenlinna, Finland), providing baseline $FEV_1 \geq 65\%$ predicted. Methacholine chloride was administered in doubling doses until a 20% reduction in FEV_1 was obtained, or until a final cumulative dose of 22.3 μmol had been given. A dose-response slope (DRS) was calculated as the ratio between the maximum percentage decline in FEV_1 from baseline and the total administered dose of methacholine (in μmol) when the test was terminated (%/μmol) (23). The DRS was $\log_{10}$ transformed (logslope) for statistical analysis. The EIA test was performed within a setting of simultaneous measurement of gas exchange and exercise flow volume loops. After establishment of a baseline FEV_1, subjects were running to exhaustion on a treadmill (Woodway ELG 70, Weil am Rhein, Germany), wearing a facemask and connected to a Vmax 29 cardiopulmonary exercise unit (SensorMedics Inc., Anaheim, USA). To facilitate familiarization with the treadmill in children with potential for various disabilities (5), a modified Bruce protocol was used. The initial phase of this protocol is slow, comfortable and reassuring, but during the last 5 min, heart rate and minute ventilation exceeded recommended levels (24) in most subjects. FEV_1 was measured after 1, 3, 6, and 10 min. The EIA test was considered positive if the largest decline in FEV_1 relative to baseline exceeded 12%. After approximately 20 min, 100 μg salbutamol per 10 kg bodyweight was administered with a metered dose inhaler (Ventoline®) via a plastic spacer (Volumatic®). FEV_1 was measured after 10 min. Reversibility to

salbutamol was considered significant if the largest increase in FEV_1 relative to baseline exceeded 12%.

The main criterion for a diagnosis of current asthma was a history of at least one episode of wheeze within the previous 12 months. In addition we required *either* a current doctor's diagnosis of asthma at inclusion to the study, *or* support from a positive EIA test or significant reversibility to salbutamol. Children were considered to have a positive family history of asthma, hay fever or atopic dermatitis if clinical symptoms or a verified diagnosis was reported in at least one first-degree relative.

Allergy and markers of airway inflammation

Skin prick tests against house dust mite (*D. Farinae* and *D. Pteronyssinus*), animal dander (cat, dog, and horse), pollens (timothy, birch, and mugwort) and moulds (*Alternaria* and *Cladosporium*) were done with standard extracts (ALK-Abello AS, Hørsholm, Denmark) in accordance with European guidelines (25). Perennial allergies are associated with decreased FEV_1 and increased AHR in the study region (26) and hence, subjects testing positive to house dust mite, animal dander or moulds were analyzed separately (perennial allergics). Early morning urine was kept cooled until aliquoting and freezing (−80 °C). Urinary creatinine was analyzed with Jaffes reaction. Urinary eosinophilic protein X (U-EPX) was analyzed with a specific radioimmunoassay (Pharmacia, Uppsala, Sweden) and expressed as μg/mmol creatinine, while urinary leukotriene E4 (U-LTE_4) was analyzed with ACE^{TM} competitive enzyme immunoassay (Cayman Chemical, Ann Arbor, USA) and expressed as ng/mmol creatinine. Serum eosinophilic cationic protein (S-ECP) was analyzed with Pharmacia CAP System ECP^{TM} and expressed in μg/l (Pharmacia Diagnostics AB, Uppsala, Sweden). Blood eosinophils (B-Eos) were counted ($\times 10^9$/l) in a Bayer Technicon H3^{TM} (Bayer, Leverkusen, Germany). Since undisputed upper reference values are difficult to establish from the literature, we chose to analyze subjects with values in the upper quartiles separately (High U-EPX, U-LTE_4, S-ECP, and B-Eos). The respective upper quartiles were determined based on data from the pre-term and term-born population, separately.

Statistical methods

Non-paired group comparisons were performed with Pearson's chi-square test, Student's *t*-test, Levene's test for equality of variances or Kruskal–Wallis' test, as appropriate. Stepwise multivariate linear regression analyses were applied to explore effects from the explanatory variables on current logslope in pre-term and control subjects. Analyses of interaction terms were used to test if explanatory variables influenced pre-terms and matched controls differently with respect to outcome variables (27). Paired data (Tables 3 and 4) were analyzed with the linear mixed model procedure of SPSS (28). Paired odds ratios (OR) and confidence intervals (CI) were calculated with special programming in Maple. The distribution of the DRS to methacholine was regarded as lognormal (23), while the variables used to describe airway inflammation were assessed non-parametrically (14). The level of significance was 0.05 and SPSS Version 11.0 was used for computations.

Results

Subjects

Altogether, 130 eligible premature subjects were born alive within the study-region, of whom 87 (67%) survived the neonatal period, and one died after hospital discharge. Five pre-terms were inaccessible and hence, 81 (94%) were examined. Sixty-one (75%) of the primarily 81 invited term-born control subjects responded positively. In cases of refusals, the next-born subject was asked, and at average 1.3 subjects had to be approached in order to recruit one willing control for each included pre-term. One control was excluded for medical reasons. All but two participating subjects were Caucasians. Mean gestational age [standard deviation (s.d.)] of the pre-terms was 27.1 (1.6) weeks and mean birth weight (s.d.) was 979 (200) g compared to 3494 (300) g for term-born control subjects. The five pre-terms not examined, had similar neonatal characteristics to those who were examined. Basic neonatal characteristics of the examined pre-terms are given in Table 1.

At examination, mean age (s.d.) was 17.7 (1.2) years in the first birth-cohort and 10.6 (0.4) years in the second. Two pre-terms with spastic tetraplegic cerebral palsy (CP) and scoliotic thorax restriction were excluded from the statistical analyses. Except for one control and one pre-term parent, the questionnaires were adequately filled in by all. Satisfactory and reproducible flow volume loops were obtained for all subjects. The coefficient of variation for repeated measurements of FEV_1 was 4.7%, and the variance was not significantly different in pre-term and term-born control subjects (Levene's test, $p = 0.370$).

Table 1. Neonatal characteristics of the prematurely born children

		BPD			
	Controls (n = 81)	Non (n = 19)	Mild (n = 38)	Moderate-severe (n = 24)	p Value
Number of females (% of group)	42 (52)	12 (63)	20 (53)	10 (42)	0.152*
Gestational age in weeks (s.d.)	–	28.3 (1.5)	26.8 (1.4)	26.4 (1.4)	<0.001†
Birth weight in grams (s.d.)	3494 (300)	1115.1 (158.5)	981.0 (200.2)	868.8 (166.0)	<0.001†
Days with ventilator treatment (range)	–	0.5 (0–4.8)	7.2 (0–40.0)	13.8 (0.7–54.5)	<0.001‡
Days with oxygen therapy (range)	–	5 (1–26)	42 (28–71)	85 (55–257)	<0.001‡
FEV_1% predicted (95% CI)	98.6 (96.3, 100.8)	94.7 (89.4, 100.1)	89.6 (86.2, 92.9)	81.4 (77.1, 85.7)	<0.001†
DRS to methacholine§ (95% CI)	1.6 (1.1, 2.3)	3.9 (1.7, 9.2)	5.9 (3.0, 11.8)	15.6 (6.6, 36.9)	0.022†
Asthma in the family (% of group)	21 (26%)	6 (32%)	12 (32%)	6 (25%)	0.889*
Intrauterine cigarette exposure (% of group)	19 (23%)	9 (47%)	18 (47%)	8 (33%)	0.378*

BPD, bronchopulmonary dysplasia; CI, confidence interval.
p Values assess trends *within* the pre-term population according to the severity of neonatal BPD.
*Chi-square test for trend.
†Linear regression.
‡Kruskal–Wallis' test.
§Dose-response slope to methacholine, expressed as geometric means (95% CI).

Assessment of PD_{20} was not done in seven pre-terms and in two control subjects for the following reasons: Six pre-terms had FEV_1 < 65%, two children could not cope, and one blind girl declined. Premature birth was associated with maternal cigarette smoking in pregnancy (paired OR: 3.0; 95% CI: 1.3, 7.7; p = 0.007) and possibly with a history of asthma reported by the mother (paired OR: 4.5; 95% CI: 0.9, 42.8; p = 0.065). At follow up, the pre-terms had significantly lower mean FEV_1% and a higher logslope compared to those born at term (Table 1) (p < 0.001, both estimates).

Characteristics of current asthma (Table 2)

There were 22 (27%) asthmatic pre-terms and ten (12%) asthmatic control subjects (p = 0.018). The asthma diagnosis was made on the basis of wheeze and a current diagnosis at inclusion in all but one control subject and four pre-terms. These five were all wheezers, responded positively to the EIA test (n = 3) or the reversibility test (n = 2), and were all offered asthma medication after the study. The distribution of asthma was similar in both birth-cohorts, i.e., respectively 26% vs. 28% in the first and second pre-term birth-cohort and 11% vs. 14% in the first and second control birth-cohort. The distribution of asthma was not related to gender. In the term-born population, a diagnosis of asthma was associated with more allergy, more eosinophilic cells and higher levels of U-EPX compared to those without asthma, and there were also non-significant numerical trends for higher S-ECP and U-LTE4 in term-born asthmatics. DRS were significantly increased in term-born asthmatics. In the pre-term population, these associations were all non-significant, and numerically either weaker or absent. The levels of DRS, B-Eos and U-EPX

Table 2. Characteristics of current asthma in pre-terms and term-born control subjects

	Pre-terms (n = 81)			Controls (n = 81)		
	Asthma (n = 22)	Non-asthma (n = 59)	p Value*	Asthma (n = 10)	Non-asthma (n = 71)	p Value*
DRS†	9.8 (3.6, 26.6)	6.2 (3.7, 10.3)	0.368	12.6 (4.7, 34.3)	1.2 (0.9, 1.7)	<0.001
Allergy‡	5 (22.7%)	5 (8.5%)	0.125	6 (60.0%)	6 (8.5%)	<0.001
B-Eos	0.130 (0.064, 0.221)	0.100 (0.065, 0.164)	0.259	0.348 (0.229, 0.501)	0.121 (0.084, 0.184)	<0.001
S-ECP	12.2 (8.8, 18.1)	10.7 (6.9, 18.8)	0.482	17.1 (12.8, 29.0)	10.7 (7.5, 19.6)	0.071
U-EPX	34.4 (27.6, 50.9)	47.5 (31.5, 86.5)	0.131	77.4 (42.3, 95.6)	37.1 (24.7, 52.8)	0.032
$U\text{-}LTE_4$	40.2 (23.0, 60.1)	27.9 (20.5, 48.2)	0.322	39.4 (22.5, 51.2)	23.2 (15.3, 33.3)	0.068

B-Eos, eosinophilic granulocytes ($\times 10^9$/l); S-ECP, serum eosinophilic cationic protein (μg/l); U-EPX, urinary eosinophilic protein X (μg/mmol creatinine); $U\text{-}LTE_4$, urinary leukotriene E4 (ng/mmol creatinine), all reported as median values (25–75 centiles).
Current asthma was principally defined by recent wheeze and a doctor's diagnosis at study inclusion.
*Relations between asthmatics and non-asthmatics in the pre-term and term-born population, tested with chi-square test (Allergy), *t*-test ($\log_{10}$ DRS) or Mann–Whitney test.
†Dose-response slope to methacholine, geometric means (95% CI).
‡Number (%) in the respective groups with perennial allergies.

Table 3. Airway hyper-responsiveness in relation to allergy and markers of airway inflammation

	DRS to methacholine, geometric means (95% CI)				Tests of interaction, p values†
	Pre-terms (n = 74)	p*	Controls (n = 79)	p*	
Allergy‡					
Yes	10.2 (2.4, 65.5)	0.317	4.4 (1.4, 13.9)	0.019	0.557
No	6.4 (3.4, 10.3)		1.4 (0.9, 2.0)		
B-Eos					
High§	8.6 (2.9, 25.8)	0.642	5.2 (2.3, 11.8)	<0.001	0.049
Low	6.7 (3.9, 11.5)		1.2 (0.8, 1.7)		
S-ECP					
High§	3.3 (1.2, 9.3)	0.088	2.4 (1.1, 5.2)	0.329	0.074
Low	8.6 (5.0, 14.7)		1.6 (1.0, 2.4)		
U-EPX					
High§	4.4 (1.6, 12.1)	0.281	2.7 (1.1, 6.6)	0.014	0.017
Low	8.1 (4.6, 14.0)		1.0 (0.7, 1.4)		
U-LTE_4					
High§	5.6 (1.9, 16.6)	0.634	2.2 (0.9, 5.2)	0.090	0.208
Low	7.3 (4.3, 12.5)		1.1 (0.8, 1.6)		

B-Eos, whole blood count of eosinophilic granulocytes; S-ECP, serum eosinophilic cationic protein; U-EPX, urinary eosinophilic protein X; U-LTE_4, urinary leukotriene E4; DRS, Dose-response slope.
*Independent samples *t*-test, assessing equality in the preceding column.
†Paired interaction analyses, testing if allergy and markers of airway inflammation were differently associated with DRS in the pre-term population compared to the matched term-born.
‡Perennial allergics (10 pre-terms and 12 term-born controls).
§*High* refers to subjects with values in the upper quartile of the pre-term and term-born population, respectively.

were significantly *less* associated with a diagnosis of asthma in the pre-term population than in the term-born population (tests of interaction, p = 0.010, 0.014, and 0.017, respectively).

Current AHR vs. allergy and markers of airway inflammation (Table 3)

The distribution of perennial allergies, U-EPX, S-ECP and B-Eos were similar in pre-terms and matched term-born controls, while U-LTE_4 was increased in pre-terms (geometric mean 36.3 ng/mmol creatinine vs. 24.6 ng/mmol creatinine; p = 0.001). In term-born control subjects, perennial allergies, high B-Eos and high U-EPX were associated with a significantly higher logslope, and for U-LTE_4 there was a non-significant but similar numerical trend. In the pre-term population, the association between these same explanatory variables and logslope were all non-significant, and numerically either weaker or absent. A stepwise multivariate linear regression model, including perennial allergies, high B-Eos, high S-ECP, high U-EPX and high U-LTE4 was applied separately in the pre-term and the term-born population. The only remaining significant explanatory variable was high B-Eos in the term-born control population, explaining 16% of the variability in logslope (p < 0.001). Paired interaction analyses revealed that high B-Eos and high U-EPX were significantly *less* associated with AHR in the pre-term population compared to the term-born.

Current AHR vs. familial atopic disease and cigarette exposures (Table 4)

In term-born adolescents, there was a significant association between self-reported smoking and increased logslope. With respect to intrauterine cigarette exposure and a family history of asthma or atopic dermatitis, there were non-significant numerical trends for increased logslope in control subjects. In the pre-terms, these associations were numerically either weaker or absent. Effects from these explanatory variables were not significantly different in the pre-term and the term-born populations (paired interaction analyses, p values not shown).

Table 4. Airway hyper-responsiveness in relation to the family history of asthma, atopic dermatitis and cigarette exposures

	Pre-terms (n = 74)			Controls (n = 79)		
	DRS (95% CI)*		p†	DRS (95% CI)*		p†
Asthma in family‡	Yes (n = 21)	9.5 (4.4, 20.4)	0.323	Yes (n = 24)	2.6 (1.1, 6.2)	0.073
	No (n = 60)	5.8 (3.3, 10.1)		No (n = 57)	1.3 (0.9, 1.8)	
Atopic dermatitis in family‡	Yes (n = 31)	5.1 (2.2, 12.0)	0.467	Yes (n = 23)	2.2 (1.2, 3.9)	0.110
	No (n = 50)	7.4 (4.3, 12.7)		No (n = 58)	1.2 (0.8, 1.8)	
Prenatal maternal cigarette exposure	Yes (n = 35)	9.1 (4.7, 17.3)	0.228	Yes (n = 19)	2.8 (1.3, 6.1)	0.056
	No (n = 46)	5.2 (2.8, 9.8)		No (n = 62)	1.3 (0.9, 1.8)	
Self-reported smoking§	Yes (n = 15)	4.3 (1.7, 10.8)	0.833	Yes (n = 14)	2.7 (1.0, 7.5)	0.038
	No (n = 31)	3.8 (1.7, 8.1)		No (n = 32)	0.9 (0.6, 1.6)	

*Dose-response slope to methacholine, geometric means (95% CI).
†Independent samples *t*-test, assessing equality in the preceding column.
‡Parents or siblings.
§Self reported cigarette smoking, assessed in the second birth-cohort.

Current AHR in pre-terms vs. the neonatal history (Fig. 1)

Pre-terms were significantly more responsive to methacholine than control subjects; geometric mean DRS (95% CI) being 7.0 (4.5, 11.0) and 1.6 (1.1, 2.3) for pre-terms and matched controls, respectively ($p < 0.001$). Within the pre-term population, there was a significant trend for increased methacholine responsiveness parallel to more severe neonatal BPD (Table 1, Fig. 1). FEV_1 decreased 20% from baseline at a cumulative dose of < 1.0 μmol methacholine in 67% of pre-terms with moderate/severe BPD, in 32% of pre-terms with mild BPD, in 26% of pre-terms without BPD and in 8% of control subjects. In stepwise multivariate linear regression models employed in the pre-term population, variables relating to familial asthma and atopic dermatitis, current allergy and markers of airway inflammation and intrauterine or self-inflicted cigarette exposures did not predict current logslope. When adding the neonatal variables *birthweight* (g), *gestational age* at birth (wk), duration (days) of *oxygen treatment* and duration (days) of *ventilator treatment* to the model, only *oxygen treatment* remained significant ($p < 0.001$), with an adjusted r^2 of 0.20. Since methacholine responsiveness is known to be related to FEV_1, and prolonged *oxygen treatment* was related to low FEV_1% (Table 1), we added FEV_1% to the model. The effect from *oxygen treatment* was reduced, but remained significant ($p = 0.032$). In this final model, also FEV1% was a significant variable ($p = 0.028$), and r^2 increased to 0.25.

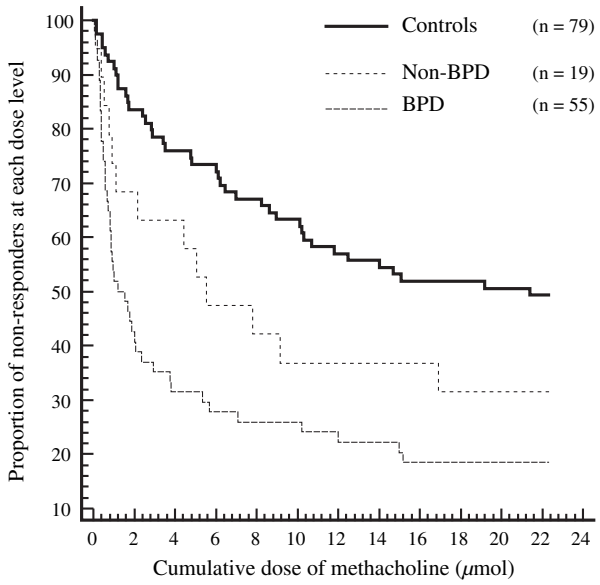

Fig. 1. Methacholine responsiveness in young people born extremely pre-term, classified by neonatal bronchopulmonary dysplasia, and in matched term-born control subjects. The x-axis represents the total cumulative dose of methacholine given to each subject, censored at the maximum administered dose of 22.3 μmol. The y-axis depicts the proportion of non-responders at any given dose. In this graph, BPD was defined as neonatal requirement for supplemental oxygen ≥28 days.

Discussion

In term-born control subjects, asthma and AHR were associated with a pattern of inheritance, allergy, and cigarette exposures and, to a varying extent, increased levels of airway inflammatory markers. In the pre-term population, these associations were either weaker or absent, but AHR was instead associated with neonatal BPD and prolonged requirement for oxygen treatment. The level of U-LTE_4 was increased in the pre-term population, but not significantly related to a clinical diagnosis of asthma or to AHR.

The study was part of an extensive regional follow up of young people who were born extremely prematurely. Despite its retrospective design, the regional organization of medical care, and an identical algorithm for determination of gestational age, ensured that the included pre-terms would have been the same subjects, had they been identified prospectively. The survival rate of 67% was similar to results reported for comparable populations in comparable institutions at the time (29). Low dropout rates ensured that the population-based sample was representative. Since outcome measures could be assessed only in surviving subjects, a relation between explanatory variables and death could theoretically introduce bias. We are, however, unaware of reports suggesting that mortality from premature birth is associated with maternal smoking of cigarettes in pregnancy or atopy in the family. It also seems unlikely that potential risk factors should influence death rates without having impact on morbidity (Table 1). Potential effects from age and gender on outcome measures were avoided through the matched design of the study. Sample size calculations had been done with respect to the major outcome variable of the study, which was FEV_1%. In this presentation, issues relating to relatively small subgroups are discussed (e.g., asthmatic control subjects), and some of the negative conclusions should therefore be treated with caution. Despite these reservations, the overall conclusions appeared statistically valid. It nevertheless seems clear, that large-scale follow-up studies should be carried out to further characterize long-term lung sequelae from extremely premature birth.

The results reported from this study, regarding asthma and AHR in term-born control subjects

and the relations to inheritance, allergy, airway inflammation and cigarette exposures, are in agreement with reports from unselected pediatric populations (2, 3). The importance and relevance of these features for asthma and AHR after premature birth, has not been established. The importance of inheritance has been addressed in several studies with diverging conclusions (9, 30, 31). In this study, the impact from familial asthma and atopy on AHR tended to be *less* in pre-terms compared to term-born controls. The relevance of concurrent allergy and markers of airway inflammation have been assessed in few studies (6, 12, 15). In this study, airway inflammation seemed to explain *less* of asthma and AHR in pre-terms compared to term-born controls. Thus, while asthma and AHR in term-born controls were characterized by a pattern consistent with inheritance, allergy and eosinophilic inflammation, this was not the case in pre-terms. Our data are supported by reports describing low levels of eNO in school aged BPD survivors (15), less atopy in pre-term wheezers compared to full term wheezers (12) and modest or no therapeutic effect from inhaled corticosteroids (16, 17). Therefore, eosinophilic airway inflammation does not appear as a likely mechanism to explain asthma and AHR after pre-term birth. Current AHR in our pre-term population was, however, strongly and significantly associated with a neonatal history of BPD and prolonged requirement for oxygen supplementation (Table 1, Fig. 1). This association was partly mediated through an association with FEV_1%, but remained significant after adjustment. Interestingly, the substantial increase in AHR in pre-terms was not confined to subjects with a diagnosis of asthma, contrasting the findings in term-born controls. Our results suggest a chronic pattern and indicate that structural sequelae in the airways or in the pulmonary interstitium may by causal factors, rather than ongoing inflammation. There are several ways in which premature birth or its treatment may injure the immature bronchi and the interstitial architecture of a developing lung (32, 33). Whether the association between current AHR and prolonged neonatal oxygen exposure represents a noxious effect of oxygen *per se* or is just a marker of other lung injury remains to be established. Brusasco recently reviewed structural factors that may modulate airway narrowing when assessing AHR (34).

The level of U-LTE_4 was significantly increased in pre-terms compared to term-born controls. We were however, unable to demonstrate significant associations with asthma or AHR, as defined in the present study. High levels of U-LTE_4 are described in the initial phase of BPD (14) and leukotriens may conceivable also play a role in long-term survivors after premature birth, and in the pathophysiology of BPD beyond infancy.

Neither intrauterine cigarette exposure nor active smoking in adolescents influenced current AHR in pre-terms. McLeod et al. (35) made a similar observation, in that post-natal maternal smoking did not influence FEV_1/FVC in a very low birth weight cohort, while a significant negative effect was observed in control children. Thus, at this stage it is difficult to argue that subjects born pre-term are more susceptible to pulmonary injury from cigarette smoking than those born at term. However, a low mean FEV_1% in pre-term adolescents along with various factors associated with a steeper age related decline in adulthood (7), should definitely encourage active guidance against all 'lung-hostile' activities.

Conclusions

Asthma and AHR occurring in young people after extremely premature birth did not fit within the established paradigm for ongoing asthma, and AHR was better explained by the extent of neonatal BPD and prolonged requirement for oxygen treatment. Structural sequelae, rather than active inflammation, appear as important mechanisms underlying these respiratory manifestations, which may represent a separate clinical entity. This knowledge should influence our approach to these young people.

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References

1. Martinez FD. Development of wheezing disorders and asthma in preschool children. Pediatrics 2002: 109: 362–7.
2. National Institute of Health. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. NHLBI/WHO Workshop Report. Publication No. 95-3659, 2002.
3. Lodrup Carlsen KC, Carlsen KH. Effects of maternal and early tobacco exposure on the development of asthma and airway hyperreactivity. Curr Opin All Clin Immunol 2001: 1: 139–43.
4. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med 2001: 163: 1723–9.
5. Allen J, Zwerdling R, Ehrenkranz R, et al. Statement on the care of the child with chronic lung disease

of infancy and childhood. Am J Respir Crit Care Med 2003: 168: 356–96.
6. Mai XM, Gaddlin PO, Nilsson L, et al. Asthma, lung function and allergy in 12-year-old children with very low birth weight: a prospective study. Pediatr All Immunol 2003: 14: 184–92.
7. Halvorsen T, Skadberg BT, Eide GE, Roksund OD, Carlsen KH, Bakke P. Pulmonary outcome in adolescents of extreme preterm birth: a regional cohort study. Acta Paediatr 2004: 93: 1294–300.
8. Pelkonen AS, Hakulinen AL, Turpeinen M. Bronchial lability and responsiveness in school children born very preterm. Am J Respir Crit Care Med 1997: 156: 1178–84.
9. Eber E, Zach MS. Long term sequelae of bronchopulmonary dysplasia (chronic lung disease of infancy). Thorax 2001: 56: 317–23.
10. Doyle LW, Cheung MM, Ford GW, Olinsky A, Davis NM, Callanan C. Birth weight <1501 g and respiratory health at age 14. Arch Dis Child 2001: 84: 40–4.
11. Gross SJ, Iannuzzi DM, Kveselis DA, Anbar RD. Effect of preterm birth on pulmonary function at school age: a prospective controlled study. J Pediatr 1998: 133: 188–92.
12. Siltanen M, Savilahti E, Pohjavuori M, Kajosaari M. Respiratory symptoms and lung function in relation to atopy in children born preterm. Pediatr Pulmonol 2004: 37: 43–9.
13. Chan KN, Noble-Jamieson CM, Elliman A, Bryan EM, Silverman M. Lung function in children of low birth weight. Arch Dis Child 1989: 64: 1284–93.
14. Sheikh S, Null D, Gentile D, et al. Urinary leukotriene E(4) excretion during the first month of life and subsequent bronchopulmonary dysplasia in premature infants. Chest 2001: 119: 1749–54.
15. Baraldi E, Bonetto G, Zacchello F, Filippone M. Low exhaled nitric oxide in school-age bronchopulmonary dysplasia children with airflow limitation. Am J Respir Crit Care Med 2005: 171: 68–72.
16. Pelkonen AS, Hakulinen AL, Hallman M, Turpeinen M. Effect of inhaled budesonide therapy on lung function in schoolchildren born preterm. Respir Med 2001: 95: 565–70.
17. Chan KN, Silverman M. Increased airway responsiveness in children of low birth weight at school age: effect of topical corticosteroids. Arch Dis Child 1993: 69: 120–4.
18. Yudkin PL, Aboualfa M, Eyre JA, Redman CW, Wilkinson AR. New birthweight and head circumference centiles for gestational ages 24 to 42 weeks. Early Hum Dev 1987: 15: 45–52.
19. ISAAC Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Lancet 1998: 351: 1225–32.
20. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests, European community for steel and coal. Official statement of the European respiratory society. Eur Respir J Suppl 1993: 16: 5–40.
21. Quanjer PH, Borsboom GJ, Brunekre B, et al. Spirometric reference values for white European children and adolescents: Polgar revisited. Pediatr Pulmonol 1995: 19: 135–42.
22. Nieminen MM, Lahdensuo A, Kellomaeki L, Karvonen J, Muittari A. Methacholine bronchial challenge using a dosimeter with controlled tidal breathing. Thorax 1988: 43: 896–900.
23. O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine. An approach suitable for population studies. Am Rev Respir Dis 1987: 136: 1412–7.
24. Carlsen KH, Engh G, Mork M. Exercise-induced bronchoconstriction depends on exercise load. Respir Med 2000: 94: 750–5.
25. The European Academy of Allergology and Clinical Immunology. Position paper: allergen standardization and skin tests. Allergy 1993: 48(Suppl.): 48–82.
26. Omenaas E, Bakke P, Eide GE, Elsayed S, Gulsvik A. Serum house dust mite antibodies: predictor of increased bronchial responsiveness in adults of a community. Eur Respir J 1996: 9: 919–25.
27. Pocock SJ, Collier TJ, Dandreo KJ, et al. Issues in the reporting of epidemiological studies: a survey of recent practice. BMJ 2004: 329: 883.
28. SPSS Inc. SPSS 11.0 Syntax Reference Guide. 2001: II: 136–51.
29. Hack M, Fanaro AA. Outcomes of children of extremely low birthweight and gestational age in the 1990's. Early Hum Dev 1999: 53: 193–218.
30. Chan KN, Noble-Jamieson CM, Elliman A, Bryan EM, Aber VR, Silverman M. Airway responsiveness in low birthweight children and their mothers. Arch Dis Child 1988: 63: 905–10.
31. Bertrand JM, Riley SP, Popkin J, Coates AL. The long-term pulmonary sequelae of prematurity: the role of familial airway hyperreactivity and the respiratory distress syndrome. N Engl J Med 1985: 312: 742–5.
32. Thibeault DW, Mabry SM, Ekekezie II, Zhang X, Truog WE. Collagen scaffolding during development and its deformation with chronic lung disease. Pediatrics 2003: 111: 766–76.
33. Denis D, Fayon MJ, Berger P, et al. Prolonged moderate hyperoxia induces hyperresponsiveness and airway inflammation in newborn rats. Pediatr Res 2001: 50: 515–9.
34. Brusasco V, Pellegrino R. Complexity of factors modulating airway narrowing in vivo: relevance to assessment of airway hyperresponsiveness. J Appl Physiol 2003: 95: 1305–13.
35. Mcleod A, Ross P, Mitchell S, et al. Respiratory health in a total very low birthweight cohort and their classroom controls. Arch Dis Child 1996: 74: 188–94.