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Nature and severity of lung function abnormalities in extremely preterm children at 11y

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Running head: EPICure11: Detailed Respiratory FU at 11

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ABSTRACT

Advances in neonatal care have resulted in increased survival of children born extremely preterm (EP). Nevertheless the incidence of bronchopulmonary dysplasia and long-term respiratory morbidity remains high. We investigated the nature of pathophysiological changes at 11 years to ascertain whether respiratory morbidity in EP children primarily reflects alterations in the lung periphery or more centralised airway function in this population.

Spirometry, plethysmography, diffusing capacity, exhaled nitric oxide, multiple-breath-washout, skin tests and methacholine challenge were used during laboratory-based assessments in a subgroup of the 1995 EPICure cohort and in controls.

Results were obtained in 49 EP and 52 control children. Lung function abnormalities were found in 78% EP children, with evidence of airway obstruction, ventilation inhomogeneity, gas trapping, and airway hyper-responsiveness. Levels of atopy and exhaled nitric oxide were similar between the groups. Prior wheeze was associated with significant reductions in forced flows and volumes. By contrast abnormalities of the lung periphery appear to be mediated primarily through EP birth per se.

Conclusions

The prevalence of lung function abnormalities, which is largely obstructive in nature and likely to have long-term implications, remains high among 11 year-old children born EP, . Spirometry proved an effective means of detecting these persistent abnormalities.

Word count: 199

INTRODUCTION

Advances in neonatal care over the past few decades have resulted in increasing survival of babies born <25 completed weeks of gestation (extremely preterm (EP))[1]. Nevertheless, the prevalence of bronchopulmonary dysplasia (BPD), remains high in such infants,[2] and our understanding of the implications of EP birth and any subsequent lung injury on lifelong lung function (LF) remains limited.

A diagnosis of BPD was initially limited to early recipients of neonatal mechanical ventilation; the associated pulmonary pathology being primarily attributed to iatrogenic damage from barotrauma and oxygen toxicity during the late saccular–early alveolar phase of lung development, with functional abnormalities persisting to adulthood[2]. Computerised tomography has revealed a high incidence of structural alterations in these early survivors of BPD[3]. By contrast, since widespread introduction of antenatal corticosteroids and neonatal surfactant therapy in the early 90s, BPD is now largely restricted to more immature infants delivered during the early saccular phase and "New" BPD has been reported to be characterised by disruption of alveolar development, with reduced alveolar number and enlarged airspaces, but less pulmonary fibrosis and lung injury than previously[4]. Nevertheless, the degree of persistent airway obstruction, as reflected by spirometry, has remained remarkably constant[2,5,6]. Given that diminished FEV_1 is a marker of all-cause premature mortality[7] and that LF is known to track throughout life,[8] there is concern that survivors of preterm birth may be at risk of early onset chronic obstructive pulmonary disease in adulthood[3].

Although a wide range of tests has been used to assess cardio-respiratory function in survivors of preterm birth,[2,5,6,9,10], relatively few studies have included the full spectrum of available techniques, and spirometric parameters, remain the most common outcomes as used during recent school assessments of survivors of the EPICure cohort at 11 years.[11] In that study, 56% of children born before 25^{+6}w gestation were found to have abnormal baseline spirometry, 27% a positive bronchodilator response (BDR) and 25% a diagnosis of asthma (twice that observed in

classmates). Apart from BPD, which had a detrimental impact on all spirometric outcomes, and "duration of postnatal steroids", none of the perinatal or maternal factors were associated with spirometric lung function at 11[11]. However, if the underlying pathophysiology of "new" BPD primarily reflects peripheral lung disease, a substantial proportion of functional abnormalities could potentially go undetected in EP children, if assessments are limited to spirometry[12,13].

The aim of the current study was to ascertain whether ongoing respiratory morbidity in EP children born in the 1990s primarily reflects alterations in the lung periphery, as assessed using multiple breath inert gas washout, plethysmography (partitioned lung volumes) and diffusion capacity or more centralised airway function,(spirometry and airway resistance), and whether there was any evidence of increased eosinophilic airway inflammation (i.e. elevated levels of fractional expired nitric oxide (FeNO) in such children. We hypothesised that LF would be diminished at 11 years of age in children born EP when compared with full-term controls, that use of spirometry alone would under-estimate the true degree of morbidity, and that changes in LF would not be accompanied by increased airway inflammation. None of the results presented in this manuscript have been reported previously except as abstracts.

Keywords: bronchopulmonary dysplasia, child, extreme prematurity, respiratory follow-up

METHODS

EPICure is a population-based study of all births at ≤25^{+6} weeks of gestation in the UK and Republic of Ireland between March and December 1995 [11,14]. The laboratory-based investigation described below was part of an extensive assessment at 11y, which included spirometric measurements in the entire cohort in school[11]. An age, sex and ethnic-group matched classmate for each EP child was sought. Classmates were excluded if <37w gestational age (GA), previously hospitalised for a respiratory complaint or had suffered TB, pneumonia or whooping cough. Asthma and atopy were not exclusion criteria. Asthma classification was based on parental report of doctor diagnosis of asthma. Our operational definition of "current asthma" was use of asthma medication or wheeze in the past 12 months by children with a doctor diagnosis of asthma *or* use of asthma medication *and* wheeze in the past 12 months even if no prior diagnosis of asthma.

Index and control children living within reasonable travelling distance of London were recruited for extensive respiratory assessments at the UCL, Institute of Child Health (ICH) in London (Figure 1). The study was approved by the ICH Research Ethics Committee. Parental written consent and assent from each child were obtained.

Lung function tests (LFTs) were performed according to ATS/ERS standards with investigators masked to birth status. Assessments included spirometry, plethysmography, diffusing capacity (DL_{CO}), fraction of exhaled nitric oxide (FeNO), multiple-breath inert-gas washout (MBW) to assess ventilation inhomogeneity, skin allergy test and methacholine challenge (MCh) (see OLS for details). Doctor-diagnosed asthma, medication use and current respiratory symptoms including wheeze were determined by parental response to the ISAAC questionnaire. Each child was requested to rate his/her own pubertal status[15]. This extensive study protocol was performed in two laboratory sessions within a three week interval.

Power of study

A sample size of 50 children in each group would provide at least 80% power to detect differences in lung function >0.57 SD between index and control groups, at the 5% significance level.

Data management and statistical analysis

Codes regarding birth status were not released until lung function data collection and analysis had been completed. Neonatal data[16] were used to identify the EP children who had had BPD, defined as those still receiving supplemental oxygen at 36w postmenstrual age (PMA)[2].

With the exception of the Lung Clearance Index (LCI) which is relatively constant throughout life in health,[12] lung function results were expressed as Z-scores to adjust for height, sex and age [17-20]. Results were classified as: *normal,* when TLC, FVC, FEV_1, FEF_{25-75} all fell within the normal range; *Obstructive,* when FEV_1/FVC was <Lower Limit of normal(LLN) and/or RV/TLC >upper limit of normal (ULN); or *Restrictive,* when TLC and/or FVC were <LLN or FEV_1 and/or FEF_{25-75} were reduced in the presence of a normal FEV_1/FVC.[21] The extent to which extreme prematurity (EP) and respiratory morbidity were associated with lung function at 11y was examined using multiple linear regression (MLR, SPSS, v15.0). Data management was undertaken using Re-Base software (J7 Group, Rickmansworth, UK).

RESULTS

Population characteristics

Successful lung function assessments and completed questionnaires were obtained from 49 EP and 52 classroom controls (Figure 1), recruited from 20/39 counties in England. No potential controls had to be excluded due to prior hospitalisation or serious respiratory morbidity (see exclusion criteria). Table 1 summarises group characteristics according to birth status.

How representative was the study population?

With the exception of exposure to maternal smoking during pregnancy, which was higher among children who were only tested in school, the subgroup of EP children attending for the extended respiratory assessments at ICH was representative of the entire cohort tested in school with respect to neonatal and maternal characteristics, neonatal history, respiratory morbidity and spirometry (Table 2). Similarly there was no significant difference between control group assessed in the lab and those at school (data not shown). The ethnic mix of the subgroup of EP children attending for laboratory testing was representative of those tested at school. However, although class-room controls were well matched for ethnic group with the EP children during school assessments of the entire cohort,[15] slightly fewer non-white controls attended the laboratory assessments (Table1).

At time of test, EP children were shorter and lighter than controls (Table 3). After adjustment for prematurity, age at test was similar between EP children and controls (10.9 vs. 11.0y respectively), while anthropometric differences remained highly significant. A similar proportion of EP and controls had reached the onset of puberty at time of test.

Prior respiratory morbidity (Table 3) was significantly more common in the EP children and both current and inactive asthma were almost twice as frequent than in controls, but there was less difference in atopic status (eczema and/or positive skin test) or prevalence of hayfever (Table 3). In

the 12 months prior to testing, significantly more EP children with prior BPD were symptomatic (i.e. wheezed or had been treated for respiratory illness), although only one child was receiving antibiotics at testing. Incidence of wheeze in the 3-month period preceding LFTs (Table 4) was generally similar in EP children *without* prior BPD to that in controls, except when associated with a cold. By contrast, prior BPD was associated with significantly more recent wheeze, irrespective of how categorised (Table 4). "Wheeze with colds" was associated with EP status (Odds ratio (OR) = 8.1 (95% CI: 1.7; 38.4; p=0.008) and among EP children, prior BPD was associated with shortness of breath during exercise (OR = 5.7 (1.4; 24.1; p = 0.017)).

Lung Function Results

Effect of extreme prematurity: With the exception of static lung volumes (FRC, TLC and VA), significant impairments in all LF variables were found among EP children when compared with controls (Table 5, Figure 2), with evidence of airway obstruction (decreased forced expiratory flows and volumes, elevated specific airways resistance), ventilation inhomogeneity, gas trapping (elevated RV/TLC), and airway hyper-responsiveness, abnormalities being most marked in EP children with BPD. By contrast, levels of FeNO and incidence of atopy were similar between EP and control children. Details of univariable analysis are presented in Table E1 (OLS).

Associations between airway function, extreme prematurity and respiratory morbidity

On multivariable analysis, after adjusting for ethnicity, being born EP was significantly associated with decrements in all LF outcomes, while respiratory morbidity (wheeze ever) was associated with further decrements of central airway function (Table 6) but not with markers of more peripheral lung function such as LCI (coefficient [95% CI]: 0.2[-0.1; 0.6]; p=0.19), gas trapping (RV/TLC: 0.4[-0.05; 0.8]; p=0.08) or diffusing capacity (0.1[-0.3; 0.5]; p=0.69).

Extent to which spirometry identified respiratory abnormalities in EP children?

In the EP children, 11(22%) had no LF abnormalities (although 2 of these had incomplete tests) and 38 had some. Spirometry identified 24 (63%) of the children with LF abnormalities, LCI 20(58%) and sR_{eff} 17(45%), while a combination of spirometry and sR_{eff} identified 30(79%) of all children with abnormalities.

Of the 47/49 EP children with acceptable spirometry and plethysmography, 21(45%) exhibited an obstructive pattern, of whom 14(67%) had a history of wheeze and 10(48%) a diagnosis of asthma, while only 5(11%) had evidence of restrictive lung disease on spirometric criteria (all of whom had a history of wheeze, but only one a diagnosis of asthma). Of these five, technically acceptable results of partitioned lung volumes were only available from two, of whom one had a reduced TLC.

DISCUSSION

This study represents the most extensive respiratory assessments in children of such low gestation to date, and reveals a wide spectrum of functional abnormalities in both the larger airways and lung periphery at 11 years of age in children born EP. After adjusting for prematurity, prior wheeze was the strongest predictor for decrements in spirometric airway function, while current asthma was associated with significant increases in airways resistance. Had assessments been limited to spirometry, 37% of LF abnormalities would have gone undetected. Nevertheless, spirometry remained the most discriminative test in this population, as well as being the most feasible for use in field studies.

Strengths and Limitations:

Strengths of this study include the fact that the EP subset who attended for laboratory tests were representative of the national cohort[11]. As measurements were limited to children who were sufficiently coordinated to perform the extensive range of lab-based tests, findings may underestimate the true extent of lung dysfunction. Investigators were masked to birth status, and

recruitment of a prospective control group that was representative of the local population, including incidence of asthma,[22] allowed us to assess the effects of EP birth over and above that of asthma[11]. By categorising functional abnormalities based on limits of normality derived from local healthy controls (Table E2), we avoided potential errors that may occur when relying simply on published reference data (www.growinglungs.org.uk)[6,23]. A reduction in spirometric parameters among Black and Asian subjects when compared with those of white European descent is well recognised,[23] and was confirmed in this study, but differences between groups remained similar whether or not the relatively few non-white children were included in the analyses.

The extensive nature of laboratory assessments, which also included exercise[14] and neuro-sensory testing,[24] and the need to travel to central London, limited the sample size and hence the power with which subgroup analyses could be reliably undertaken. Nevertheless, the trends towards increased morbidity and functional deficits in those with prior BPD in those who participated in the laboratory assessments mirrored the significant changes found in the entire cohort[11]. As reported previously,[11] maternal smoking in pregnancy was not associated with changes in any LF outcome, the EP children possibly having been delivered before anticipated adverse effects occurred. We could not examine the potential impact of intrauterine growth retardation in this population[10] due to extremely low survival of such infants at these gestations.

Respiratory Morbidity

While 29% of children with prior BPD had doctor-diagnosed asthma and recent asthma medication, there was no increase in either the prevalence of atopy or levels of FeNO in these children, suggesting a different underlying pathophysiology to that usually observed in childhood asthma[25]. The fact that the airways obstruction observed in these children was only partially reversible raises the issue as to whether these children have been correctly diagnosed, or optimally treated.[11] Consistent with previous reports,[9,10] airway responsiveness was increased among

the EP children, although had we not undertaken identical challenges in prospective controls, the extent of such hyper-responsiveness would have been over-estimated (see OLS). Children with BPD are known to be at increased risk for symptomatic respiratory illnesses in infancy and childhood. This may largely reflect the effects of diminished airway calibre, as indicated by the increased sR_{eff} and decreased expiratory flows and volumes found in this population, such that wheeze can be invoked with minimal further airway narrowing. However, neonatal hyperoxia may also exacerbate inflammatory responses, leading to long-term disruption of key innate immuno-regulatory pathways in such subjects[26].

Nature of underlying pathophysiology

Initial descriptions of 'new BPD' pathology suggested that EP delivery could result in an *'arrest'* of alveolar development[2] but later reviews suggest *impaired* alveolisation,[4] since there is evidence of continued postnatal alveolar formation despite early lung insults.[27] The reduced gas mixing efficiency (elevated LCI) observed in EP children may reflect some parenchymal or small airway changes, secondary to disruption of the interstitial collagen network that has been reported following neonatal positive pressure ventilation [28]. While the LCI has been shown to be an early indicator of airway disease in those with cystic fibrosis[29] it has not been found to be particularly discriminative when assessing BPD or prematurity either during infancy[30,31] or in the current study. A simplified bronchial tree with fewer generations of peripheral airways may have contributed to the relatively normal LCI observed in EP children at 11 years and this could potentially have masked some ventilation inhomogeneity. However it is equally possible that following BPD there may be relatively homogenous airway narrowing and hence minimal impact on the LCI. Whatever the underlying mechanism, it appears that the LCI would not be the method of choice when trying to identify airway abnormalities in children born EP, unless used in conjunction with spirometry. The reduced diffusion capacity amongst those born EP in this study has been previously reported[6] and probably reflects disruption of alveolar development (larger but

fewer alveoli), decreased surface area for gas exchange and disrupted angiogenesis[32]. Nevertheless, very few EP children demonstrated evidence of restrictive lung disease, even when using a liberal definition based on both reduced FVC with normal FEV_1/FVC. Of the 37 (76%) EP children with technically acceptable plethysmographic lung volumes, only one (3%) had evidence of restriction. By contrast, evidence of obstructive airway disease was observed in 45% of these children, 91% of whom were correctly identified by spirometry alone. Although it is recognised that plethysmography should be used to confirm restrictive lung disease, this technique may be difficult for young children especially if there is any developmental delay. We therefore adopted the more liberal definition including spirometry to ensure we did not underestimate any restriction in this population.

Indeed, although the aetiology of BPD has changed during the past four decades, a similar degree of respiratory dysfunction continues to be observed, albeit in increasingly immature survivors[2]. The increase in sR_{eff} among children with prior BPD, but not in those without, may be associated with increased bronchial smooth muscle and airway narrowing associated with the more prolonged and intense ventilatory support received by these children[33]. These findings of functional impairments are consistent with morphometric data from survivors with BPD[34].

The question of whether respiratory dysfunction following preterm birth is a) due to disruption of normal lung development following premature exposure of an immature lung to extra-uterine conditions, b) related to factors contributing to preterm delivery or c) subsequent to lung injury incurred during resuscitation and subsequent ventilatory support during the neonatal period remains controversial due to the close interaction of these factors. While there is clear evidence of disrupted lung development following preterm birth *per se*,[35,36] both respiratory morbidity and reductions in LF were far more severe in EP children with prior BPD. Indeed, the minority of EP children who survived without BPD were generally functioning remarkably well at 11y[11]. The fact that these

functional abnormalities have persisted into early adolescence among those born preterm suggests that the damage observed during infancy[37] may be permanent[2,3].

CONCLUSIONS

There is a high incidence of persistent LF abnormalities among EP children born in the mid-1990s, which is largely obstructive in nature and likely to have long-term implications for future lung health. Spirometry proved to be an effective means of detecting these persistent abnormalities in survivors of EP birth and BPD, although discrimination could be improved in lab-based assessments by including measures of specific resistance and/or ventilation inhomogeneity. To minimise the risk of early onset chronic obstructive lung disease in adulthood, efforts should be made to preserve existing lung reserves by encouraging these children to lead a healthy life style with respect to diet, exercise and avoidance of smoking.

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FIGURE TITLES AND LEGENDS

Figure 1: Study population: subject recruitment and accrual

* Includes one not identified in 1995, but eligible to be in the 1995 EPICure cohort

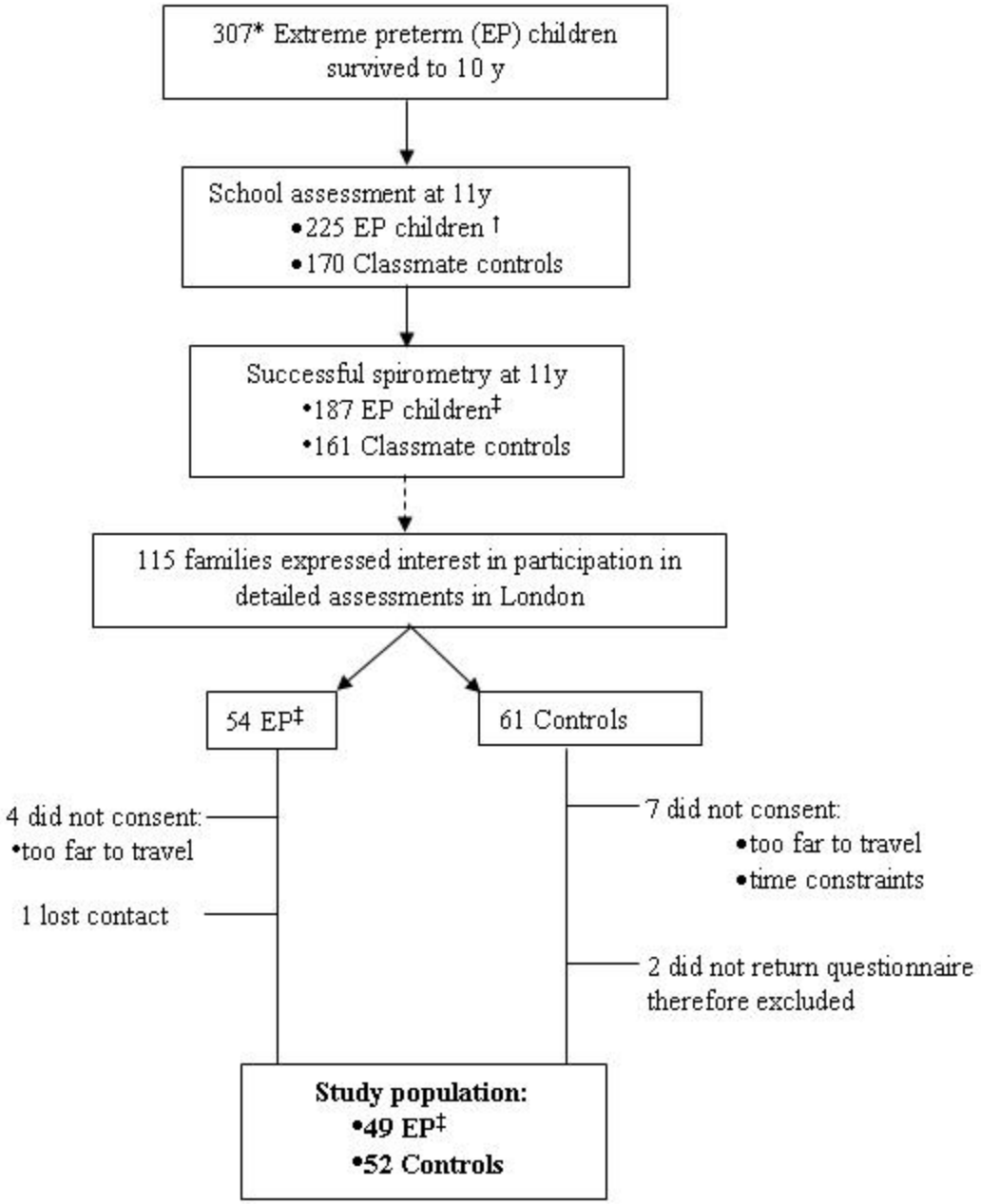
† Includes six born in *January* 1996 who were recruited to the EPICure study but not included in the cohort analyses, as born after December 31st 1995

‡ including two of the six children in '†'

Figure 2: Comparison of lung function in children born extremely preterm (± BPD, and full-term controls according to lung disease categories.

Horizontal line within each subgroup denotes the mean value for that group. Filled squares denote children classified as having obstructive airways disease while those with filled triangles represent those with restrictive lung disease.

Evidence of airway obstruction was only evident in one control with a history of asthma, whose zFEF was -3.9. The remaining control with FEV_1 and FEF_{25-75} just below LLN was not asthmatic but had had a lower respiratory tract infection requiring medication in the last 3 months. Unfortunately, technically acceptable LCI results were not obtained in either of these controls.


307* Extreme preterm (EP) children survived to 10 y
School assessment at 11y
•225 EP children †
•170 Classmate controls
Successful spirometry at 11y
•187 EP children‡
•161 Classmate controls
115 families expressed interest in participation in detailed assessments in London
54 EP‡
61 Controls
4 did not consent:
•too far to travel
1 lost contact
7 did not consent:
•too far to travel
•time constraints
2 did not return questionnaire therefore excluded
Study population:
•49 EP‡
•52 Controls

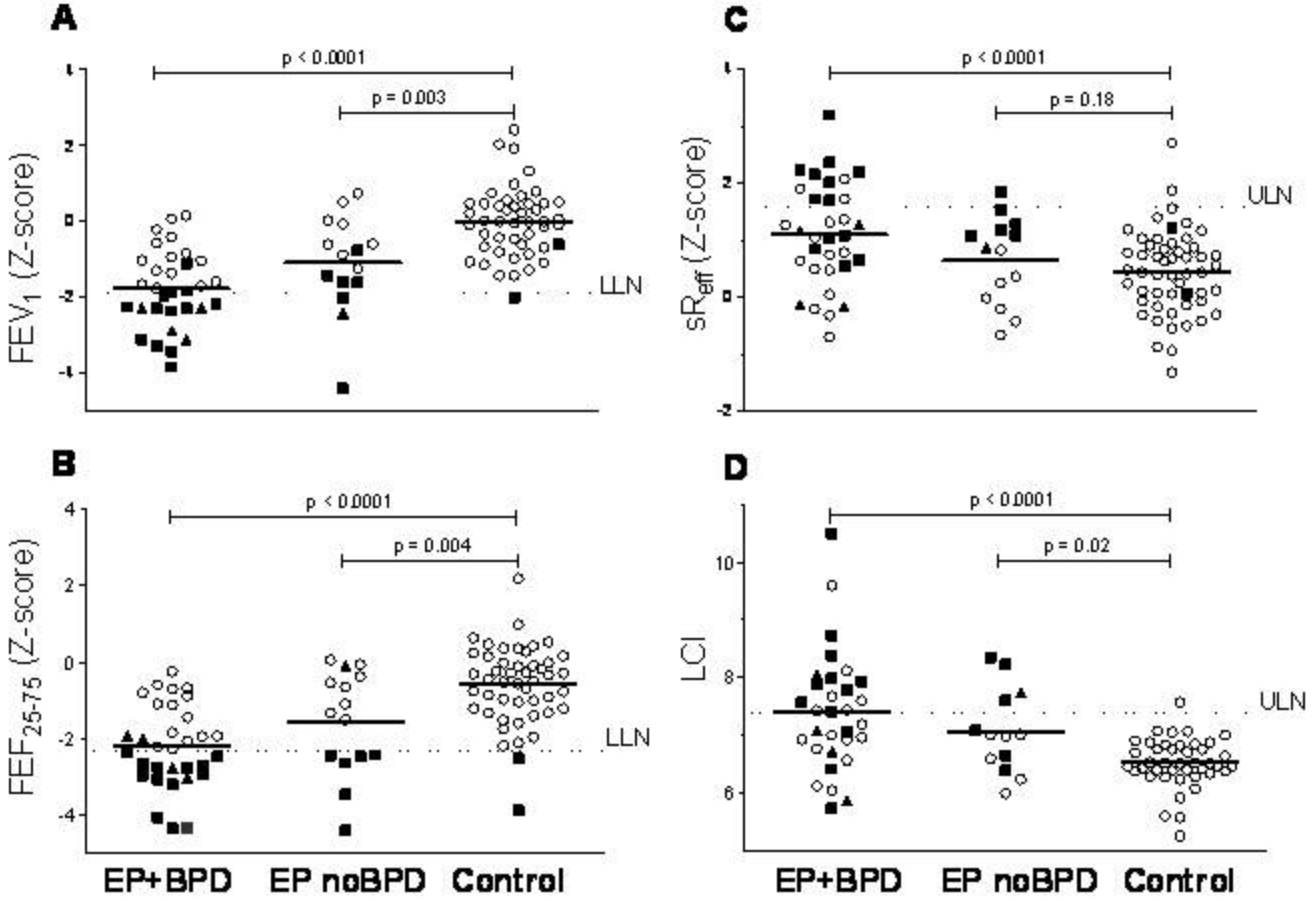

A
p < 0.0001
p = 0.003
FEV1 (Z-score)
LLN
B
p < 0.0001
p = 0.004
FEF25-75 (Z-score)
LLN
C
p < 0.0001
p = 0.18
sReff (Z-score)
ULN
D
p < 0.0001
p = 0.02
LCI
ULN
EP+BPD
EP noBPD
Control
EP+BPD
EP noBPD
Control

Table 1 Group characteristics of EP children with and without prior BPD compared to classmate controls

	EP: BPD	EP:no BPD	All EP	Control (C)	95% CI of Diff (EP-C)
Neonatal details:					
n	34	15	49	52	-
Boys (%)	35%	40%	37%	37%	-18%; 18%
Gestational age (w)	24.8 (0.8)	25.0 (0.6)	24.9 (0.7)	40.1 (1.6)	**-15.7; -14.7 *** **
Birthweight (kg)	0.740 (0.101)	0.762 (0.093)	0.747 (0.098)	3.361 (0.450)	**-2.75; -2.48 *** **
Birthweight Z-score#	-0.06 (0.71)	-0.03 (0.80)	-0.05 (0.74)	-0.24 (1.14)	-0.57; 0.20
BPD (%)	100%	0%	69%	-	-
Postnatal steroids	70%	67%	69%	-	-
Maternal/family details:					
Antenatal steroids	85%	87%	86%	-	-
PROM	15%	31%	19%	-	-
Chorioamnionitis	21%	39%	26%	-	-
Smoking in pregnancy	25%	14%	22%	22%	-16%; 17%
Current smoking exposure	18%	7%	15%	26%	-27%; 4%
Maternal asthma	12%	0%	8%	12%	-16%; 9%
FH of asthma	38%	33%	37%	33%	-14%; 22%
Maternal ethnicity: %White	74%	87%	78%	94%	**-30%; -3% ***
Maternal education after age 16	52%	57%	53%	73%	**-38%; 0% ***
Non-manual occupation	74%	79%	76%	69%	-10%; 29%

Data presented as Mean (SD) or as indicated; *** $p < 0.0001$; ** $p < 0.01$; * $p < 0.05$;

According to Child Growth Foundation algorithms (Cole et al, *Stat Med* 1998)

Abbreviations: EP: Extremely preterm; BPD: Bronchopulmonary dysplasia (defined as oxygen given at or beyond 36w post menstrual age);

PROM: Prolonged rupture of membranes (> 24hr)

Classification of Non-manual occupation: based on either parent with a non-manual occupation

Table 2 Comparison of children who did and did not attend the respiratory lab at ICH for extended assessments

	EP: School tested (not ICH)	EP: ICH tested	95% CI of difference (School-ICH)
n (% boys)	140 (44%)	49 (37%)	-9%, 22%
Gestational age (w)	25.0 (0.7)	24.9 (0.7)	-0.13, 0.35
Birthweight (kg)	0.749 (0.126)	0.747 (0.098)	-0.033, 0.037
Birthweight Z-score[38]	-0.17 (0.78)	-0.05 (0.73)	-0.36, 0.13
BPD (%)	71%	69%	-12%, 16%
Received ANS (%)	80%	86%	-16%, 8%
h/o chorioamnionitis (%)	22%	26%	-18%, 10%
Wheeze in last 12m (%)	23%	22%	-13%, 14%
Current asthma (%)	26%	27%	-16%, 13%
Ethnicity: White (%)	84%	78%	-8%, 19%
Smoking in pregnancy (%)	39%	22%	**-31%, -2%***
FEV_1 Z-score[20]	-1.4 (1.2)	-1.6 (1.1)	-0.3, 0.5
FEF_{25-75} Z-score[20]	-2.0 (1.3)	-2.0 (1.2)	-0.4, 0.4

Data presented as Mean (SD) unless otherwise indicated. * $p<0.05$

Comparison of FEV_1 and FEF_{25-75} z-scores was undertaken by using data obtained from both groups during school assessments.

Abbreviations: BPD: Bronchopulmonary dysplasia (defined as receiving supplementary O_2 ≥ 36 w postmenstrual age); ANS: Antenatal steroid; h/o: history of

Current asthma: defined as currently symptomatic and/or with doctor-diagnosis of asthma and on medication during past 12 months.

Table 3 Group characteristics of EP children with and without prior BPD compared to classmate controls at 11 years of age.

	EP: BPD	EP: no BPD	All EP	Control	95% CI (EP-C)
n (% boys)	34 (35%)	15 (40%)	49 (37%)	52 (37%)	-18%; 18%
Test age (y)	11.2 (0.4)	11.2 (0.5)	11.2 (0.4)	11.0 (0.5)	**0.0; 0.4 ***
Height (cm)	141.5 (5.7)	143.0 (8.5)	142.0 (6.6)	146.1 (6.7)	**-6.7; -1.5 ***
Height Z[#]	-0.47 (0.92)	-0.29 (1.08)	-0.41 (0.97)	0.35 (0.96)	**-1.14; -0.38 *****
Weight (kg)	35.5 (8.7)	38.0 (8.8)	36.3 (8.7)	39.8 (8.8)	**-7.04; -0.10 ***
Weight Z[#]	-0.34 (1.19)	-0.05 (1.42)	-0.25 (1.26)	0.43 (1.05)	**-1.14; -0.22 ****
BMI Z[#]	-0.13 (1.33)	0.27 (1.12)	-0.01 (1.27)	0.35 (1.08)	-0.82; 0.11
Puberty (%)[15]	29%	29%	29%	29%	-18%; 18%
Respiratory morbidity: (%)					
Bronchiolitis (%)	44%	36%	42%	2%	**25%; 54% *****
Pneumonia (%)[§]	19%	9%	17%	-	-
Wheeze ever (%)	**62%**	**27%**	51%	21%	**12%; 48% ****
Wheeze last 12m[‖]	**29%**	**7%**	23%	10%	-2%; 27%
Inactive asthma	15%	13%	14%	8%	-6%; 20%
Current asthma	29%	20%	27%	13%	-3%;28%
Hayfever ever(%)	32%	33%	33%	30%	-15% 20%
Eczema ever (%)	41%	40%	41%	33%	-11%; 25%
Skin test positive	6%	13%	9%	4%	-6%; 16%
Medication in last 12m: n (%)					
B-Agonist	9 (26%)	3 (20%)	12 (25%)	5 (10%)	**0%; 30% ***
Inhaled steroids	9 (26%)	2 (13%)	11 (22%)	6 (12%)	-4%; 26%
Antibiotics	8 (24%)	1 (7%)	9 (18%)	2 (4%)	**2%; 28% ***
Current Medication: n (%)					
B-Agonist	8 (24%)	1 (7%)	9 (18%)	4 (8%)	-3%; 24%
Inhaled steroids	3 (9%)	2 (13%)	5 (10%)	4 (8%)	-9%; 15%

Data presented as Mean (SD) or otherwise as stated. *** $p < 0.0001$;** $p < 0.01$; * $p < 0.05$

[#] According to Child Growth Foundation algorithms (Cole et al, *Stat Med* 1998)

[‖] detailed description of wheeze 3m prior to test is given in Table 3.

[§] Exclusion criteria for controls.

Onset of puberty defined as having reached Tanner Stage 3 in their physical and pubic hair development.[15]

Inactive asthma: defined as those who had been diagnosed with asthma by a doctor but not symptomatic over past 12m.

Current asthma: defined as currently symptomatic and/or with doctor diagnosis of asthma and on medication for past 12 months.

Inhaled steroids given were: Flixotide, Becotide or Pulmicort

Table 4 Pattern of wheeze in the past 3 months

	EP: BPD	EP: no BPD	Control
n	34	15	34
Wheeze during the day	24%	7%	8%
Wheeze with colds	**29%****	13%	4%
Wheeze without colds	15%	7%	6%
Wheeze with exercise	**26%***	7%	6%
SoB with exercise	**59%****	20%	21%#

Abbreviation: SoB: shortness of breath

Exact test for comparison between the 3 subgroups: ** $p < 0.01$; * $p < 0.05$;

Objective assessments of physical activity showed that all children undertook considerably less physical activity (PA) than current recommendations of at least 60 minutes of moderate-vigorous PA per day which may explain why 21% of controls experience SoB with exercise

Table 5 Lung function results in EP children compared to classmate controls at 11 years

	EP: BPD	EP: no BPD	All EP	Control (All)	95% CI of difference (EP – Control)
n	34	15	49	52	
$zFEV_1$[20]	-1.76 (1.02)	-1.08 (1.29)	-1.55 (1.14)	-0.02 (0.90)	**-1.94, -1.10** ***
$zFEF_{25-75}$[20]	-2.18 (1.07)	-1.55 (1.38)	-1.98 (1.19)	-0.59 (1.02)	**-1.85, -0.94** ***
zFVC[20]	-1.02 (0.85)	-0.43 (1.26)	-0.84 (1.02)	0.22 (0.97)	**-1.46, -0.65** ***
$zFEV_1$/FVC[20]	-1.27 (0.99)	-0.98 (1.23)	-1.17 (1.07)	-0.40 (0.91)	**-1.18, -0.37** ***
zRV[18]	1.84 (1.30)	1.25 (0.57)	1.63 (1.13)	1.17 (0.71)	**0.05, 0.88 ***
zTLC[18]	0.16 (0.41)	0.41 (0.57)	0.25 (0.66)	0.31 (0.56)	-0.29, 0.17
zRV/TLC[19]	1.26 (1.07)	0.86 (0.86)	1.12 (1.0)	0.44 (0.80)	**0.27, 1.08 ****
$zFRC_{pleth}$[18]	0.47 (1.13)	0.17 (0.72)	0.36 (1.0)	0.16 (0.89)	-0.20, 0.60
$FRC_{pleth-MBW}$ ($mL.kg^{-1}$)	12.7 (8.8)	12.0 (6.3)	12.4 (7.9)	8.5 (5.3)	**0.8, 7.1 ***
Z_sReff[17]	1.11 (0.91)	0.70 (0.76)	0.98 (0.88)	0.42 (0.73)	**0.24, 0.88 ****
LCI	7.4 (1.0)	7.1 (0.7)	7.3 (0.9)	6.5 (0.4)	**0.46, 1.10** ***
zDL_{CO}[18]	-1.08 (0.90)	-1.40 (0.91)	-1.20 (0.91)	-0.42 (0.91)	**-1.19, -0.37** *
zVA[18]	1.28 (1.24)	1.36 (0.68)	1.31 (1.05)	1.41 (1.17)	-0.60, 0.41
zDL_{CO}/VA[18]	-2.15 (0.80)	-2.58 (0.67)	-2.31 (0.77)	-1.69 (0.78)	**-0.97, -0.27 ****
PC_{20} ($mg.mL^{-1}$) †	0.41 (2.90)	0.39 (2.64)	0.41 (2.75)	1.72 (5.31)	**0.12, 0.46**
FeNO (ppb) †	4.6 (2.0)	5.5 (2.5)	4.9 (2.2)	5.9 (2.1)	0.6, 1.15

Data expressed as mean (SD) Z-scores unless otherwise stated.

95% CI of difference in **BOLD** denote statistically significant difference found (EP-C):

*** $p < 0.0001$; ** $p < 0.01$; * $p < 0.05$

† Data presented as Geometric mean (SD) and 95% CIs of the Geometric mean were calculated from the Log_e of PC_{20} and eNO

Abbreviations: FEV_1: forced expired volume in 1 second; FEF_{25-75}: forced expiratory flow between 25-75% FVC; FVC: forced vital capacity; RV: residual volume; TLC: total lung capacity; MBW: multiple breath washout; FRC_{pleth}: plethysmographic functional residual capacity; $FRC_{pleth-MBW}$: difference in FRC measured using plethysmography and MBW (a measure of gas trapping); sReff: specific effective airway resistance; LCI: Lung clearance index; DL_{CO}: Diffusing Capacity of the

lung for Carbon Monoxide; VA: Alveolar volume; PC_{20}: Provocation concentration; FeNO: Fraction of exhaled nitric oxide.

Table 6: Associations between spirometric outcomes and specific airway resistance with extremely preterm birth and other factors

	$zFEV_1$	**$zFEF_{25-75}$**	**$zFEV_1/FVC$**	**Z sReff**
n	94	94	94	100
% variability explained[#]	41%	36%	20%	13%
Constant	-0.11	-0.43	0.28	0.34
EP	**-1.31 (-1.73; -0.89)*** **	**-1.14 (-1.59; -0.69)*** **	**-0.58 (-0.99; -0.17)** **	**0.45 (0.12; 0.78) ** **
Wheeze: ever	**-0.71 (-1.16; -0.26)** **	**-0.84 (-1.32; -0.36)** **	**-0.66 (-1.09; -0.22)** **	**0.38 (0.04; 0.73) ***
Other variables of interest which were not significantly associated with the LF outcomes after adjusting for the above variables				
BPD	-0.47 (-1.10; 0.15)	-0.38 (-1.05; 0.29)	-0.08 (-0.69; 0.53)	0.30 (-0.20; 0.81)
White mother	0.43 (-0.19; 1.05)	NA	NA	NA

Data presented as coefficient (95% CI); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$

Abbreviations: # adjusted R^2 derived from MLR using the whole dataset (EP + Controls); NA = Not applicable: factor not included in the model as it was not significantly associated with the lung function variable.

"Wheeze: ever" was most significantly associated with these lung function outcomes. With the exception of sR_{eff}, once accounted for this variable, additional symptoms and current asthma did not add significantly to the models. Current asthma status was significantly associated with a further increase in sR_{eff} by an average of 0.48 Z-score (95% CI: 0.07; 0.90; $p = 0.02$) while the contribution from the "wheeze: ever" variable was no longer significant (Mean [95%CI]: 0.26 Z-score [-0.10; 0.61]).

Although the univariate associations of BPD with lung function and of ethnicity with FEV_1 (but not $FEF_{\%}$, FEV_1/FVC or sR_{eff}) (Table E1) were no longer significant during MLR, they are reported as additional variables of interest since they show identical trends to those found when analysing the entire cohort.[11]