The relationship between infant lung function and the risk of wheeze in the preschool years

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Summary

Rationale

There is evidence that lung function in infancy predicts childhood wheeze. However, very few studies have both measured premorbid lung function in early infancy and considered clinically significant childhood wheeze phenotypes. Consequently, it is unclear whether early lung development is most closely associated with atopic or non-atopic preschool wheeze.

Objective

To examine the association between premorbid infant lung function and preschool wheeze according to atopic or non-atopic wheeze phenotype.

Methods

Infant lung function was measured in 147 healthy term infants aged 5-14 weeks. Rapid thoracoabdominal compression was performed during tidal breathing and at raised volume to measure maximal expiratory flow at functional residual capacity (V'_{maxFRC}) and forced expiratory volume in 0.4 sec ($FEV_{0.4}$). Atopic status was determined by skin prick testing at 3 years and wheeze ascertained from parental questionnaires (1 and 3 years).

Measurements and Main Results

Lower early infancy V'_{maxFRC} was associated with wheeze in both the first and third years of life (p=0.002 and p=0.006, respectively). Lower early infancy $FEV_{0.4}$ was associated with wheeze in infancy (p=0.03). Compared to non-atopic children who did not wheeze, non-atopic children who wheezed in their third year of life had lower $FEV_{0.4}$, (p=0.02), whilst atopic children who did and did not wheeze had similar $FEV_{0.4}$ (p=0.4).

Conclusions

Lower premorbid infant lung function was present in infants who subsequently wheezed during the first and third years of life. Lower $FEV_{0.4}$ in early infancy was associated with non-atopic wheeze but not atopic wheeze at 3 years of age.

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Introduction

Lower infant lung function is a risk factor for wheeze in infancy and early childhood[1-5]. Previous studies have demonstrated abnormalities of infant lung function are present in those children who subsequently experience wheeze and these abnormalities can be detected prior to the first wheezing illness[1-4]. The results of longer-term follow up studies are less consistent. Although tracking of lung function from infancy, through childhood[6] and into adult life[7] is well described, the relationship between early lung function and phenotypes of wheezing illness is less clear. The Tucson study, for example, found reduced V'_{maxFRC} to be associated with a transient wheeze phenotype in 125 infants assessed at a mean age 2.4 months (SD 2 months), but no association was found with wheezing after 3 years of age[8]. In contrast, other studies suggest early impairment of lung function is a risk factor for wheeze which persists into later childhood[6;9].

The original 'wheeze phenotypes' described in the Tuscon study were defined retrospectively according to age of onset and persistence of symptoms[8]. More recently, work has been undertaken to determine clinical phenotypes[10;11] and to consider whether early abnormalities of lung function are associated with childhood wheeze phenotypes based upon clinical features. Infants with recurrent wheeze considered at high risk of subsequent asthma due to either a parental history of asthma or personal history of eczema, allergic rhinitis, wheezing without a cold and/or serum eosinophilia have been found to have lower forced expiratory volumes at 8-20 months than healthy controls or wheezy infants at lower risk of subsequent asthma[12]. This study suggests early impairment of lung function is associated with preschool wheeze and particularly so in the presence of markers of atopic sensitisation. However, premorbid infant lung function was not measured and the relationship between lung function and clinical wheeze phenotype according to atopy was not established in an unselected

population. Whilst it is clear that both early lung function and atopic status are important determinants of preschool wheeze it is not clear how premorbid infant lung function relates to the atopic and non-atopic wheeze phenotypes.

Premorbid infant lung function may reflect early environmental or genetic influences. Common genetic polymorphism is known to impact neonatal disease and outcome[13]. ADAM33 is a positionally cloned asthma susceptibility gene that shows strongest linkage with a combined asthma and bronchial hyperresponsiveness phenotype[14]. ADAM33 is expressed in lung fibroblasts and bronchial smooth muscle and is expressed during embryonic lung development[15]. Previously, ADAM33 polymorphisms have been associated with impaired lung function at 3 years of age, specifically deficits in FEV_1 and carriers homozygous for the A allele of F+1 SNP had double the risk of transient early wheeze[16]. Associations between a number of individual SNPs and both baseline lung function and non atopic asthma have also been demonstrated in a population of German school children[17].

The Southampton Women's Survey (SWS) was the first to measure premorbid $FEV_{0.4}$ in a longitudinal pregnancy cohort study[18]. The rapid thoracoabdominal compression (RTC) technique has been used for many years to measure V'_{maxFRC} in infants. Concerns about intersubject and intrasubject variability led to a modification of the method, performing the manoeuvre at raised volume (RV-RTC), removing the use of a variable volume landmark[19]. It has been suggested that measurement of $FEV_{0.5}$ using RVRTC is more sensitive at detecting impaired airway function than V'_{maxFRC} in infants with CF prior to respiratory symptoms[20;21]. This is the first study to investigate the predictive value of infant $FEV_{0.4}$ in healthy term infants in relation to future wheeze. Using this outcome measure, we aimed to test the hypothesis that lower infant lung function is associated with preschool wheeze and to explore

the associations between premorbid infant lung function and preschool wheeze according to atopic phenotype. As a secondary aim, we explored the relationship between ADAM33 polymorphism, lung function in infancy and preschool wheeze.

Methods

Participants

The Southampton and South West Hampshire Local Research Ethics Committee approved the protocol and written consent was obtained from the children's mothers. Detailed information regarding the participants and infant lung function testing have been described[18]. In brief, Caucasian infants, born at least 37 weeks gestation without major congenital anomalies and prior to any respiratory infection were recruited from the SWS, a population-based pregnancy cohort[22]. 362+ women were approached about the study; 219+ (60%) declined. Of those who agreed to participate 12+ (3%) were excluded because of recent upper respiratory tract infection or other illness; the majority of those declining did so due to concerns about sedation and respiratory infection was not a common reason for declining to participate. Lung function was measured, between May 1999 and October 2002, when the infants were 5-14 weeks old.

Lung function was measured, with infants lying supine, in quiet sleep augmented with chloral hydrate (75-100 mg/kg). An inflatable jacket connected to a rapid inflation system was placed around the infant's chest and abdomen and a leak-free facemask with Fleisch pneumotachograph (Dynasciences, Blue Bell, CA) was held over the nose and mouth. Data were collected using RASP software (Physiologic Ltd, Newbury, Berks, UK) and analysed in SQUEEZE (Paul Dixon, London).

As previously described[18], respiratory rate was measured during tidal breathing. To record partial expiratory flow volume curves, a stable end expiratory level was established before performing an RTC at the lowest pressure to achieve the best V'_{maxFRC}, calculated from the partial expiratory flow volume curve. Passive, relaxed inflations to 30cm water were recorded

using a resuscitator connected to the pneumotachograph. The airway opening pressure was measured during occlusion which causes the respiratory muscles to relax by the Hering Breuer reflex and airway pressure to equilibrate with alveolar pressure; this in turn represents the summed elastic recoil pressure of the lung and chest wall. Compliance of the respiratory system (Crs) was calculated using SQUEEZE from the resultant passive flow–volume curves as change in volume for unit of airway outlet pressure (ml/mm water). Raised volume RTC curves were recorded at the optimal jacket pressure at the end of a passive inspiration using a technique adapted from Feher and colleagues[19], and $FEV_{0.4}$ and FVC were measured from the forced expiratory flow–volume curve.

For each infant, $FEV_{0.4}$ and Crs were calculated from the 'best' raised volume loop (greatest sum of $FEV_{0.4}$ and FVC) and the best V'_{maxFRC} was used. At least two acceptable, reproducible (within 10%) raised volume and partial expiratory curves were obtained. It was not possible to record valid data in every infant for each measure of lung function, there were approximately 100 infants with acceptable lung function data for each measurement.

Symptoms

Mothers completed contemporaneous respiratory symptom diaries during their child's first year of life. Symptoms were explained to each mother by the infant lung function nurses. A new diary was posted every 3 months, followed up by a reminder phone call from a nurse of the infant lung function study team. For each 24hr period parents recorded daytime and night time wheeze. At ages one and three years mothers completed a nurse-administered questionnaire which included the question 'has your child experienced any episodes of chestiness associated with wheezing or whistling in his/her chest since they were last seen?'. Skin reactivity to cat[a], dog[a], house dust mite[a] (Dermatophagoides pteronyssinus), milk[a], grass

pollens[a], and egg[b] was assessed at age 3 years (Hollister-Stier, Spokane, WA[a], Alyostal, Antony, France[b]). Atopy was defined as a wheal to any allergen at least 3mm in diameter in the presence of a wheal of at least 3 mm in diameter to 10 mg/ml histamine[a] solution and no response to 50% glycerin[a].

Genotyping

DNA was isolated from cord blood using a salting-out procedure[23]. The following five SNPs were chosen for genotyping as they span the ADAM33 gene region (based on the LDU map from Simpson *et al*[16]). ADAM33 SNPs Bp1 (rs487377), Fp1 (rs511898), STp7 (rs574174), Vm3 (rs628977), V4 (rs2787094) were genotyped by TaqMan SNP genotyping assays (E-Table1).

Statistical Analysis

The infant lung function data were logarithmically transformed to achieve normality and adjusted for age and sex where necessary. t-tests were used to assess differences between geometric mean group values of infant lung function measurements according to wheeze status at 1 and 3 years, and after subdividing the 3-year wheeze group according to atopic status. Agreement between the parent-completed symptom diaries and the nurse-administered questionnaires was assessed by weighted Kappa (κ) analysis. ADAM33 haplotype quantitative trait and power analysis were performed using Haploscore (Haplo.stats R package version 1.3.8.)[24] in R (version 2.5.1)[25] with the additive haplotype effect model.

Assuming 50 children in each outcome group, there was 80% power at the 5% level of significance to detect a difference of 0.57 SDs in any infant lung function measurement between the two outcome groups.

Results

147 infants had lung function measurements, of which 146 provided questionnaire data at age one year, 141 provided questionnaire data at 3 years of age and 113 had skin prick testing. Symptom diaries were returned by 102 mothers. There was strong concordance between the prospective symptom diaries and retrospective questionnaires with respect to classification of children according to presence or absence of wheeze during the first year of life, (κ =0.71, $p<0.001$). Given this, all subsequent analyses are based upon questionnaire data. A total of 114 DNA samples were available for ADAM33 SNP genotyping; genotype assignment approached 99% (6 failed PCR reactions out of a total 570) with a maximum of two unassigned genotypes for each SNP and all genotype frequencies were in Hardy-Weinberg Equilibrium. Matched genotype and lung function data were available for 103 children for respiratory rate, 82 for Crs, 105 for V'_{maxFRC} and for 76 children for $FEV_{0.4}$. (See online supplement).

During the first year of life, 74 infants had at least one episode of wheezing. Thirty three children experienced wheeze in the third year of life, of these 23 had also experienced wheeze in the first year of life whilst 10 had not. Of the 113 children who were skin prick tested for allergy, 20 (17.7%) had at least one positive reaction and were classed as atopic. Of the children who experienced wheeze 25% were atopic and 75% were not.

The demographic characteristics of those children reporting wheeze at 3 years and those that did not are listed in table 1. Comparing children who did and did not wheeze, there were no differences in gender or the mother's age, atopy but those who wheezed at 3 years were more likely to have a mother who smoked during pregnancy. However, those who wheezed in the

first year of life were more likely to be born to younger mothers and have mothers who had smoked in pregnancy than those who did not wheeze (data not shown).

Associations between wheeze or wheeze phenotype and lung function were examined after adjusting for factors that were found to influence measures of lung function on univariate analysis. $FEV_{0.4}$, and V'_{maxFRC} were adjusted for gender and Crs for gender and age at testing.

Relationship between infant lung function and respiratory symptoms reported at age 1 year

V'_{maxFRC}, $FEV_{0.4}$ and Crs were lower in infants who subsequently wheezed in their first year than in those who did not by 22.5% (p=0.002), 8.1% (p=0.03) and 6.8% (p=0.04) respectively (Table 2). The group mean respiratory rate was 6.8% higher in the wheeze group than in the non-wheeze group (p=0.03).

Relationship between infant lung function and respiratory symptoms reported at age 3 years

V'_{maxFRC} was 23.2% lower in infants who wheezed in their third year than in those who did not (Table 2) (p=0.006). However, there were only small non-significant differences in $FEV_{0.4}$, Crs and respiratory rate between the two groups. When non-atopic children were considered separately from atopic individuals, $FEV_{0.4}$ was 11% lower in those who wheezed (p=0.02) (Table 3). There were no significant differences in any measure of infant lung function when atopic children with wheeze in the third year were compared to non-atopic children without wheeze (Table 3).

ADAM33 SNP haplotype analysis

No ADAM33 SNP haplotype was significantly associated with infant lung function measurements after adjusting for multiple testing by Bonferroni correction (E-Table3).

Haplopower analysis (Online Data Supplement) revealed that with the small sample size (n = 82) we were underpowered (Power = 19%) to detect a common haplotype (freq = 18%) association accounting for 5% of the variance in the mean of the Crs measurement.

Discussion

This study demonstrates an association between impaired premorbid infant lung function and preschool wheezing illness. This confirms the results of previous studies[4;5]. Our analyses show for the first time that lower lung function in early infancy is a risk factor for non-atopic wheeze, rather than atopic wheeze. Additionally, this study is the first to report reduced $FEV_{0.4,}$ prior to lower respiratory infection, as a risk factor for wheeze in the preschool years. This is particularly important in view of recent work demonstrating that diminished timed forced expiratory volumes are an important predictor of all-cause mortality in adult life[26]. As with other studies of infant lung function, this study is of low power due to the heavy technical investment required to produce high quality measurements.

Premorbid measures of forced expiratory volume in 0.4 seconds ($FEV_{0.4}$) during infancy as a predictor of wheeze have not previously been studied, though reduced $FEV_{0.5}$ following symptoms has been reported in wheezy children aged 8 – 20 months[12]. Lung function measures derived from raised volume expiratory manoeuvres are believed to discriminate better between healthy individuals and those with respiratory disease when compared to tidal breathing measures[19;20]. $FEV_{0.4}$ is derived from measurement of expiratory flow from close to total lung capacity and is not dependent upon a volume landmark. This is more repeatable than measurement at FRC as the FRC of infants can vary on a breath by breath basis, $FEV_{0.4}$ provides a more robust measure of infant lung function than V'_{maxFRC}[27]. Furthermore, $FEV_{0.4}$ is well suited to longitudinal study as it is intuitively more comparable to the measures of lung function, such as FEV_1, which are used in older children and adults.

The results of this study are of relevance to the general population. We recruited healthy infants and only excluded those born before 37 weeks gestation to prevent bias due to effects

of prematurity upon lung development. Our results demonstrate that variations in measures of expiratory flow measured in unselected populations are associated with preschool wheeze.

Small study numbers limited the exploration of lung function measures according to wheeze phenotype. However, a significant difference in $FEV_{0.4}$ was noted in non-atopic children who wheezed during the third year of life compared to non-atopic children without wheeze. There was no difference in any measure of infant lung function when atopic children with wheeze in the third year were compared to non-atopic children without wheeze. This may have been partly related to greater power associated with a more balanced distribution of non-atopic than atopic individuals between the wheeze and no wheeze groups. However, others have speculated that wheezing during the first year of life is often a transient condition due to reduced airway caliber and that wheeze beginning or persisting into later childhood is more likely to be due to the wheeze phenotype recognised as asthma[4].

Due to the number of subjects studied it was not possible to analyse our data according to age of onset. However, the broader confidence intervals and divergence of results between atopic and non-atopic individuals at age three years may be evidence of comparative heterogeneity of wheeze phenotypes later in childhood. The strong association between reduced measures of forced expiratory flow and wheeze at one year may reflect a common underlying mechanism, similar to that proposed by Martinez, whereby initial airway diameter, length or other characteristics might predispose certain infants to wheezing with viral respiratory infections[3]. With growth this may resolve in some infants as airway size increases but for those with the smallest airway dimensions the risk of non-atopic wheeze may persist. In contrast, airway dimensions at birth may be less significant in the aetiology of atopic wheeze compared to other factors acquired in association with an atopic phenotype.

As described earlier, ADAM33 is expressed in the embryonic lung[15] and SNPs in the gene encoding ADAM33 were previously found to predict impaired lung function at age 3 and 5 years[16]. However, it is unclear whether ADAM33 polymorphism is associated with abnormal lung function at birth (reflecting altered *in utero* lung development) or whether post-natal gene-environment interaction alters lung function and increases risk of asthma. Recent observations from the PIAMA birth cohort show that *in utero*, but not post natal, cigarette smoke exposure interacts with ADAM33 polymorphism to determine childhood lung function and BHR would suggest that ADAM33 polymorphism may alter *in utero* lung development[28]. In the current study, no ADAM33 haplotype was associated at the 5% level with lung function or symptoms in the first or third years of life after correction for multiple testing. Simpson *et al.* described that the F+1 polymorphism explained 3% of the variance in lung function (sRaw) at 3 years of age[16]; power calculations show that we only had 19% power to detect an association that predicts 5% variance in Crs measurements. Given the observation of interaction with *in utero* smoke exposure and the trend towards association seen in the current study, the association between ADAM33 haplotypes and infant lung function needs to be examined in a meta-analysis of data from all available infant lung function cohorts.

Strengths and limitations of this study

We successfully measured infant lung function in 147 infants; the robust $FEV_{0.4}$ measurement was recorded in 98 infants, and 141 children (96%) were followed up to age 3 years. Although the number of infants recruited to this study was small it is comparable to the numbers recruited to similar cohort studies[4;5]. The numbers involved precluded adjustment for multiple confounders or subgroup analysis. For example, although some studies have found evidence of reduced early lung function in infants whose mothers smoked[29;30], it was not

feasible to stratify for maternal smoking. Moreover, maternal smoking was not considered a confounder of the relationship between infant lung function and later wheeze as we have previously demonstrated that infant lung function is not significantly associated with maternal smoking in our cohort[18]. A second source of potential bias relates to exclusion of children on the basis of respiratory infection prior to lung function measurement. This exclusion was used to ensure that the effects of premorbid lung function upon later wheeze status were explored and to avoid bias by postnatal effects. This would only bias the result if the relationship between infant lung function and later wheeze differed between those infants who experienced early infection and those who did not. There is no reason to believe that this relationship would differ in a manner which would decrease the strength of the association between poor infant lung function and later wheeze; that is to say it is unlikely infants suffering early respiratory infection would have a better outcome in terms of later wheeze than those that did not experience early infections.

In summary this study has shown a significant relationship between early life lung function and respiratory symptoms in the first and third years of life. Lower $FEV_{0.4}$ in early infancy was associated with non-atopic wheeze but not atopic wheeze at 3 years of age. This is further evidence suggesting that pre-natal factors contribute to the development of wheeze in early life.

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Figures

Figure 1 Flow diagram of recruitment process and follow up

Tables

Table 1 Characteristics of those children reported to wheeze at 3 years of age compared to those who were not

	Children not reported to wheeze at 3 years (n=108)	Children reported to wheeze at 3 years (n=33)	P-value
Maternal age at child's birth (years), mean (SD)	30.3 (3.3)	29.7 (4.2)	0.3
Maternal smoking during pregnancy, (%)	12.3	27.3	0.04
Male gender, (%)	49.1	54.6	0.6
Maternal atopy, (%)	43.3	48.3	0.6

Table 2 Infant lung function measures by whether the infant wheezed or not in the first and in the third year of life

	One year of age					Three years of age				
	No wheeze		Wheeze		Reduction/increase in those who wheezed	No wheeze		Wheeze		Reduction/increase in those who wheezed
	Mean, (95% CI)	n	Mean, (95% CI)	n	%, (95% CI)	Mean, (95% CI)	n	Mean, (95% CI)	n	%, (95% CI)
V'_{maxFRC}*	149.0, (133.3 to 166.5)	70	115.4, (103.3 to 129.0)	72	-22.5%, (-33.7% to -9.5%)	139.2, (126.5 to 153.1)	106	106.8, (93.0 to 122.7)	32	-23.2%, (-36.4 % to -7.3%)
$FEV_{0.4}$**	143.3, (136.4 to 150.7)	49	131.8, (124.0 to 140.1)	49	-8.1%, (-15.0% to -0.6%)	139.6, (133.8 to 145.7)	73	128.6, (118.4 to 139.6)	25	-7.9%, (-15.5% to 0.3%)
Crs***	49.4, (47.3 to 51.6)	52	46.0, (43.7 to 48.5)	56	-6.8%, (-12.8% to -0.3%)	48.0, (46.2 to 49.8)	79	46.8, (43.1 to 50.7)	29	-2.5%, (-9.8% to 5.4%)
Respiratory rate (breaths/min)	42.8, (41.1 to 44.5)	69	45.7, (43.8 to 47.7)	72	6.8%, (0.8% to 13.1%)	44.1, (42.6 to 45.7)	106	45.2, (42.9 to 47.7)	31	2.5%, (-4.6% to 10.1%)

* Maximal expiratory flow at functional residual capacity (ml/s), adjusted for age

** Forced expiratory volume in the first 0.4 seconds of expiration (ml), adjusted for age

*** Respiratory system compliance (ml/mm water), adjusted for age and sex

Results are geometric mean values. Percentage increase is calculated from the logged values of the respiratory measures

Table 3 Infant lung function measures by whether the infant wheezed and their atopy status in the third year of life

	No wheeze and no atopy		Wheeze and no atopy		Reduction/increase in those who wheezed but were not atopic	Wheeze and atopy		Reduction/increase in those who wheezed and were atopic
	Mean, (95% CI)	n	Mean, (95% CI)	n	%, (95% CI)	Mean, (95% CI)	n	%, (95% CI)
V'_{maxFRC}*	135.0, (119.8 to 152.1)	71	107.1, (87.5 to 131.1)	20	-20.6%, (-37.9% to 1.5%)	108.5, (87.1 to 135.2)	7	-19.6%, (-45.3% to 18.1%)
$FEV_{0.4}$*	139.0, (132.2 to 146.2)	49	123.7, (114.6 to 133.5)	16	-11.0%, (-19.2% to -2.0%)	148.9, (110.1 to 201.3)	5	7.1%, (-9.6% to 26.9%)
Crs**	48.6, (46.4 to 51.0)	56	45.8, (41.3 to 50.9)	19	-5.8%, (-14.7% to 4.1%)	51.8, (45.5 to 59.0)	6	6.5%, (-8.2% to 23.6%)
Respiratory rate (breaths/min)	45.5, (43.4 to 47.6)	71	43.8, (40.6 to 47.2)	20	-3.7%, (-12.3% to 5.7%)	49.1, (45.0 to 53.5)	6	7.9%, (-7.8% to 26.3%)

* Maximal expiratory flow at functional residual capacity (ml/s), adjusted for age

** Forced expiratory volume in the first 0.4 seconds of expiration (ml), adjusted for age

*** Respiratory system compliance (ml/mm water), adjusted for age and sex

Results are geometric mean values. Percentage increase is calculated from the logged values of the respiratory measures