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Pediatric Pulmonology

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

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3 4	37	Summary
5 6 7	38	Rationale
7 8 9	39	There is evidence that perinatal lung development predicts childhood wheeze. However, very
10 11	40	few studies have examined whether preschool wheeze is associated with lower premorbid
12 13	41	lung function in early infancy, and as yet there is no information relating atopic and non-
14 15 16	42	atopic preschool wheeze to early lung development.
17 18	43	Objective
19 20 21	44	To examine the association between premorbid infant lung function and preschool wheeze,
21 22 23	45	and to explore associations with atopic and non-atopic wheeze phenotypes.
24 25	46	Methods
26 27 28	47	Infant lung function was measured in 147 healthy term infants aged 5-14 weeks. Rapid
29 30	48	thoracoabdominal compression was performed during tidal breathing and at raised volume to
31 32	49	measure maximal expiratory flow at functional residual capacity (V'_{maxFRC}) and forced
33 34 35	50	expiratory volume in 0.4 sec (FEV $_{0.4}$). Atopic status was determined by skin prick testing at 3
36 37	51	years and wheeze ascertained from parental questionnaires (1 and 3 years).
38 39 40	52	Measurements and Main Results
40 41 42	53	Lower early infancy V'_{maxFRC} was associated with wheeze in both the first and third years of
43 44	54	life (p=0.002 and p=0.006, respectively). Lower early infancy $FEV_{0.4}$ was associated with
45 46 47	55	wheeze in the first year (p=0.03). Compared to non-atopic children who did not wheeze, non-
48 49	56	atopic children who wheezed in their third year of life had lower $FEV_{0.4}$, (p=0.02), whilst
50 51 52	57	$FEV_{0.4}$ values of atopic children who wheezed were not significantly different (p=0.4).
52 53 54	58	Conclusions
55 56	59	Lower premorbid infant lung function was present in infants who subsequently wheezed
57 58 59	60	during the first and third years of life. Lower $FEV_{0.4}$ in early infancy was associated with non-
60	61	atopic wheeze but not atopic wheeze at 3 years of age.

62 Word count 248

63 Keywords: wheeze, asthma, infant lung function, preschool.

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65 Introduction

Previous studies have demonstrated that reduced infant lung function is present in those children who subsequently experience wheeze¹⁻⁵. Although tracking of lung function from infancy, through childhood⁶ and into adult life⁷ is well described, the relationship between early lung function and phenotypes of wheezing illness is less clear; for example, in 125 infants assessed at a mean age 2.4 months (SD 2 months) within the Tucson study, a reduced V'_{maxFRC} was associated with a transient wheeze phenotype, but no association was found with wheezing after 3 years of age⁸. 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 symptoms⁸. More recently, work has been undertaken to determine clinical phenotypes^{10;11} and to consider whether early impairment of lung function is 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 were found to have lower forced expiratory volumes at 8-20 months than healthy controls or wheezy infants at lower risk of subsequent asthma¹². This study suggested that 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, that is lung function measured prior to any respiratory infection, was not measured and the relationship between lung function and clinical wheeze phenotype according to atopy was not established in an unselected population. Whilst there is evidence that both early lung function and atopic status are determinants of preschool wheeze it is not

clear how premorbid infant lung function relates to the atopic and non-atopic wheezephenotypes.

Premorbid infant lung function is likely to reflect both early environmental influences and common genetic polymorphisms. ADAM33 is a positionally cloned asthma susceptibility gene that shows strongest linkage with a combined asthma and bronchial hyperresponsiveness phenotype¹³. ADAM33 is expressed in lung fibroblasts and bronchial smooth muscle and is expressed during embryonic lung development¹⁴. Previously, ADAM33 polymorphisms have been associated with impaired lung function at 3 years of age, specifically deficits in sRaw, and carriers homozygous for the A allele of F+1 SNP have been shown to have double the risk of transient early wheeze¹⁵. 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 children¹⁶.

The Southampton Women's Survey (SWS) was the first to measure premorbid $FEV_{0.4}$ in a longitudinal pregnancy cohort study¹⁷. In SWS infants, a rapid thoracoabdominal compression (RTC) technique was used to measure V'_{maxFRC} at raised volume (RV-RTC), reducing intersubject and intrasubject variability by removing the use of a variable volume landmark¹⁸. It has been suggested that measurement of timed forced expiratory volumes using RVRTC is a more sensitive method of detecting impaired airway function than V'_{maxFRC}^{19:20}. The SWS study was also the first 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 examine the hypothesis that lower infant lung function is associated with preschool wheeze and to then explore the associations between premorbid infant lung function and preschool atopic and

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2 3 4	113	non-atopic wheeze phenotypes. As a secondary aim, we explored the relationship between
5 6	114	ADAM33 polymorphism, lung function in infancy and preschool wheeze.
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115 Methods

116 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 has been described¹⁷. In brief, Caucasian infants, born at at least 37 weeks' gestation without major congenital anomalies and prior to any respiratory infection were recruited from the SWS, a population-based pregnancy cohort²¹. 429 women were approached about the study after the birth of their babies; 264 (62%) declined before testing. Of those who agreed to participate 18 (10%) were excluded because of recent upper respiratory tract infection or other illness (Figure 1). The majority of subjects declining did so due to concerns about sedation, 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 by mouth). 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 described¹⁷, 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

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performed using a resuscitator connected to the pneumotachograph, inducing the respiratory muscles to relax via the Hering-Breuer reflex. Compliance of the respiratory system (Crs ml/mm water) was calculated using SQUEEZE software from the resultant passive flow-volume curves as change in volume for unit of airway outlet pressure (an estimate of alveolar pressure). 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 colleagues¹⁸, and FEV_{0.4} and FVC were calculated using SQUEEZE from the forced expiratory flow-volume curve. For each infant, FEV_{0.4} was calculated from the 'best' raised volume loop (greatest sum of FEV_{0.4} and FVC) and the highest technically acceptable 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 study nurses. A new diary was posted every 3 months and followed up by reminder phone calls from the study team. For

159 each 24 hr 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

161 your child experienced any episodes of chestiness associated with wheezing or whistling in

162 his/her chest since they were last seen?'. Skin reactivity to cat^a, dog^a, house dust mite^a

 $\binom{6}{8}$ 163 (Dermatophagoides pteronyssinus), milk^a, grass pollens^a, and egg^b was assessed at age 3 years

60 164 (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.

168 <u>Genotyping</u>

169 DNA was isolated from cord blood using a salting-out procedure²³. ADAM33 SNPs Bp1 170 (rs487377), Fp1 (rs511898), STp7 (rs574174), Vm3 (rs628977), V4 (rs2787094) were 171 genotyped by TaqMan SNP genotyping assays (E-Table1). The SNPs were chosen for 172 genotyping as they were determined to be haplotype tagging SNPs, (equivalent to $r^2 \ge 0.8$, 173 minor allele frequency > 5%) based on the LDU map from Simpson *et al*¹⁶, thereby providing 174 more genetic information as they tag a further 12 SNPs spanning the *ADAM33* gene region.

176 <u>Statistical Analysis</u>

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.)²³ in R (version 2.5.1)²⁴ 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. This equates to a 10% change in $FEV_{0.4}$, a 12% change in

0 189 Crs or respiratory rate, or a 33% change in V'_{maxFRC} .

Results

191	147 infants had lung function measurements, of which 146 provided questionnaire data at age
192	one year, 141 provided questionnaire data at 3 years of age and 113 had skin prick testing.
193	Symptom diaries were returned by 102 mothers. There was strong concordance between the
194	prospective symptom diaries and retrospective questionnaires with respect to classification of
195	children according to presence or absence of wheeze during the first year of life, (κ =0.71,
196	p<0.001). Given this, all subsequent analyses are based upon questionnaire data. A total of
197	114 DNA samples were available for ADAM33 SNP genotyping; genotype assignment
198	approached 99% (6 failed PCR reactions out of a total 570) with a maximum of two
199	unassigned genotypes for each SNP and all genotype frequencies were in Hardy-Weinberg
200	Equilibrium. Matched genotype and lung function data were available for 103 children for
201	respiratory rate, 82 for Crs, 105 for V' _{maxFRC} and for 76 children for FEV _{0.4} . (See online
202	supplement).
203	

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 included in the study of infant lung function and those that who were approached but did not participate are listed in table 1. Comparing children who did and did not participate, there were no differences in gender, maternal smoking during pregnancy or history of asthma in either parent, but those who did

not participate were more likely to have a younger mother with lower educational qualification and to be of lower birth weight (Table 1).

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 atopic status was also taken into account, $FEV_{0.4}$ was found to be 11% lower in those who wheezed but were not atopic compared to participants with no wheeze nor atopy (p=0.02) (Table 3). There were no significant differences in any measure of infant lung function when either atopic children with wheeze in the third year or atopic children who did not wheeze in the third year were compared to non-atopic children without wheeze (Table 3).

ADAM33 SNP and haplotype analysis

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3 4	238	No ADAM33 SNP or haplotype was significantly associated with infant lung function
5 6	239	measurements after adjusting for multiple testing by Bonferroni correction (E-Table 3).
7 8 9	240	Haplopower analysis (Online Data Supplement) revealed that with the small sample size (n =
10 11	241	82) we were underpowered (Power = 19%) to detect a common haplotype (freq = 18%)
12 13 14	242	association accounting for 5% of the variance in the mean of the Crs measurement.
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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 life²⁵. Although we found no associations with ADAM33 polymorphisms this may have resulted from the low power of our study resulting from the challenge of recruiting large numbers of participants into a study assessing infant lung function.

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 months¹². 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, thus FEV_{0.4} provides a more robust measure of infant lung function than V'_{maxFRC}^{26} . Furthermore, FEV_{0.4} is well suited to longitudinal study as it is intuitively more comparable to measures of lung function, such as FEV_1 , which are used in older children and adults.

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268 The results of this study are likely to be of relevance to the general population. We recruited 269 healthy infants and only excluded those born before 37 weeks gestation to prevent bias due to 270 effects of prematurity upon lung development. Our results demonstrate that variations in 271 measures of expiratory flow measured in unselected populations are associated with preschool 272 wheeze.

274 Small study numbers limited the exploration of lung function measures according to wheeze 275 phenotype. However, non-atopic children who wheezed during the third year of life had a 276 significantly lower FEV_{0.4} in infancy compared to non-atopic children without wheeze. There 277 was no difference in any measure of infant lung function when atopic children with wheeze in 278 the third year were compared to non-atopic children without wheeze. This may have partly 279 related to greater power associated with a more balanced distribution of non-atopic than 280 atopic individuals between the wheeze and no wheeze groups. However, others have 281 speculated that wheezing during the first year of life is often a transient condition due to 282 reduced airway caliber and that wheeze beginning or persisting into later childhood is more 283 closely aligned with the wheeze phenotype recognised as asthma⁴.

Due to the number of subjects studied it was not possible to stratify our data according to age 285 286 of onset. However, the broader confidence intervals and divergence of results between atopic 287 and non-atopic individuals at age three years may be evidence of comparative heterogeneity 288 of wheeze phenotypes later in childhood. The strong association between reduced measures of 289 forced expiratory flow and wheeze at one year may reflect a common underlying mechanism, 290 similar to that proposed by Martinez, whereby initial airway diameter, length or other 291 characteristics might predispose certain infants to wheezing with viral respiratory infections³. 292 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 lung¹⁴ and SNPs in the gene encoding ADAM33 were previously found to predict impaired lung function at age 3 and 5 years¹⁵. 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 showing that in utero, but not post natal, cigarette smoke exposure interacts with ADAM33 polymorphism to determine childhood lung function and BHR suggests that ADAM33 polymorphism may alter *in utero* lung development²⁷. 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 age¹⁵; 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.

60 317 Although the number of infants recruited to this study was small it is comparable to the

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numbers recruited to similar cohort studies^{4;5}. The numbers involved limited 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^{28;29}, it was not feasible to stratify for maternal smoking. 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 this cohort¹⁷. 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 influence the findings 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 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.

3 341 Acknowledgements

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2 3 4	443	Figures
5 6	444	Figure 1 Flow diagram of recruitment process and follow up
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2 3 4	1	The relationship between infant lung function and the risk of wheeze in the preschool
5 6	2	years
7 8 9	3	
10 11	4	¹ Developmental Origins of Health and Disease Division, ² Human Genetics Division and
12 13	5	³ Infection, Inflammation & Immunity Division, , School of Medicine, University of
14 15 16	6	Southampton, ⁴ Medical Research Council Epidemiology Resource Centre, University of
17 18	7	Southampton, ⁵ Respiratory and ⁶ Nutrition, Diet & Lifestyle Biomedical Research Units,
19 20 21	8	Southampton University Hospitals NHS Trust
22 23	9	
24 25	10	
26 27 28 29 30	11	KC Pike MRCPCH ¹ , MJ Rose-Zerilli PhD ² , E Caffrey Osvald BM BS ³ , HM Inskip PhD ⁴ ,
	12	KM Godfrey FRCP ^{4,6} , SR Crozier PhD ⁴ , G Roberts MRCPCH ^{3,5} , JB Clough DM ³ , JW
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34	Running title: Infant lung	function and later wheeze
35	Word count: 3187	
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2 3	27	Summary .				
4	37	Summary				
5 6 7	38	Rationale				
8 9	39	There is evidence that perinatal lung development predicts childhood wheeze. However, very				
10 11	40	few studies have examined whether preschool wheeze is associated with lower premorbid				
12 13 14	41	lung function in early infancy, and as yet there is no information relating atopic and non-				
15 16	42	atopic preschool wheeze to early lung development.				
17 18	43	<u>Objective</u>				
19 20 21	44	To examine the association between premorbid infant lung function and preschool wheeze,				
22 23	45	and to explore associations with atopic and non-atopic wheeze phenotypes.				
24 25	46	Methods				
26 27 28	47	Infant lung function was measured in 147 healthy term infants aged 5-14 weeks. Rapid				
29 30	48	thoracoabdominal compression was performed during tidal breathing and at raised volume to				
31 32	49	measure maximal expiratory flow at functional residual capacity (V'_{maxFRC}) and forced				
33 34 35	50	expiratory volume in 0.4 sec (FEV $_{0.4}$). Atopic status was determined by skin prick testing at 3				
36 37	51	years and wheeze ascertained from parental questionnaires (1 and 3 years).				
38 39 40	52	Measurements and Main Results				
40 41 42	53	Lower early infancy V'_{maxFRC} was associated with wheeze in both the first and third years of				
43 44	54	life (p=0.002 and p=0.006, respectively). Lower early infancy $FEV_{0.4}$ was associated with				
45 46 47	55	wheeze in the first year (p=0.03). Compared to non-atopic children who did not wheeze, non-				
48 49	56	atopic children who wheezed in their third year of life had lower $FEV_{0.4}$, (p=0.02), whilst				
50 51	57	$FEV_{0.4}$ values of atopic children who wheezed were not significantly different (p=0.4).				
52 53 54	58	Conclusions				
55 56	59	Lower premorbid infant lung function was present in infants who subsequently wheezed				
57 58	60	during the first and third years of life. Lower $FEV_{0,4}$ in early infancy was associated with non-				
ว9 60	61	atopic wheeze but not atopic wheeze at 3 years of age.				

62 Word count 248

63 Keywords: wheeze, asthma, infant lung function, preschool.

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65 Introduction

Previous studies have demonstrated that reduced infant lung function is present in those children who subsequently experience wheeze¹⁻⁵. Although tracking of lung function from infancy, through childhood⁶ and into adult life⁷ is well described, the relationship between early lung function and phenotypes of wheezing illness is less clear; for example, in 125 infants assessed at a mean age 2.4 months (SD 2 months) within the Tucson study, a reduced V'_{maxFRC} was associated with a transient wheeze phenotype, but no association was found with wheezing after 3 years of age⁸. 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 symptoms⁸. More recently, work has been undertaken to determine clinical phenotypes^{10;11} and to consider whether early impairment of lung function is 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 were found to have lower forced expiratory volumes at 8-20 months than healthy controls or wheezy infants at lower risk of subsequent asthma¹². This study suggested that 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, that is lung function measured prior to any respiratory infection, was not measured and the relationship between lung function and clinical wheeze phenotype according to atopy was not established in an unselected population. Whilst there is evidence that both early lung function and atopic status are determinants of preschool wheeze it is not

clear how premorbid infant lung function relates to the atopic and non-atopic wheezephenotypes.

Premorbid infant lung function is likely to reflect both early environmental influences and common genetic polymorphisms. ADAM33 is a positionally cloned asthma susceptibility gene that shows strongest linkage with a combined asthma and bronchial hyperresponsiveness phenotype¹³. ADAM33 is expressed in lung fibroblasts and bronchial smooth muscle and is expressed during embryonic lung development¹⁴. Previously, ADAM33 polymorphisms have been associated with impaired lung function at 3 years of age, specifically deficits in sRaw, and carriers homozygous for the A allele of F+1 SNP have been shown to have double the risk of transient early wheeze¹⁵. 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 children¹⁶.

The Southampton Women's Survey (SWS) was the first to measure premorbid $FEV_{0.4}$ in a longitudinal pregnancy cohort study¹⁷. In SWS infants, a rapid thoracoabdominal compression (RTC) technique was used to measure V'_{maxFRC} at raised volume (RV-RTC), reducing intersubject and intrasubject variability by removing the use of a variable volume landmark¹⁸. It has been suggested that measurement of timed forced expiratory volumes using RVRTC is a more sensitive method of detecting impaired airway function than V'_{maxFRC}^{19:20}. The SWS study was also the first 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 examine the hypothesis that lower infant lung function is associated with preschool wheeze and to then explore the associations between premorbid infant lung function and preschool atopic and

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2 3 4	113	non-atopic wheeze phenotypes. As a secondary aim, we explored the relationship between
5 6	114	ADAM33 polymorphism, lung function in infancy and preschool wheeze.
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115 Methods

116 <u>Participants</u>

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 has been described¹⁷. In brief, Caucasian infants, born at at least 37 weeks' gestation without major congenital anomalies and prior to any respiratory infection were recruited from the SWS, a population-based pregnancy cohort²¹. 429 women were approached about the study after the birth of their babies; 264 (62%) declined before testing. Of those who agreed to participate 18 (10%) were excluded because of recent upper respiratory tract infection or other illness (Figure 1). The majority of subjects declining did so due to concerns about sedation, 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 by mouth). 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 described¹⁷, 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

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performed using a resuscitator connected to the pneumotachograph, inducing the respiratory muscles to relax via the Hering-Breuer reflex. Compliance of the respiratory system (Crs ml/mm water) was calculated using SQUEEZE software from the resultant passive flow-volume curves as change in volume for unit of airway outlet pressure (an estimate of alveolar pressure). 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 colleagues¹⁸, and FEV_{0.4} and FVC were calculated using SQUEEZE from the forced expiratory flow-volume curve. For each infant, FEV_{0.4} was calculated from the 'best' raised volume loop (greatest sum of FEV_{0.4} and FVC) and the highest technically acceptable 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 study nurses. A new diary was posted every 3 months and followed up by reminder phone calls from the study team. For each 24 hr 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.

168 <u>Genotyping</u>

DNA was isolated from cord blood using a salting-out procedure²³. ADAM33 SNPs Bp1 (rs487377), Fp1 (rs511898), STp7 (rs574174), Vm3 (rs628977), V4 (rs2787094) were genotyped by TaqMan SNP genotyping assays (E-Table1). The SNPs were chosen for genotyping as they were determined to be haplotype tagging SNPs, (equivalent to $r^2 \ge 0.8$, minor allele frequency > 5%) based on the LDU map from Simpson *et al*¹⁶, thereby providing more genetic information as they tag a further 12 SNPs spanning the *ADAM33* gene region.

176 <u>Statistical Analysis</u>

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.)²³ in R (version 2.5.1)²⁴ 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. This equates to a 10% change in $FEV_{0.4}$, a 12% change in

Crs or respiratory rate, or a 33% change in V'_{maxFRC}.

Results

191	147 infants had lung function measurements, of which 146 provided questionnaire data at age
192	one year, 141 provided questionnaire data at 3 years of age and 113 had skin prick testing.
193	Symptom diaries were returned by 102 mothers. There was strong concordance between the
194	prospective symptom diaries and retrospective questionnaires with respect to classification of
195	children according to presence or absence of wheeze during the first year of life, (κ =0.71,
196	p<0.001). Given this, all subsequent analyses are based upon questionnaire data. A total of
197	114 DNA samples were available for ADAM33 SNP genotyping; genotype assignment
198	approached 99% (6 failed PCR reactions out of a total 570) with a maximum of two
199	unassigned genotypes for each SNP and all genotype frequencies were in Hardy-Weinberg
200	Equilibrium. Matched genotype and lung function data were available for 103 children for
201	respiratory rate, 82 for Crs, 105 for V' _{maxFRC} and for 76 children for FEV _{0.4} . (See online
202	supplement).
203	
204	During the first year of life, 74 infants had at least one episode of wheezing. Thirty three
205	children experienced wheeze in the third year of life of these 23 had also experienced wheeze

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 included in the study of infant lung function and those that who were approached but did not participate are listed in table 1. Comparing children who did and did not participate, there were no differences in gender, maternal smoking during pregnancy or history of asthma in either parent, but those who did

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not participate were more likely to have a younger mother with lower educationalqualification and to be of lower birth weight (Table 1).

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217 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.

221 Relationship between infant lung function and respiratory symptoms reported at age 1 year 222 V'_{maxFRC} , FEV_{0.4} and Crs were lower in infants who subsequently wheezed in their first year 223 than in those who did not by 22.5% (p=0.002), 8.1% (p=0.03) and 6.8% (p=0.04) respectively 224 (Table 2). The group mean respiratory rate was 6.8% higher in the wheeze group than in the 225 non-wheeze group (p=0.03).

226

Relationship between infant lung function and respiratory symptoms reported at age 3 years 227 228 V'_{maxFRC} was 23.2% lower in infants who wheezed in their third year than in those who did 229 not (Table 2) (p=0.006). However, there were only small non-significant differences in 230 $FEV_{0.4}$, Crs and respiratory rate between the two groups. When atopic status was also taken 231 into account, $FEV_{0.4}$ was found to be 11% lower in those who wheezed but were not atopic 232 compared to participants with no wheeze nor atopy (p=0.02) (Table 3). There were no 233 significant differences in any measure of infant lung function when either atopic children with 234 wheeze in the third year or atopic children who did not wheeze in the third year were 235 compared to non-atopic children without wheeze (Table 3). 236

237 ADAM33 SNP and haplotype analysis

2 3	238	No ADAM33 SNP or haplotype was significantly associated with infant lung function
4 5	220	massurements after adjusting for multiple testing by Ronferroni correction (F. Table 3)
6 7	239	measurements after adjusting for multiple testing by Bomertoin correction (E-Table 5).
8 9	240	Haplopower analysis (Online Data Supplement) revealed that with the small sample size (n =
10 11	241	82) we were underpowered (Power = 19%) to detect a common haplotype (freq = 18%)
12 13 14	242	association accounting for 5% of the variance in the mean of the Crs measurement.
14 15 16 17 18 19 20 22 23 24 25 26 27 28 29 31 22 34 25 26 27 28 29 31 22 34 25 26 27 28 29 31 23 34 35 36 37 38 90 41 22 34 45 56 57 58 59 60	243	For peer periew

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 life²⁵. Although we found no associations with ADAM33 polymorphisms this may have resulted from the low power of our study resulting from the challenge of recruiting large numbers of participants into a study assessing infant lung function.

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 months¹². 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, thus FEV_{0.4} provides a more robust measure of infant lung function than V'_{maxFRC}^{26} . Furthermore, FEV_{0.4} is well suited to longitudinal study as it is intuitively more comparable to measures of lung function, such as FEV_1 , which are used in older children and adults.

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268 The results of this study are likely to be of relevance to the general population. We recruited 269 healthy infants and only excluded those born before 37 weeks gestation to prevent bias due to 270 effects of prematurity upon lung development. Our results demonstrate that variations in 271 measures of expiratory flow measured in unselected populations are associated with preschool 272 wheeze.

274 Small study numbers limited the exploration of lung function measures according to wheeze 275 phenotype. However, non-atopic children who wheezed during the third year of life had a 276 significantly lower FEV_{0.4} in infancy compared to non-atopic children without wheeze. There 277 was no difference in any measure of infant lung function when atopic children with wheeze in 278 the third year were compared to non-atopic children without wheeze. This may have partly 279 related to greater power associated with a more balanced distribution of non-atopic than 280 atopic individuals between the wheeze and no wheeze groups. However, others have 281 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 282 283 closely aligned with the wheeze phenotype recognised as asthma⁴.

Due to the number of subjects studied it was not possible to stratify our data according to age 285 286 of onset. However, the broader confidence intervals and divergence of results between atopic 287 and non-atopic individuals at age three years may be evidence of comparative heterogeneity 288 of wheeze phenotypes later in childhood. The strong association between reduced measures of 289 forced expiratory flow and wheeze at one year may reflect a common underlying mechanism, 290 similar to that proposed by Martinez, whereby initial airway diameter, length or other 291 characteristics might predispose certain infants to wheezing with viral respiratory infections³. 292 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 lung¹⁴ and SNPs in the gene encoding ADAM33 were previously found to predict impaired lung function at age 3 and 5 years¹⁵. 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 showing that in utero, but not post natal, cigarette smoke exposure interacts with ADAM33 polymorphism to determine childhood lung function and BHR suggests that ADAM33 polymorphism may alter *in utero* lung development²⁷. 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 age¹⁵; 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

316 was recorded in 98 infants, and 141 children (96%) were followed up to age 3 years.

60 317 Although the number of infants recruited to this study was small it is comparable to the

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numbers recruited to similar cohort studies^{4;5}. The numbers involved limited 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^{28;29}, it was not feasible to stratify for maternal smoking. 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 this cohort¹⁷. 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 influence the findings 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 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.

Acknowledgements

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343 They are grateful to Claire Foreman and the staff of the Wellcome Trust Clinical Research

344 Facility for collection of the infant lung function measurements and the staff of the

345 Southampton Women's Survey for collecting and processing the questionnaire data.

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2 3 4	443	Figures
5 6	444	Figure 1 Flow diagram of recruitment process and follow up
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KC Pike, MJ Rose-Zerilli, E Caffrey Osvald, HM Inskip, KM Godfrey, SR Crozier, GC Roberts, JB Clough, JW Holloway, JS Lucas and the Southampton Women's Survey Study Group

Online Data Supplement

plement

E-Table 1 ADAM33 TaqMan SNP genotyping assay primer and probe sequences.

Assay ID	Forward primer sequence (5'-3')	Reverse primer sequence	VIC Probe Sequence MGB-NFQ	6-Fam Probe Sequence MGB-NFQ
Bp1	GCCAAGATGGTACAGAAGAAGAAGAGT	GTGAGTGTTTCCTGCCTTTTGG	AGGCTAGGTgTCCCCT	AGGCTAGGTaTCCCCT
FP1	AATGCTGTATCTATAGCCCTCCAAA	GGAACTTGTTTCTTCAGACTTCAATAAA	AGAAGAGACgGGAATT	AGAGACaGGAATTCA
STp7	AAGGAACATCACAGGAAATGACAA	CCTGCTGCCCTTGATGATTC	CCATCCCATCcCC	CCATCCCATCtCC
Vm3	CTGTGGCTTTTCCTGGATCACT	GGCCCCAGGCTGCAA	AGCCCAcCCTCACT	AGCCCAtCCTCACTC
V4	GGCCCTATGGTTCGACTGAGT	AGGAAGGTCCCCAAAATTATGTTT	CTCCCCTGcAGCCT	CCCTGgAGCCTGG

^aSNP positions are denoted by lower case bases.

<text>

Haploscore Analysis

E-Table 2 ADAM33 Genotype Frequencies

		ADAM33 Bp1	ADAM33 Fp1	ADAM33 STp7	ADAM33 V4	ADAM33 Vm3
Genotypes		G>A	G>A	C>T	C>G	C>T
	11	73 (65%)	52 (46%)	79 (71%)	81 (72%)	49 (44%)
	12	38 (34%)	50 (44%)	32 (29%)	30 (27%)	46 (41%)
	22	2 (2%)	12 (11%)	1 (1%)	2 (2%)	17 (15%)
Undetermined		1 (1%)	0 (0%)	2 (2%)	1 (1%)	2 (2%)
Total genotypes	5					
(n=114)		113/114	114/114	112/114	113/114	112/114
HWE χ^2		1.40	0.00	1.35	0.17	1.25
HWE χ^2 = Hardy-Weinberg Equilibrium Chi-squared values; genotype frequencies with Chi-squared values <						

3.84 have not significantly deviated from HWE at the 5% level of significance.

HaploPower analysis of Crs measurement[1]

With a sample size of 82 we have the following statistical power when the second most

common (GGCTC, freq = 0.179) haplotype association accounts for 25% ($r^2 = 0.25$), 10% (r^2

= 0.10) and 5% (r^2 = 0.05) of the variance in the mean of the Crs measurement:

 $R^2 = 0.25$ Power (phased haplotype) = 0.93

 $R^2 = 0.10$ Power (phased haplotype) = 0.40

 $R^2 = 0.05$ Power (phased haplotype) = 0.19

HaploScore analysis settings

All haplotype analysis was performed under the additive genetic model, with a haplotype frequency cut off set to .005.

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E-Table 3 Summary of results of haplotype analysis of ADAM33 with uncorrected P

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values	<	0.1.
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								Uncorrected	Corrected
Phenotype	adam33bp1	adam33fp1	adam33stp7	adam33vm3	adam33v4	Hap-Freq ^a	Hap-Score ^b	P-value ^c	P-value ^d
Respiratory rate	G	G	С	Т	G	0.02351	-2.13291	0.0329	0.43
(n= 103)									
Crs									
(n = 82)	G	А	С	Т	C	0.0137	2.8227	0.0048	0.06
V'max _{FRC}	А	G	С	Т	G	0.07069	-1.85387	0.0638	
(n= 105)	А	А	Т	Т	С	0.03186	1.65231	0.0985	
Atopy									
(n= 82)	G	Α	Т	С	G	0.04798	2.40585	0.0161	0.24
Atopic wheeze									
(n= 57)	А	G	С	Т	G	0.0539	1.89707	0.0578	

^aOnly haplotypes with a greater then 1% frequency presented. ^bPositive z-score means the haplotype associates with increased measurement (continuous variable) or presence of a trait (binomial). ^cP values <0.1 are highlighted in bold. ^dSignificant P-values (< 0.05) were adjusted for multiple testing by Bonferroni correction (p-value multiplied by number of ADAM33 haplotypes with frequency > 0.005). Full analyses are presented in the on-line data supplement (Tables E4-E12).

E-Table 4 ADAM33 Haplotyping and FEV_{0.4} measurements (log transformed). (n= 76)

ADAM33	ADAM33	ADAM33	ADAM33	ADAM33	Нар	Hap-	Uncorrected	Corrected
Bp1	Fp1	STp7	Vm3	V4	Freq	Score ^a	P-value ^b	P-value ^c
G	А	С	С	С	0.09703	-1.14117	0.2538	N/A
А	А	Т	Т	G	0.00617	-0.96107	0.33652	N/A
G	А	Т	С	С	0.05153	-0.70109	0.48324	N/A
А	А	С	С	С	0.05347	-0.66507	0.50601	N/A
G	G	С	С	С	0.39818	-0.15671	0.87547	N/A
G	G	С	Т	G	0.01924	-0.02244	0.9821	N/A
G	А	Т	С	G	0.05769	0.35257	0.72441	N/A
G	G	С	Т	С	0.18731	0.48028	0.63103	N/A
G	А	Т	Т	С	0.01058	0.69738	0.48556	N/A
G	А	с	Т	С	0.01209	0.808	0.41909	N/A
А	G	С	Т	G	0.07291	0.81405	0.41562	N/A
А	А	Т	Т	С	0.03193	1.29756	0.19444	N/A

ADAM33	ADAM33	ADAM33	ADAM33	ADAM33	Hap	Нар-	Uncorrected	Corrected
Bp1	Fp1	STp7	Vm3	V4	Freq	Score ^a	P-value ^b	P-value ^c
G	G	С	С	С	0.4076	-0.3144	0.7533	N/A
G	G	С	Т	С	0.1794	0.4225	0.6727	N/A
G	А	С	С	С	0.0971	-0.2820	0.7780	N/A
А	G	С	Т	G	0.0729	-0.4183	0.6757	N/A
А	А	С	С	С	0.0539	0.8150	0.4151	N/A
G	А	Т	С	G	0.0476	-0.9151	0.3602	N/A
G	А	Т	С	С	0.0475	-0.4860	0.6270	N/A
А	А	Т	Т	С	0.0291	1.0337	0.3013	N/A
G	G	С	Т	G	0.0201	0.4818	0.6300	N/A
G	А	с	Т	С	0.0137	2.8227	0.0048	0.06
А	G	С	С	С	0.0111	-0.5687	0.5696	N/A
G	А	Т	Т	С	0.0104	0.6350	0.5254	N/A
А	А	Т	Т	G	0.0058	-0.5949	0.5519	N/A

E-Table 5 ADAM33 SNP haplotypes and Crs measurement (log transformed). (n = 82)

E-Table 6 ADAM33 Haplotyping and Respiratory Rate (log transformed). (n= 103)

ADAM33	ADAM33	ADAM33	ADAM33	ADAM33	Hap	Hap-	Uncorrected	Corrected
Bp1	Fp1	STp7	Vm3	V4	Freq	Score ^a	P-value ^b	P-value ^c
G	G	С	С	С	0.36409	-0.25286	0.80038	N/A
G	G	С	Т	С	0.18429	1.30254	0.19273	N/A
G	А	С	С	С	0.11114	-0.08324	0.93366	N/A
А	G	С	Т	G	0.07049	-0.19078	0.8487	N/A
G	А	Т	С	G	0.05388	1.40582	0.15978	N/A
А	А	С	С	С	0.0511	-1.05761	0.29024	N/A
G	А	Т	С	С	0.04914	0.12155	0.90325	N/A
А	А	Т	Т	С	0.0388	-0.49199	0.62273	N/A
А	G	С	Т	С	0.02461	-0.22106	0.82504	N/A
G	G	С	Т	G	0.02351	-2.13291	0.03293	0.43
А	А	Т	Т	G	0.00746	-0.96629	0.3339	N/A
А	G	С	С	С	0.00657	0.15884	0.87379	N/A
G	G	Т	Т	С	0.00605	-0.6583	0.51034	N/A

ADAM33	ADAM33	ADAM33	ADAM33	ADAM33	Нар	Hap-	Uncorrected	Corrected
Bp1	Fp1	STp7	Vm3	V4	Freq	Score ^a	P-value ^b	P-value ^c
G	G	С	С	С	0.37142	0.71104	0.47706	N/A
G	G	С	Т	С	0.1737	-0.40202	0.68767	N/A
G	А	С	С	С	0.11878	-0.16644	0.86781	N/A
А	G	С	Т	G	0.07069	-1.85387	0.06376	N/A
G	А	Т	С	G	0.05323	0.42974	0.66738	N/A
А	А	С	С	С	0.04967	-0.4796	0.63151	N/A
G	А	Т	С	С	0.04328	0.54817	0.58358	N/A
А	А	Т	Т	С	0.03186	1.65231	0.09847	N/A
А	G	С	Т	С	0.02568	0.04928	0.9607	N/A
G	G	c	Т	G	0.02236	-0.13254	0.89456	N/A
G	А	Т	Т	С	0.01204	0.10082	0.91969	N/A
G	А	С	Т	С	0.00885	0.2224	0.82401	N/A
А	G	С	С	С	0.00648	-0.77756	0.43683	N/A
А	А	Т	Т	G	0.0061	-0.52333	0.60075	N/A
G	G	Т	Т	С	0.00587	-0.11012	0.91231	N/A

E-Table 7 ADAM33 Haplotyping and V'max_{FRC} (log transformed). (n= 105)

^aPositive z-score means the haplotype associates with increased measurement (continuous variable) or presence of a trait (binomial). ^bP values <0.1 are highlighted in bold. ^cSignificant P-values (< 0.05) were adjusted for multiple testing by Bonferroni correction (p-value multiplied by number of ADAM33 haplotypes with frequency > 0.005).

ADAM33	ADAM33	ADAM33	ADAM33	ADAM33	Нар	Hap-	Uncorrected	Corrected
Bp1	Fp1	STp7	Vm3	V4	Freq	Score ^a	P-value ^b	P-value ^c
G	G	С	С	С	0.3926	-1.46574	0.14272	N/A
G	G	С	Т	С	0.18496	0.14124	0.88768	N/A
G	А	С	С	С	0.1147	-0.385	0.70023	N/A
А	G	С	Т	G	0.05167	1.36539	0.17213	N/A
G	А	Т	С	G	0.04798	2.40585	0.01613	0.24
G	А	Т	С	С	0.04363	1.0211	0.30721	N/A
А	А	Т	Т	С	0.04069	0.59387	0.5526	N/A
А	А	С	С	С	0.03277	1.18788	0.23488	N/A
G	G	С	Т	G	0.02062	-0.88568	0.37579	N/A
А	G	С	Т	С	0.01725	-0.82196	0.4111	N/A
А	А	Т	Т	G	0.0137	-0.41561	0.6777	N/A
G	А	С	Т	С	0.01206	-0.43907	0.59618	N/A
G	А	Т	Т	С	0.01136	-0.65321	0.51362	N/A
А	G	С	С	С	0.00855	-0.55888	0.57624	N/A
G	G	Т	Т	с	0.00727	-0.5299	0.57624	N/A

ADAM33	ADAM33	ADAM33	ADAM33	ADAM33	Нар	Hap-	Uncorrected	Corrected
Bp1	Fp1	STp7	Vm3	V4	Freq	Score ^a	P-value ^b	P-value ^c
G	G	С	С	С	0.37169	0.50102	0.61636	N/A
G	G	С	Т	С	0.17366	-0.21277	0.83151	N/A
G	А	С	С	С	0.11872	0.45715	0.64756	N/A
А	G	С	Т	G	0.06577	0.25515	0.79861	N/A
G	А	Т	С	G	0.05341	0.00583	0.99535	N/A
А	А	С	С	С	0.04969	-0.24647	0.80532	N/A
G	А	Т	С	С	0.04286	0.3942	0.69343	N/A
А	А	Т	Т	С	0.03695	-0.27592	0.78261	N/A
А	G	С	Т	С	0.02556	-0.85731	0.39127	N/A
G	G	с	Т	G	0.02242	-1.40217	0.16087	N/A
G	А	Т	Т	С	0.01206	-0.59158	0.55413	N/A
G	А	С	Т	С	0.00886	-0.55052	0.58196	N/A
А	G	С	С	С	0.00648	-0.65254	0.51405	N/A
А	А	Т	Т	G	0.00602	0.18489	0.85332	N/A
G	G	Т	Т	С	0.00584	1.54596	0.12211	N/A

E-Table 9 ADAM33 Haplotyping and wheeze in first year of life. (n= 105)

^aPositive z-score means the haplotype associates with increased measurement (continuous variable) or presence of a trait (binomial). ^bP values <0.1 are highlighted in bold. ^cSignificant P-values (< 0.05) were adjusted for multiple testing by Bonferroni correction (p-value multiplied by number of ADAM33 haplotypes with frequency > 0.005).

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E-Table 10 ADAM3.	B Haplotyping and	wheeze in the th	nird year of life.	(n= 101)
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ADAM33	ADAM33	ADAM33	ADAM33	ADAM33	Нар	Нар-	Uncorrected	Corrected
Bp1	Fp1	STp7	Vm3	V4	Freq	Score ^a	P-value ^b	P-value ^c
G	G	С	С	С	0.37754	0.2363	0.8132	N/A
G	G	С	Т	С	0.17981	-0.47127	0.63745	N/A
G	А	С	С	С	0.10778	0.57664	0.56418	N/A
А	G	С	Т	G	0.06289	-0.03682	0.97063	N/A
G	А	Т	С	G	0.05556	0.16215	0.87119	N/A
G	А	Т	С	С	0.04453	1.42632	0.15378	N/A
А	А	С	С	С	0.04166	1.19777	0.23101	N/A
А	А	Т	Т	С	0.03834	-0.96884	0.33263	N/A
А	G	С	Т	С	0.027	-0.82586	0.40889	N/A
G	G	С	Т	G	0.02338	-1.32961	0.18365	N/A
G	А	Т	Т	С	0.01231	-0.57808	0.60513	N/A
G	А	С	Т	С	0.00997	-0.12154	0.90327	N/A
А	А	Т	Т	G	0.00669	0.02954	0.97644	N/A
А	G	С	С	С	0.00659	-0.51704	0.56321	N/A
G	G	Т	Т	с	0.00595	-0.61456	0.53885	N/A

ADAM33	ADAM33	ADAM33	ADAM33	ADAM33	Нар	Нар-	Uncorrected	Corrected
Bp1	Fp1	STp7	Vm3	V4	Freq	Score ^a	P-value ^b	P-value ^c
G	G	С	С	С	0.44182	0.66335	0.50711	N/A
G	G	С	Т	С	0.16215	-0.31129	0.75558	N/A
G	А	С	С	С	0.10858	-0.69978	0.48406	N/A
А	G	С	Т	G	0.0539	1.89707	0.05782	N/A
G	А	Т	С	G	0.03574	0.9594	0.33735	N/A
А	А	Т	Т	С	0.03392	-0.70422	0.4813	N/A
G	G	С	Т	G	0.02821	-0.60566	0.54474	N/A
G	А	Т	Т	С	0.02623	-0.61918	0.5358	N/A
А	G	С	Т	С	0.02581	-0.61498	0.53857	N/A
G	А	С	Т	С	0.02252	0.47068	0.63787	N/A
А	G	С	с	С	0.01424	-0.40237	0.68741	N/A
G	А	Т	С	С	0.01307	-0.4319	0.66581	N/A
А	А	Т	Т	G	0.0119	-0.41454	0.67848	N/A
G	G	Т	Т	С	0.01073	-0.38745	0.69842	N/A
А	А	С	С	с	0.00937	-0.36681	0.71376	N/A

E-Table 11_ADAM33 Haplotyping and atopic wheeze in the third year of life. (n= 57)

^aPositive z-score means the haplotype associates with increased measurement (continuous variable) or presence of a trait (binomial). ^bP values <0.1 are highlighted in bold. ^cSignificant P-values (< 0.05) were adjusted for multiple testing by Bonferroni correction (p-value multiplied by number of ADAM33 haplotypes with frequency > 0.005).

E-Table 12_ADAM33 Haplotyping and non-atopic wheeze in the third year of life. (n=

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ADAM33	ADAM33	ADAM33	ADAM33	ADAM33	Нар	Hap-	Uncorrected	Corrected
Bp1	Fp1	STp7	Vm3	V4	Freq	Score ^a	P-value ^b	P-value ^c
G	G	С	С	С	0.42518	0.12058	0.90403	N/A
G	G	С	Т	С	0.17242	-0.29578	0.7674	N/A
G	А	С	С	С	0.12302	0.28399	0.77641	N/A
А	G	С	Т	G	0.03561	-0.17169	0.86368	N/A
А	А	Т	Т	С	0.03399	-0.19726	0.84362	N/A
G	А	Т	С	С	0.03181	1.38403	0.16635	N/A
G	А	Т	С	G	0.03003	0.15309	0.87833	N/A
А	G	С	Т	С	0.02838	-0.27028	0.78694	N/A
G	G	С	Т	G	0.02674	-1.0183	0.30853	N/A
А	А	С	С	С	0.0203	1.4343	0.15149	N/A
А	А	Т	Т	G	0.01953	0.3155	0.75238	N/A
G	А	Т	Т	С	0.01726	-0.83726	0.40245	N/A
G	А	С	Т	С	0.01509	-0.49991	0.61714	N/A
А	G	С	с	С	0.01145	-0.4895	0.62449	N/A
G	G	Т	Т	С	0.00918	-0.64524	0.51877	N/A

Online Supplement References

1. Schaid DJ. Power and Sample Size for Testing Associations of Haplotypes with Complex Traits. *Annals of Human Genetics* 2005;70:116-130.



Figure 1 Flow diagram of recruitment process and follow up 275x190mm (96 x 96 DPI)

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Table 1 Characteristics of those children who performed the lung function test

compared with those who were eligible but did not participate

	Performed lung	Did not perform lung	P-
	function test (n=147)	function test (n=327)	value
Mother's age at child's birth (mean(SD))	30.1 (3.5)	29.3 (3.9)	0.048
Mother's Educational attainment (n(%))			
None	3 (2.0)	15 (4.6)	
CSE/GCSE D-G	15 (10.2)	47 (14.4)	
O levels/GCSE A*-C	35 (23.8)	119 (36.4)	
A levels	52 (35.4)	92 (28.1)	
HND	7 (4.8)	20 (6.1)	
Degree	35 (23.8)	34 (10.4)	< 0.001
Mother has had asthma (n(%))			
No	114 (78.6)	252 (78.3)	
Yes	31 (21.4)	70 (21.7)	0.9
Father has had asthma $(n(\%))$			
No	123 (84.8)	263 (82.7)	
Yes	22 (15.2)	55 (17.3)	0.6
Mother smoked in pregnancy (n(%))			
No	121 (83.4)	241 (76.3)	
Yes	24 (16.6)	75 (23.7)	0.08
Sex (n(%))			
Male	75 (51.0)	182 (55.7)	
Female	72 (49.0)	145 (44.3)	0.3
Birth weight (kg) (mean(SD))	3.59 (0.47)	3.47 (0.47)	0.007

Table 1 Characteristics of those children who performed the lung function test

compared with those who were eligible but did not participate

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	function test (n=147)	function test (n=327)	value
Mother's age at child's birth (mean(SD))	30.1 (3.5)	29.3 (3.9)	0.048
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CSE/GCSE D-G	15 (10.2)	47 (14.4)	
O levels/GCSE A*-C	35 (23.8)	119 (36.4)	
A levels	52 (35.4)	92 (28.1)	
HND	7 (4.8)	20 (6.1)	
Degree	35 (23.8)	34 (10.4)	<0.001
Mother has had asthma (n(%))			
No	114 (78.6)	252 (78.3)	
Yes	31 (21.4)	70 (21.7)	0.9
Father has had asthma $(n(\%))$			
No	123 (84.8)	263 (82.7)	
Yes	22 (15.2)	55 (17.3)	0.6
Mother smoked in pregnancy (n(%))			
No	121 (83.4)	241 (76.3)	
Yes	24 (16.6)	75 (23.7)	0.08
Sex (n(%))			
Male	75 (51.0)	182 (55.7)	
Female	72 (49.0)	145 (44.3)	0.3
Birth weight (kg) (mean(SD))	3.59 (0.47)	3.47 (0.47)	0.007

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	No wheeze		Wheeze		Reduction/increase in
					those who wheezed					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	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%)
(breaths/min)										

* 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 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	No wheeze		Wheeze		Reduction/increase in
					those who wheezed					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	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

(breaths/min)

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	Wheeze and atopy		Reduction/increase in those	No wheeze but atopic		Reduction/increase in those
					those who wheezed but			who wheezed and were			who did not wheeze but
					were not atopic			ıtopic			were atopic
	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)	Mean (95% CI)	n	% (95% CI)	Mean (95% CI)	n	Mean (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%)	160.3 (120.4 to 213.5)	13	-18.8% (-60.2% to 12.0%)
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%)	138.7 (123.4 to 155.9)	8	0.2% (-13.6% to 12.4%)
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	5.5% (-8.2% to 23.6%)	47.4 (39.7 to 56.5)	7	2.6% (-12.3% to 15.6%)
Respiratory rate	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%)	45.1 (40.5 to 50.2)	13	0.9% (-11.0% to 11.5%)
(breaths/min)					664						
* Maxi	mal expiratory flo	ow a	t functional resid	ual	capacity (ml/s), adj	usted 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

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

	No wheeze and no atopy	ý	Wheeze and no atopy		Reduction/increase in those who wheezed but	Wheeze and atopy	Reduction/increase in those who wheezed and were	No wheeze but atopic		Reduction/increase in tho who did not wheeze but
	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)	Mean (95% CI)	1001c	Mean (95% CI)	n	were atopic Mean (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%)	160.3 (120.4 to 213.5)	13	-18.8% (-60.2% to 12.0%
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%)	138.7 (123.4 to 155.9)	8	0.2% (-13.6% to 12.4%)
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 5.5% (-8.2% to 23.6%)	47.4 (39.7 to 56.5)	7	2.6% (-12.3% to 15.6%)
Respiratory rate	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%)	45.1 (40.5 to 50.2)	13	0.9% (-11.0% to 11.5%)
(
* Maxie** Force*** Respin	mal expiratory flo d expiratory volu ratory system cor	ow a .me npli	t functional resid in the first 0.4 sec ance (ml/mm wat	ual conc ter),	capacity (ml/s), adj ds of expiration (ml , adjusted for age ai	usted for age), adjusted for ag nd sex	ge			
Results are g	eometric mean va	alues	s. Percentage incr	ease	e is calculated from	the logged value	es of the respiratory m	easures		