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



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3 37 **Summary**  
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5 38 Rationale  
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8 39 There is evidence that perinatal lung development predicts childhood wheeze. However, very  
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10 40 few studies have examined whether preschool wheeze is associated with lower premorbid  
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12 41 lung function in early infancy, and as yet there is no information relating atopic and non-  
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14 42 atopic preschool wheeze to early lung development.  
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17 43 Objective  
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20 44 To examine the association between premorbid infant lung function and preschool wheeze,  
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22 45 and to explore associations with atopic and non-atopic wheeze phenotypes.  
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25 46 Methods  
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28 47 Infant lung function was measured in 147 healthy term infants aged 5-14 weeks. Rapid  
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30 48 thoracoabdominal compression was performed during tidal breathing and at raised volume to  
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32 49 measure maximal expiratory flow at functional residual capacity ( $V'_{\max\text{FRC}}$ ) and forced  
33  
34 50 expiratory volume in 0.4 sec ( $\text{FEV}_{0.4}$ ). Atopic status was determined by skin prick testing at 3  
35  
36 51 years and wheeze ascertained from parental questionnaires (1 and 3 years).  
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39 52 Measurements and Main Results  
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41 53 Lower early infancy  $V'_{\max\text{FRC}}$  was associated with wheeze in both the first and third years of  
42  
43 54 life ( $p=0.002$  and  $p=0.006$ , respectively). Lower early infancy  $\text{FEV}_{0.4}$  was associated with  
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45 55 wheeze in the first year ( $p=0.03$ ). Compared to non-atopic children who did not wheeze, non-  
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47 56 atopic children who wheezed in their third year of life had lower  $\text{FEV}_{0.4}$ , ( $p=0.02$ ), whilst  
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49 57  $\text{FEV}_{0.4}$  values of atopic children who wheezed were not significantly different ( $p=0.4$ ).  
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52 58 Conclusions  
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55 59 Lower premorbid infant lung function was present in infants who subsequently wheezed  
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57 60 during the first and third years of life. Lower  $\text{FEV}_{0.4}$  in early infancy was associated with non-  
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59 61 atopic wheeze but not atopic wheeze at 3 years of age.  
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62 Word count 248

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

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For Peer Review

## 65 Introduction

66 Previous studies have demonstrated that reduced infant lung function is present in those  
67 children who subsequently experience wheeze<sup>1-5</sup>. Although tracking of lung function from  
68 infancy, through childhood<sup>6</sup> and into adult life<sup>7</sup> is well described, the relationship between  
69 early lung function and phenotypes of wheezing illness is less clear; for example, in 125  
70 infants assessed at a mean age 2.4 months (SD 2 months) within the Tucson study, a reduced  
71  $V'_{\max\text{FRC}}$  was associated with a transient wheeze phenotype, but no association was found  
72 with wheezing after 3 years of age<sup>8</sup>. In contrast, other studies suggest early impairment of  
73 lung function is a risk factor for wheeze which persists into later childhood<sup>6,9</sup>.

74  
75 The original 'wheeze phenotypes' described in the Tucson study were defined retrospectively  
76 according to age of onset and persistence of symptoms<sup>8</sup>. More recently, work has been  
77 undertaken to determine clinical phenotypes<sup>10,11</sup> and to consider whether early impairment of  
78 lung function is associated with childhood wheeze phenotypes based upon clinical features.  
79 Infants with recurrent wheeze considered at high risk of subsequent asthma due to either a  
80 parental history of asthma or personal history of eczema, allergic rhinitis, wheezing without a  
81 cold and/or serum eosinophilia were found to have lower forced expiratory volumes at 8-20  
82 months than healthy controls or wheezy infants at lower risk of subsequent asthma<sup>12</sup>. This  
83 study suggested that early impairment of lung function is associated with preschool wheeze  
84 and particularly so in the presence of markers of atopic sensitisation. However, premorbid  
85 infant lung function, that is lung function measured prior to any respiratory infection, was not  
86 measured and the relationship between lung function and clinical wheeze phenotype  
87 according to atopy was not established in an unselected population. Whilst there is evidence  
88 that both early lung function and atopic status are determinants of preschool wheeze it is not

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3 89 clear how premorbid infant lung function relates to the atopic and non-atopic wheeze  
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5 90 phenotypes.  
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10 92 Premorbid infant lung function is likely to reflect both early environmental influences and  
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12 93 common genetic polymorphisms. ADAM33 is a positionally cloned asthma susceptibility  
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14 94 gene that shows strongest linkage with a combined asthma and bronchial hyperresponsiveness  
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16 95 phenotype<sup>13</sup>. ADAM33 is expressed in lung fibroblasts and bronchial smooth muscle and is  
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18 96 expressed during embryonic lung development<sup>14</sup>. Previously, ADAM33 polymorphisms have  
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20 97 been associated with impaired lung function at 3 years of age, specifically deficits in sRaw,  
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22 98 and carriers homozygous for the A allele of F+1 SNP have been shown to have double the  
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24 99 risk of transient early wheeze<sup>15</sup>. Associations between a number of individual SNPs and both  
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26 100 baseline lung function and non atopic asthma have also been demonstrated in a population of  
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28 101 German school children<sup>16</sup>.  
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36 103 The Southampton Women's Survey (SWS) was the first to measure premorbid FEV<sub>0.4</sub> in a  
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38 104 longitudinal pregnancy cohort study<sup>17</sup>. In SWS infants, a rapid thoracoabdominal compression  
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40 105 (RTC) technique was used to measure V'<sub>maxFRC</sub> at raised volume (RV-RTC), reducing  
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42 106 intersubject and intrasubject variability by removing the use of a variable volume landmark<sup>18</sup>.  
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44 107 It has been suggested that measurement of timed forced expiratory volumes using RVRTC is  
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46 108 a more sensitive method of detecting impaired airway function than V'<sub>maxFRC</sub><sup>19:20</sup>. The SWS  
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48 109 study was also the first to investigate the predictive value of infant FEV<sub>0.4</sub> in healthy term  
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50 110 infants in relation to future wheeze. Using this outcome measure, we aimed to examine the  
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52 111 hypothesis that lower infant lung function is associated with preschool wheeze and to then  
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54 112 explore the associations between premorbid infant lung function and preschool atopic and  
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113 non-atopic wheeze phenotypes. As a secondary aim, we explored the relationship between  
114 ADAM33 polymorphism, lung function in infancy and preschool wheeze.

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3 115 **Methods**

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5 116 Participants

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8 117 The Southampton and South West Hampshire Local Research Ethics Committee approved the  
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10 118 protocol and written consent was obtained from the children's mothers. Detailed information  
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12 119 regarding the participants and infant lung function testing has been described<sup>17</sup>. In brief,  
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14 120 Caucasian infants, born at at least 37 weeks' gestation without major congenital anomalies  
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16 121 and prior to any respiratory infection were recruited from the SWS, a population-based  
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18 122 pregnancy cohort<sup>21</sup>. 429 women were approached about the study after the birth of their  
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20 123 babies; 264 (62%) declined before testing. Of those who agreed to participate 18 (10%) were  
21  
22 124 excluded because of recent upper respiratory tract infection or other illness (Figure 1). The  
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24 125 majority of subjects declining did so due to concerns about sedation, respiratory infection was  
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26 126 not a common reason for declining to participate. Lung function was measured, between May  
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28 127 1999 and October 2002, when the infants were 5-14 weeks old.  
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36 129 Lung function was measured, with infants lying supine, in quiet sleep augmented with chloral  
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38 130 hydrate (75-100 mg/kg by mouth). An inflatable jacket connected to a rapid inflation system  
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40 131 was placed around the infant's chest and abdomen and a leak-free facemask with Fleisch  
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42 132 pneumotachograph (Dynasciences, Blue Bell, CA) was held over the nose and mouth. Data  
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44 133 were collected using RASP software (Physiologic Ltd, Newbury, Berks, UK) and analysed in  
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46 134 SQUEEZE (Paul Dixon, London).  
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53 136 As previously described<sup>17</sup>, respiratory rate was measured during tidal breathing. To record  
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55 137 partial expiratory flow volume curves, a stable end expiratory level was established before  
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57 138 performing an RTC at the lowest pressure to achieve the best  $V'_{\max\text{FRC}}$ , calculated from the  
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59 139 partial expiratory flow volume curve. Passive, relaxed inflations to 30cm water were  
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3 140 performed using a resuscitator connected to the pneumotachograph, inducing the respiratory  
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5 141 muscles to relax via the Hering-Breuer reflex. Compliance of the respiratory system (Crs  
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7 142 ml/mm water) was calculated using SQUEEZE software from the resultant passive flow–  
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9 143 volume curves as change in volume for unit of airway outlet pressure (an estimate of alveolar  
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11 144 pressure). Raised volume RTC curves were recorded at the optimal jacket pressure at the end  
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13 145 of a passive inspiration using a technique adapted from Feher and colleagues<sup>18</sup>, and FEV<sub>0.4</sub>  
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15 146 and FVC were calculated using SQUEEZE from the forced expiratory flow–volume curve.  
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22 148 For each infant, FEV<sub>0.4</sub> was calculated from the ‘best’ raised volume loop (greatest sum of  
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24 149 FEV<sub>0.4</sub> and FVC) and the highest technically acceptable V’<sub>maxFRC</sub> was used. At least two  
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26 150 acceptable, reproducible (within 10%) raised volume and partial expiratory curves were  
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28 151 obtained. It was not possible to record valid data in every infant for each measure of lung  
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30 152 function; there were approximately 100 infants with acceptable lung function data for each  
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32 153 measurement.  
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### 39 155 Symptoms

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41 156 Mothers completed contemporaneous respiratory symptom diaries during their child’s first  
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43 157 year of life. Symptoms were explained to each mother by the study nurses. A new diary was  
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45 158 posted every 3 months and followed up by reminder phone calls from the study team. For  
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47 159 each 24 hr period parents recorded daytime and night-time wheeze. At ages one and three  
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49 160 years mothers completed a nurse-administered questionnaire which included the question ‘has  
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51 161 your child experienced any episodes of chestiness associated with wheezing or whistling in  
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53 162 his/her chest since they were last seen?’. Skin reactivity to cat<sup>a</sup>, dog<sup>a</sup>, house dust mite<sup>a</sup>  
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55 163 (Dermatophagoides pteronyssinus), milk<sup>a</sup>, grass pollens<sup>a</sup>, and egg<sup>b</sup> was assessed at age 3 years  
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57 164 (Hollister-Stier, Spokane, WA<sup>a</sup>, Alyostal, Antony, France<sup>b</sup>). Atopy was defined as a wheal to  
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3 165 any allergen at least 3mm in diameter in the presence of a wheal of at least 3 mm in diameter  
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5 166 to 10 mg/ml histamine<sup>a</sup> solution and no response to 50% glycerin<sup>a</sup>.  
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10 168 Genotyping

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12 169 DNA was isolated from cord blood using a salting-out procedure<sup>23</sup>. ADAM33 SNPs Bp1  
13 (rs487377), Fp1 (rs511898), STp7 (rs574174), Vm3 (rs628977), V4 (rs2787094) were  
14 170 (rs487377), Fp1 (rs511898), STp7 (rs574174), Vm3 (rs628977), V4 (rs2787094) were  
15 171 genotyped by TaqMan SNP genotyping assays (E-Table1). The SNPs were chosen for  
16 172 genotyping as they were determined to be haplotype tagging SNPs, (equivalent to  $r^2 \geq 0.8$ ,  
17 173 minor allele frequency > 5%) based on the LDU map from Simpson *et al*<sup>16</sup>, thereby providing  
18 174 more genetic information as they tag a further 12 SNPs spanning the ADAM33 gene region.  
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29 176 Statistical Analysis

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31 177 The infant lung function data were logarithmically transformed to achieve normality and  
32 178 adjusted for age and sex where necessary. t-tests were used to assess differences between  
33 179 geometric mean group values of infant lung function measurements according to wheeze  
34 180 status at 1 and 3 years, and after subdividing the 3-year wheeze group according to atopic  
35 181 status. Agreement between the parent-completed symptom diaries and the nurse-administered  
36 182 questionnaires was assessed by weighted Kappa ( $\kappa$ ) analysis. ADAM33 haplotype  
37 183 quantitative trait and power analysis were performed using HaploScore (Haplo.stats R  
38 184 package version 1.3.8.)<sup>23</sup> in R (version 2.5.1)<sup>24</sup> with the additive haplotype effect model.  
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52 186 Assuming 50 children in each outcome group, there was 80% power at the 5% level of  
53 187 significance to detect a difference of 0.57 SDs in any infant lung function measurement  
54 188 between the two outcome groups. This equates to a 10% change in FEV<sub>0.4</sub>, a 12% change in  
55 189 Crs or respiratory rate, or a 33% change in V'<sub>maxFRC</sub>.  
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**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, ( $\kappa = 0.71$ ,  $p < 0.001$ ). 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  $V'_{\max FRC}$  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 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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3 214 not participate were more likely to have a younger mother with lower educational  
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5 215 qualification and to be of lower birth weight (Table 1).  
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10 217 Associations between wheeze or wheeze phenotype and lung function were examined after  
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12 218 adjusting for factors that were found to influence measures of lung function on univariate  
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15 219 analysis. FEV<sub>0.4</sub>, and V'<sub>maxFRC</sub> were adjusted for gender and Crs for gender and age at testing.  
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20 221 Relationship between infant lung function and respiratory symptoms reported at age 1 year

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22 222 V'<sub>maxFRC</sub>, FEV<sub>0.4</sub> and Crs were lower in infants who subsequently wheezed in their first year  
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24 223 than in those who did not by 22.5% (p=0.002), 8.1% (p=0.03) and 6.8% (p=0.04) respectively  
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26 224 (Table 2). The group mean respiratory rate was 6.8% higher in the wheeze group than in the  
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28 225 non-wheeze group (p=0.03).  
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34 227 Relationship between infant lung function and respiratory symptoms reported at age 3 years

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36 228 V'<sub>maxFRC</sub> was 23.2% lower in infants who wheezed in their third year than in those who did  
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38 229 not (Table 2) (p=0.006). However, there were only small non-significant differences in  
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40 230 FEV<sub>0.4</sub>, Crs and respiratory rate between the two groups. When atopic status was also taken  
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42 231 into account, FEV<sub>0.4</sub> was found to be 11% lower in those who wheezed but were not atopic  
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44 232 compared to participants with no wheeze nor atopy (p=0.02) (Table 3). There were no  
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46 233 significant differences in any measure of infant lung function when either atopic children with  
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48 234 wheeze in the third year or atopic children who did not wheeze in the third year were  
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50 235 compared to non-atopic children without wheeze (Table 3).  
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57 237 ADAM33 SNP and haplotype analysis  
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3 238 No ADAM33 SNP or haplotype was significantly associated with infant lung function  
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6 239 measurements after adjusting for multiple testing by Bonferroni correction (E-Table 3).  
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8 240 Haplopower analysis (Online Data Supplement) revealed that with the small sample size (n =  
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10 241 82) we were underpowered (Power = 19%) to detect a common haplotype (freq = 18%)  
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12 242 association accounting for 5% of the variance in the mean of the Crs measurement.  
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For Peer Review

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3 244 **Discussion**  
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5 245 This study demonstrates an association between impaired premorbid infant lung function and  
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7 246 preschool wheezing illness. This confirms the results of previous studies<sup>4;5</sup>. Our analyses  
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10 247 show for the first time that lower lung function in early infancy is a risk factor for non-atopic  
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12 248 wheeze, rather than atopic wheeze. Additionally, this study is the first to report reduced  
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15 249 FEV<sub>0.4</sub>, prior to lower respiratory infection, as a risk factor for wheeze in the preschool years.  
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17 250 This is particularly important in view of recent work demonstrating that diminished timed  
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19 251 forced expiratory volumes are an important predictor of all-cause mortality in adult life<sup>25</sup>.  
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22 252 Although we found no associations with ADAM33 polymorphisms this may have resulted  
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24 253 from the low power of our study resulting from the challenge of recruiting large numbers of  
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27 254 participants into a study assessing infant lung function.  
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31 256 Premorbid measures of forced expiratory volume in 0.4 seconds (FEV<sub>0.4</sub>) during infancy as a  
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33 257 predictor of wheeze have not previously been studied, though reduced FEV<sub>0.5</sub> following  
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35 258 symptoms has been reported in wheezy children aged 8 – 20 months<sup>12</sup>. Lung function  
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38 259 measures derived from raised volume expiratory manoeuvres are believed to discriminate  
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41 260 better between healthy individuals and those with respiratory disease when compared to tidal  
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43 261 breathing measures<sup>19;20</sup>. FEV<sub>0.4</sub> is derived from measurement of expiratory flow from close to  
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45  
46 262 total lung capacity and is not dependent upon a volume landmark. This is more repeatable  
47  
48 263 than measurement at FRC as the FRC of infants can vary on a breath by breath basis, thus  
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50 264 FEV<sub>0.4</sub> provides a more robust measure of infant lung function than V'<sub>maxFRC</sub><sup>26</sup>. Furthermore,  
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53 265 FEV<sub>0.4</sub> is well suited to longitudinal study as it is intuitively more comparable to measures of  
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55 266 lung function, such as FEV<sub>1</sub>, which are used in older children and adults.  
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3 268 The results of this study are likely to be of relevance to the general population. We recruited  
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5 269 healthy infants and only excluded those born before 37 weeks gestation to prevent bias due to  
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7 270 effects of prematurity upon lung development. Our results demonstrate that variations in  
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9 271 measures of expiratory flow measured in unselected populations are associated with preschool  
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11 272 wheeze.  
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15 273  
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17 274 Small study numbers limited the exploration of lung function measures according to wheeze  
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19 275 phenotype. However, non-atopic children who wheezed during the third year of life had a  
20  
21 276 significantly lower FEV<sub>0.4</sub> in infancy compared to non-atopic children without wheeze. There  
22  
23 277 was no difference in any measure of infant lung function when atopic children with wheeze in  
24  
25 278 the third year were compared to non-atopic children without wheeze. This may have partly  
26  
27 279 related to greater power associated with a more balanced distribution of non-atopic than  
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29 280 atopic individuals between the wheeze and no wheeze groups. However, others have  
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31 281 speculated that wheezing during the first year of life is often a transient condition due to  
32  
33 282 reduced airway caliber and that wheeze beginning or persisting into later childhood is more  
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35 283 closely aligned with the wheeze phenotype recognised as asthma<sup>4</sup>.  
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41 284  
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43 285 Due to the number of subjects studied it was not possible to stratify our data according to age  
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45 286 of onset. However, the broader confidence intervals and divergence of results between atopic  
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47 287 and non-atopic individuals at age three years may be evidence of comparative heterogeneity  
48  
49 288 of wheeze phenotypes later in childhood. The strong association between reduced measures of  
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51 289 forced expiratory flow and wheeze at one year may reflect a common underlying mechanism,  
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53 290 similar to that proposed by Martinez, whereby initial airway diameter, length or other  
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55 291 characteristics might predispose certain infants to wheezing with viral respiratory infections<sup>3</sup>.  
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58 292 With growth this may resolve in some infants as airway size increases but for those with the  
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3 293 smallest airway dimensions the risk of non-atopic wheeze may persist. In contrast, airway  
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5 294 dimensions at birth may be less significant in the aetiology of atopic wheeze compared to  
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8 295 other factors acquired in association with an atopic phenotype.  
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12 297 As described earlier, ADAM33 is expressed in the embryonic lung<sup>14</sup> and SNPs in the gene  
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15 298 encoding ADAM33 were previously found to predict impaired lung function at age 3 and 5  
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17 299 years<sup>15</sup>. However, it is unclear whether ADAM33 polymorphism is associated with abnormal  
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20 300 lung function at birth (reflecting altered *in utero* lung development) or whether post-natal  
21  
22 301 gene-environment interaction alters lung function and increases risk of asthma. Recent  
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24  
25 302 observations from the PIAMA birth cohort showing that *in utero*, but not post natal, cigarette  
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27 303 smoke exposure interacts with ADAM33 polymorphism to determine childhood lung function  
28  
29 304 and BHR suggests that ADAM33 polymorphism may alter *in utero* lung development<sup>27</sup>. In  
30  
31 305 the current study, no ADAM33 haplotype was associated at the 5% level with lung function  
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33  
34 306 or symptoms in the first or third years of life after correction for multiple testing. Simpson *et*  
35  
36 307 *al.* described that the F+1 polymorphism explained 3% of the variance in lung function  
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38 308 (sRaw) at 3 years of age<sup>15</sup>; power calculations show that we only had 19% power to detect an  
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41 309 association that predicts 5% variance in Crs measurements. Given the observation of  
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44 310 interaction with *in utero* smoke exposure and the trend towards association seen in the current  
45  
46 311 study, the association between ADAM33 haplotypes and infant lung function needs to be  
47  
48 312 examined in a meta-analysis of data from all available infant lung function cohorts.  
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53 314 Strengths and limitations of this study

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55 315 We successfully measured infant lung function in 147 infants; the robust FEV<sub>0.4</sub> measurement  
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57 316 was recorded in 98 infants, and 141 children (96%) were followed up to age 3 years.

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

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3 318 numbers recruited to similar cohort studies<sup>4;5</sup>. The numbers involved limited adjustment for  
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5 319 multiple confounders or subgroup analysis. For example, although some studies have found  
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7 320 evidence of reduced early lung function in infants whose mothers smoked<sup>28;29</sup>, it was not  
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9  
10 321 feasible to stratify for maternal smoking. Maternal smoking was not considered a confounder  
11  
12 322 of the relationship between infant lung function and later wheeze as we have previously  
13  
14 323 demonstrated that infant lung function is not significantly associated with maternal smoking  
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16 324 in this cohort<sup>17</sup>. A second source of potential bias relates to exclusion of children on the basis  
17  
18 325 of respiratory infection prior to lung function measurement. This exclusion was used to ensure  
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20 326 that the effects of premorbid lung function upon later wheeze status were explored and to  
21  
22 327 avoid bias by postnatal effects. This would only influence the findings if the relationship  
23  
24 328 between infant lung function and later wheeze differed between those infants who  
25  
26 329 experienced early infection and those who did not. There is no reason to believe that this  
27  
28 330 relationship would differ in a manner which would decrease the strength of the association  
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30 331 between poor infant lung function and later wheeze; that is to say it is unlikely infants  
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32 332 suffering early respiratory infection would have a better outcome in terms of later wheeze  
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34 333 than those that did not experience early infections.  
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43 335 In summary this study has shown a relationship between early life lung function and  
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45 336 respiratory symptoms in the first and third years of life. Lower FEV<sub>0.4</sub> in early infancy was  
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47 337 associated with non-atopic wheeze but not atopic wheeze at 3 years of age. This is further  
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49 338 evidence suggesting that pre-natal factors contribute to the development of wheeze in early  
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51 339 life.  
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For Peer Review

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443 Figures

444 Figure 1 Flow diagram of recruitment process and follow up

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For Peer Review

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3 1 **The relationship between infant lung function and the risk of wheeze in the preschool**  
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5 2 **years**  
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10 4 <sup>1</sup>Developmental Origins of Health and Disease Division, <sup>2</sup>Human Genetics Division and  
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33 14 Group  
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3 37 **Summary**

4  
5 38 Rationale

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8 39 There is evidence that perinatal lung development predicts childhood wheeze. However, very  
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10 40 few studies have examined whether preschool wheeze is associated with lower premorbid  
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12 41 lung function in early infancy, and as yet there is no information relating atopic and non-  
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14 42 atopic preschool wheeze to early lung development.

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17 43 Objective

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20 44 To examine the association between premorbid infant lung function and preschool wheeze,  
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22 45 and to explore associations with atopic and non-atopic wheeze phenotypes.

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24 46 Methods

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27 47 Infant lung function was measured in 147 healthy term infants aged 5-14 weeks. Rapid  
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29 48 thoracoabdominal compression was performed during tidal breathing and at raised volume to  
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31 49 measure maximal expiratory flow at functional residual capacity ( $V'_{\max\text{FRC}}$ ) and forced  
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33 50 expiratory volume in 0.4 sec ( $\text{FEV}_{0.4}$ ). Atopic status was determined by skin prick testing at 3  
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35 51 years and wheeze ascertained from parental questionnaires (1 and 3 years).

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38 52 Measurements and Main Results

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41 53 Lower early infancy  $V'_{\max\text{FRC}}$  was associated with wheeze in both the first and third years of  
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43 54 life ( $p=0.002$  and  $p=0.006$ , respectively). Lower early infancy  $\text{FEV}_{0.4}$  was associated with  
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45 55 wheeze in the first year ( $p=0.03$ ). Compared to non-atopic children who did not wheeze, non-  
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47 56 atopic children who wheezed in their third year of life had lower  $\text{FEV}_{0.4}$ , ( $p=0.02$ ), whilst  
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49 57  $\text{FEV}_{0.4}$  values of atopic children who wheezed were not significantly different ( $p=0.4$ ).

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52 58 Conclusions

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55 59 Lower premorbid infant lung function was present in infants who subsequently wheezed  
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57 60 during the first and third years of life. Lower  $\text{FEV}_{0.4}$  in early infancy was associated with non-  
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59 61 atopic wheeze but not atopic wheeze at 3 years of age.

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62 Word count 248

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

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For Peer Review

## 65 Introduction

66 Previous studies have demonstrated that reduced infant lung function is present in those  
67 children who subsequently experience wheeze<sup>1-5</sup>. Although tracking of lung function from  
68 infancy, through childhood<sup>6</sup> and into adult life<sup>7</sup> is well described, the relationship between  
69 early lung function and phenotypes of wheezing illness is less clear; for example, in 125  
70 infants assessed at a mean age 2.4 months (SD 2 months) within the Tucson study, a reduced  
71  $V'_{\max\text{FRC}}$  was associated with a transient wheeze phenotype, but no association was found  
72 with wheezing after 3 years of age<sup>8</sup>. In contrast, other studies suggest early impairment of  
73 lung function is a risk factor for wheeze which persists into later childhood<sup>6:9</sup>.

74  
75 The original 'wheeze phenotypes' described in the Tucson study were defined retrospectively  
76 according to age of onset and persistence of symptoms<sup>8</sup>. More recently, work has been  
77 undertaken to determine clinical phenotypes<sup>10:11</sup> and to consider whether early impairment of  
78 lung function is associated with childhood wheeze phenotypes based upon clinical features.  
79 Infants with recurrent wheeze considered at high risk of subsequent asthma due to either a  
80 parental history of asthma or personal history of eczema, allergic rhinitis, wheezing without a  
81 cold and/or serum eosinophilia were found to have lower forced expiratory volumes at 8-20  
82 months than healthy controls or wheezy infants at lower risk of subsequent asthma<sup>12</sup>. This  
83 study suggested that early impairment of lung function is associated with preschool wheeze  
84 and particularly so in the presence of markers of atopic sensitisation. However, premorbid  
85 infant lung function, that is lung function measured prior to any respiratory infection, was not  
86 measured and the relationship between lung function and clinical wheeze phenotype  
87 according to atopy was not established in an unselected population. Whilst there is evidence  
88 that both early lung function and atopic status are determinants of preschool wheeze it is not

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3 89 clear how premorbid infant lung function relates to the atopic and non-atopic wheeze  
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5 90 phenotypes.  
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10 92 Premorbid infant lung function is likely to reflect both early environmental influences and  
11  
12 93 common genetic polymorphisms. ADAM33 is a positionally cloned asthma susceptibility  
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14 94 gene that shows strongest linkage with a combined asthma and bronchial hyperresponsiveness  
15  
16 95 phenotype<sup>13</sup>. ADAM33 is expressed in lung fibroblasts and bronchial smooth muscle and is  
17  
18 96 expressed during embryonic lung development<sup>14</sup>. Previously, ADAM33 polymorphisms have  
19  
20 97 been associated with impaired lung function at 3 years of age, specifically deficits in sRaw,  
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22 98 and carriers homozygous for the A allele of F+1 SNP have been shown to have double the  
23  
24 99 risk of transient early wheeze<sup>15</sup>. Associations between a number of individual SNPs and both  
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26 100 baseline lung function and non atopic asthma have also been demonstrated in a population of  
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28 101 German school children<sup>16</sup>.  
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34 102  
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36 103 The Southampton Women's Survey (SWS) was the first to measure premorbid FEV<sub>0.4</sub> in a  
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38 104 longitudinal pregnancy cohort study<sup>17</sup>. In SWS infants, a rapid thoracoabdominal compression  
39  
40 105 (RTC) technique was used to measure V'<sub>maxFRC</sub> at raised volume (RV-RTC), reducing  
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42 106 intersubject and intrasubject variability by removing the use of a variable volume landmark<sup>18</sup>.  
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44 107 It has been suggested that measurement of timed forced expiratory volumes using RVRTC is  
45  
46 108 a more sensitive method of detecting impaired airway function than V'<sub>maxFRC</sub><sup>19:20</sup>. The SWS  
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48 109 study was also the first to investigate the predictive value of infant FEV<sub>0.4</sub> in healthy term  
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50 110 infants in relation to future wheeze. Using this outcome measure, we aimed to examine the  
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52 111 hypothesis that lower infant lung function is associated with preschool wheeze and to then  
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54 112 explore the associations between premorbid infant lung function and preschool atopic and  
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113 non-atopic wheeze phenotypes. As a secondary aim, we explored the relationship between  
114 ADAM33 polymorphism, lung function in infancy and preschool wheeze.

For Peer Review

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3 115 **Methods**

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5 116 Participants

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8 117 The Southampton and South West Hampshire Local Research Ethics Committee approved the  
9  
10 118 protocol and written consent was obtained from the children's mothers. Detailed information  
11  
12 119 regarding the participants and infant lung function testing has been described<sup>17</sup>. In brief,  
13  
14 120 Caucasian infants, born at at least 37 weeks' gestation without major congenital anomalies  
15  
16 121 and prior to any respiratory infection were recruited from the SWS, a population-based  
17  
18 122 pregnancy cohort<sup>21</sup>. 429 women were approached about the study after the birth of their  
19  
20 123 babies; 264 (62%) declined before testing. Of those who agreed to participate 18 (10%) were  
21  
22 124 excluded because of recent upper respiratory tract infection or other illness (Figure 1). The  
23  
24 125 majority of subjects declining did so due to concerns about sedation, respiratory infection was  
25  
26 126 not a common reason for declining to participate. Lung function was measured, between May  
27  
28 127 1999 and October 2002, when the infants were 5-14 weeks old.  
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36 129 Lung function was measured, with infants lying supine, in quiet sleep augmented with chloral  
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38 130 hydrate (75-100 mg/kg by mouth). An inflatable jacket connected to a rapid inflation system  
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40 131 was placed around the infant's chest and abdomen and a leak-free facemask with Fleisch  
41  
42 132 pneumotachograph (Dynasciences, Blue Bell, CA) was held over the nose and mouth. Data  
43  
44 133 were collected using RASP software (Physiologic Ltd, Newbury, Berks, UK) and analysed in  
45  
46 134 SQUEEZE (Paul Dixon, London).  
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53 136 As previously described<sup>17</sup>, respiratory rate was measured during tidal breathing. To record  
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55 137 partial expiratory flow volume curves, a stable end expiratory level was established before  
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57 138 performing an RTC at the lowest pressure to achieve the best  $V'_{\max\text{FRC}}$ , calculated from the  
58  
59 139 partial expiratory flow volume curve. Passive, relaxed inflations to 30cm water were  
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3 140 performed using a resuscitator connected to the pneumotachograph, inducing the respiratory  
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5 141 muscles to relax via the Hering-Breuer reflex. Compliance of the respiratory system (Crs  
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7 142 ml/mm water) was calculated using SQUEEZE software from the resultant passive flow–  
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10 143 volume curves as change in volume for unit of airway outlet pressure (an estimate of alveolar  
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12 144 pressure). Raised volume RTC curves were recorded at the optimal jacket pressure at the end  
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15 145 of a passive inspiration using a technique adapted from Feher and colleagues<sup>18</sup>, and FEV<sub>0.4</sub>  
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17 146 and FVC were calculated using SQUEEZE from the forced expiratory flow–volume curve.  
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22 148 For each infant, FEV<sub>0.4</sub> was calculated from the ‘best’ raised volume loop (greatest sum of  
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24 149 FEV<sub>0.4</sub> and FVC) and the highest technically acceptable V’<sub>maxFRC</sub> was used. At least two  
25  
26 150 acceptable, reproducible (within 10%) raised volume and partial expiratory curves were  
27  
28 151 obtained. It was not possible to record valid data in every infant for each measure of lung  
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30 152 function; there were approximately 100 infants with acceptable lung function data for each  
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32 153 measurement.  
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### 37 38 39 155 Symptoms

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41 156 Mothers completed contemporaneous respiratory symptom diaries during their child’s first  
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43 157 year of life. Symptoms were explained to each mother by the study nurses. A new diary was  
44  
45 158 posted every 3 months and followed up by reminder phone calls from the study team. For  
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47 159 each 24 hr period parents recorded daytime and night-time wheeze. At ages one and three  
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49 160 years mothers completed a nurse-administered questionnaire which included the question ‘has  
50  
51 161 your child experienced any episodes of chestiness associated with wheezing or whistling in  
52  
53 162 his/her chest since they were last seen?’. Skin reactivity to cat<sup>a</sup>, dog<sup>a</sup>, house dust mite<sup>a</sup>  
54  
55 163 (Dermatophagoides pteronyssinus), milk<sup>a</sup>, grass pollens<sup>a</sup>, and egg<sup>b</sup> was assessed at age 3 years  
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57 164 (Hollister-Stier, Spokane, WA<sup>a</sup>, Alyostal, Antony, France<sup>b</sup>). Atopy was defined as a wheal to  
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3 165 any allergen at least 3mm in diameter in the presence of a wheal of at least 3 mm in diameter  
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5 166 to 10 mg/ml histamine<sup>a</sup> solution and no response to 50% glycerin<sup>a</sup>.  
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10 168 Genotyping  
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12 169 DNA was isolated from cord blood using a salting-out procedure<sup>23</sup>. ADAM33 SNPs Bp1  
13 170 (rs487377), Fp1 (rs511898), STp7 (rs574174), Vm3 (rs628977), V4 (rs2787094) were  
14 171 genotyped by TaqMan SNP genotyping assays (E-Table1). The SNPs were chosen for  
15 172 genotyping as they were determined to be haplotype tagging SNPs, (equivalent to  $r^2 \geq 0.8$ ,  
16 173 minor allele frequency > 5%) based on the LDU map from Simpson *et al*<sup>16</sup>, thereby providing  
17 174 more genetic information as they tag a further 12 SNPs spanning the *ADAM33* gene region.  
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29 176 Statistical Analysis  
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31 177 The infant lung function data were logarithmically transformed to achieve normality and  
32 178 adjusted for age and sex where necessary. t-tests were used to assess differences between  
33 179 geometric mean group values of infant lung function measurements according to wheeze  
34 180 status at 1 and 3 years, and after subdividing the 3-year wheeze group according to atopic  
35 181 status. Agreement between the parent-completed symptom diaries and the nurse-administered  
36 182 questionnaires was assessed by weighted Kappa ( $\kappa$ ) analysis. ADAM33 haplotype  
37 183 quantitative trait and power analysis were performed using Haploscore (Haplo.stats R  
38 184 package version 1.3.8.)<sup>23</sup> in R (version 2.5.1)<sup>24</sup> with the additive haplotype effect model.  
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53 186 Assuming 50 children in each outcome group, there was 80% power at the 5% level of  
54 187 significance to detect a difference of 0.57 SDs in any infant lung function measurement  
55 188 between the two outcome groups. This equates to a 10% change in FEV<sub>0.4</sub>, a 12% change in  
56 189 Crs or respiratory rate, or a 33% change in V'<sub>maxFRC</sub>.  
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3 190 **Results**  
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5 191 147 infants had lung function measurements, of which 146 provided questionnaire data at age  
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8 192 one year, 141 provided questionnaire data at 3 years of age and 113 had skin prick testing.  
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10 193 Symptom diaries were returned by 102 mothers. There was strong concordance between the  
11  
12 194 prospective symptom diaries and retrospective questionnaires with respect to classification of  
13  
14 195 children according to presence or absence of wheeze during the first year of life, ( $\kappa = 0.71$ ,  
15  
16 196  $p < 0.001$ ). Given this, all subsequent analyses are based upon questionnaire data. A total of  
17  
18 197 114 DNA samples were available for ADAM33 SNP genotyping; genotype assignment  
19  
20 198 approached 99% (6 failed PCR reactions out of a total 570) with a maximum of two  
21  
22 199 unassigned genotypes for each SNP and all genotype frequencies were in Hardy-Weinberg  
23  
24 200 Equilibrium. Matched genotype and lung function data were available for 103 children for  
25  
26 201 respiratory rate, 82 for Crs, 105 for  $V'_{\max FRC}$  and for 76 children for  $FEV_{0.4}$ . (See online  
27  
28 202 supplement).  
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36 204 During the first year of life, 74 infants had at least one episode of wheezing. Thirty three  
37  
38 205 children experienced wheeze in the third year of life, of these 23 had also experienced wheeze  
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40 206 in the first year of life whilst 10 had not. Of the 113 children who were skin prick tested for  
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42 207 allergy, 20 (17.7%) had at least one positive reaction and were classed as atopic. Of the  
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44 208 children who experienced wheeze 25% were atopic and 75% were not.  
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50 210 The demographic characteristics of those children included in the study of infant lung  
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52 211 function and those that who were approached but did not participate are listed in table 1.  
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54 212 Comparing children who did and did not participate, there were no differences in gender,  
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56 213 maternal smoking during pregnancy or history of asthma in either parent, but those who did  
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3 214 not participate were more likely to have a younger mother with lower educational  
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5 215 qualification and to be of lower birth weight (Table 1).  
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10 217 Associations between wheeze or wheeze phenotype and lung function were examined after  
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12 218 adjusting for factors that were found to influence measures of lung function on univariate  
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15 219 analysis. FEV<sub>0.4</sub>, and V'<sub>maxFRC</sub> were adjusted for gender and Crs for gender and age at testing.  
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20 221 Relationship between infant lung function and respiratory symptoms reported at age 1 year

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22 222 V'<sub>maxFRC</sub>, FEV<sub>0.4</sub> and Crs were lower in infants who subsequently wheezed in their first year  
23  
24 223 than in those who did not by 22.5% (p=0.002), 8.1% (p=0.03) and 6.8% (p=0.04) respectively  
25  
26 224 (Table 2). The group mean respiratory rate was 6.8% higher in the wheeze group than in the  
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29 225 non-wheeze group (p=0.03).  
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34 227 Relationship between infant lung function and respiratory symptoms reported at age 3 years

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36 228 V'<sub>maxFRC</sub> was 23.2% lower in infants who wheezed in their third year than in those who did  
37  
38 229 not (Table 2) (p=0.006). However, there were only small non-significant differences in  
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41 230 FEV<sub>0.4</sub>, Crs and respiratory rate between the two groups. When atopic status was also taken  
42  
43 231 into account, FEV<sub>0.4</sub> was found to be 11% lower in those who wheezed but were not atopic  
44  
45 232 compared to participants with no wheeze nor atopy (p=0.02) (Table 3). There were no  
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48 233 significant differences in any measure of infant lung function when either atopic children with  
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50 234 wheeze in the third year or atopic children who did not wheeze in the third year were  
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53 235 compared to non-atopic children without wheeze (Table 3).  
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58 237 ADAM33 SNP and haplotype analysis  
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3 238 No ADAM33 SNP or haplotype was significantly associated with infant lung function  
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6 239 measurements after adjusting for multiple testing by Bonferroni correction (E-Table 3).  
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8 240 Haplopower analysis (Online Data Supplement) revealed that with the small sample size (n =  
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10 241 82) we were underpowered (Power = 19%) to detect a common haplotype (freq = 18%)  
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12 242 association accounting for 5% of the variance in the mean of the Crs measurement.  
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For Peer Review

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3 244 **Discussion**

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5 245 This study demonstrates an association between impaired premorbid infant lung function and  
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8 246 preschool wheezing illness. This confirms the results of previous studies<sup>4;5</sup>. Our analyses  
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10 247 show for the first time that lower lung function in early infancy is a risk factor for non-atopic  
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12 248 wheeze, rather than atopic wheeze. Additionally, this study is the first to report reduced  
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15 249 FEV<sub>0.4</sub>, prior to lower respiratory infection, as a risk factor for wheeze in the preschool years.  
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17 250 This is particularly important in view of recent work demonstrating that diminished timed  
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19 251 forced expiratory volumes are an important predictor of all-cause mortality in adult life<sup>25</sup>.  
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21 252 Although we found no associations with ADAM33 polymorphisms this may have resulted  
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23 253 from the low power of our study resulting from the challenge of recruiting large numbers of  
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27 254 participants into a study assessing infant lung function.  
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31 256 Premorbid measures of forced expiratory volume in 0.4 seconds (FEV<sub>0.4</sub>) during infancy as a  
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33 257 predictor of wheeze have not previously been studied, though reduced FEV<sub>0.5</sub> following  
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35 258 symptoms has been reported in wheezy children aged 8 – 20 months<sup>12</sup>. Lung function  
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38 259 measures derived from raised volume expiratory manoeuvres are believed to discriminate  
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40 260 better between healthy individuals and those with respiratory disease when compared to tidal  
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42 261 breathing measures<sup>19;20</sup>. FEV<sub>0.4</sub> is derived from measurement of expiratory flow from close to  
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45 262 total lung capacity and is not dependent upon a volume landmark. This is more repeatable  
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48 263 than measurement at FRC as the FRC of infants can vary on a breath by breath basis, thus  
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50 264 FEV<sub>0.4</sub> provides a more robust measure of infant lung function than V'<sub>maxFRC</sub><sup>26</sup>. Furthermore,  
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52 265 FEV<sub>0.4</sub> is well suited to longitudinal study as it is intuitively more comparable to measures of  
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55 266 lung function, such as FEV<sub>1</sub>, which are used in older children and adults.  
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3 268 The results of this study are likely to be of relevance to the general population. We recruited  
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5 269 healthy infants and only excluded those born before 37 weeks gestation to prevent bias due to  
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7 270 effects of prematurity upon lung development. Our results demonstrate that variations in  
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9 271 measures of expiratory flow measured in unselected populations are associated with preschool  
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11 272 wheeze.  
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16 274 Small study numbers limited the exploration of lung function measures according to wheeze  
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18 275 phenotype. However, non-atopic children who wheezed during the third year of life had a  
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20 276 significantly lower FEV<sub>0.4</sub> in infancy compared to non-atopic children without wheeze. There  
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22 277 was no difference in any measure of infant lung function when atopic children with wheeze in  
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24 278 the third year were compared to non-atopic children without wheeze. This may have partly  
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26 279 related to greater power associated with a more balanced distribution of non-atopic than  
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28 280 atopic individuals between the wheeze and no wheeze groups. However, others have  
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30 281 speculated that wheezing during the first year of life is often a transient condition due to  
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32 282 reduced airway caliber and that wheeze beginning or persisting into later childhood is more  
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34 283 closely aligned with the wheeze phenotype recognised as asthma<sup>4</sup>.  
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43 285 Due to the number of subjects studied it was not possible to stratify our data according to age  
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45 286 of onset. However, the broader confidence intervals and divergence of results between atopic  
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47 287 and non-atopic individuals at age three years may be evidence of comparative heterogeneity  
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49 288 of wheeze phenotypes later in childhood. The strong association between reduced measures of  
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51 289 forced expiratory flow and wheeze at one year may reflect a common underlying mechanism,  
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53 290 similar to that proposed by Martinez, whereby initial airway diameter, length or other  
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55 291 characteristics might predispose certain infants to wheezing with viral respiratory infections<sup>3</sup>.  
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58 292 With growth this may resolve in some infants as airway size increases but for those with the  
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3 293 smallest airway dimensions the risk of non-atopic wheeze may persist. In contrast, airway  
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5 294 dimensions at birth may be less significant in the aetiology of atopic wheeze compared to  
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8 295 other factors acquired in association with an atopic phenotype.  
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12 297 As described earlier, ADAM33 is expressed in the embryonic lung<sup>14</sup> and SNPs in the gene  
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14 298 encoding ADAM33 were previously found to predict impaired lung function at age 3 and 5  
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17 299 years<sup>15</sup>. However, it is unclear whether ADAM33 polymorphism is associated with abnormal  
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20 300 lung function at birth (reflecting altered *in utero* lung development) or whether post-natal  
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22 301 gene-environment interaction alters lung function and increases risk of asthma. Recent  
23  
24 302 observations from the PIAMA birth cohort showing that *in utero*, but not post natal, cigarette  
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26 303 smoke exposure interacts with ADAM33 polymorphism to determine childhood lung function  
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29 304 and BHR suggests that ADAM33 polymorphism may alter *in utero* lung development<sup>27</sup>. In  
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31 305 the current study, no ADAM33 haplotype was associated at the 5% level with lung function  
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33  
34 306 or symptoms in the first or third years of life after correction for multiple testing. Simpson *et*  
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36 307 *al.* described that the F+1 polymorphism explained 3% of the variance in lung function  
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38 308 (sRaw) at 3 years of age<sup>15</sup>; power calculations show that we only had 19% power to detect an  
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41 309 association that predicts 5% variance in Crs measurements. Given the observation of  
42  
43 310 interaction with *in utero* smoke exposure and the trend towards association seen in the current  
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46 311 study, the association between ADAM33 haplotypes and infant lung function needs to be  
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48 312 examined in a meta-analysis of data from all available infant lung function cohorts.  
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52  
53 314 Strengths and limitations of this study

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55 315 We successfully measured infant lung function in 147 infants; the robust FEV<sub>0.4</sub> measurement  
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57 316 was recorded in 98 infants, and 141 children (96%) were followed up to age 3 years.

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

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3 318 numbers recruited to similar cohort studies<sup>4;5</sup>. The numbers involved limited adjustment for  
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5 319 multiple confounders or subgroup analysis. For example, although some studies have found  
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7 320 evidence of reduced early lung function in infants whose mothers smoked<sup>28;29</sup>, it was not  
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10 321 feasible to stratify for maternal smoking. Maternal smoking was not considered a confounder  
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12 322 of the relationship between infant lung function and later wheeze as we have previously  
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15 323 demonstrated that infant lung function is not significantly associated with maternal smoking  
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17 324 in this cohort<sup>17</sup>. A second source of potential bias relates to exclusion of children on the basis  
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20 325 of respiratory infection prior to lung function measurement. This exclusion was used to ensure  
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22 326 that the effects of premorbid lung function upon later wheeze status were explored and to  
23  
24 327 avoid bias by postnatal effects. This would only influence the findings if the relationship  
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26  
27 328 between infant lung function and later wheeze differed between those infants who  
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29 329 experienced early infection and those who did not. There is no reason to believe that this  
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31 330 relationship would differ in a manner which would decrease the strength of the association  
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34 331 between poor infant lung function and later wheeze; that is to say it is unlikely infants  
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36 332 suffering early respiratory infection would have a better outcome in terms of later wheeze  
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38 333 than those that did not experience early infections.  
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43 335 In summary this study has shown a relationship between early life lung function and  
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46 336 respiratory symptoms in the first and third years of life. Lower FEV<sub>0.4</sub> in early infancy was  
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48 337 associated with non-atopic wheeze but not atopic wheeze at 3 years of age. This is further  
49  
50 338 evidence suggesting that pre-natal factors contribute to the development of wheeze in early  
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52  
53 339 life.

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56  
57 341 Acknowledgements  
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3 342 The authors acknowledge the help of the parents and infants who participated in this study.  
4  
5 343 They are grateful to Claire Foreman and the staff of the Wellcome Trust Clinical Research  
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7  
8 344 Facility for collection of the infant lung function measurements and the staff of the  
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10 345 Southampton Women's Survey for collecting and processing the questionnaire data.  
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443 Figures

444 Figure 1 Flow diagram of recruitment process and follow up

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3 The relationship between infant lung function and the risk of wheeze in the preschool years  
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**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	GCCAAGATGGTACAGAAGAAAGAGT	GTGAGTGTTTCCTGCCTTTTGG	AGGCTAGGT <sub>g</sub> TCCCCT	AGGCTAGGT <sub>a</sub> TCCCCT
FP1	AATGCTGTATCTATAGCCCTCCAAA	GGAACTTGTTTCTCAGACTTCAATAAA	AGAAGAGAC <sub>g</sub> GGAATT	AGAGAC <sub>a</sub> GGAATTCA
STp7	AAGGAACATCACAGGAAATGACAA	CCTGCTGCCCTTGATGATTC	CCATCCCAT <sub>c</sub> CC	CCATCCCAT <sub>i</sub> CC
Vm3	CTGTGGCTTTTCCTGGATCACT	GGCCCCAGGCTGCAA	AGCCCA <sub>c</sub> CCTCACT	AGCCCA <sub>i</sub> CCTCACTC
V4	GGCCCTATGGTTCGACTGAGT	AGGAAGGTCCCCAAAATTATGTTT	CTCCCCTG <sub>c</sub> AGCCT	CCCTG <sub>g</sub> AGCCTGG

<sup>a</sup>SNP positions are denoted by lower case bases.

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## Haploscore Analysis

**E-Table 2 ADAM33 Genotype Frequencies**

Genotypes	ADAM33 Bp1 G>A	ADAM33 Fp1 G>A	ADAM33 STp7 C>T	ADAM33 V4 C>G	ADAM33 Vm3 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 (n=114)	113/114	114/114	112/114	113/114	112/114
HWE $\chi^2$	1.40	0.00	1.35	0.17	1.25

HWE  $\chi^2$  = 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.

**E-Table 3 Summary of results of haplotype analysis of ADAM33 with uncorrected P values < 0.1.**

Phenotype	adam33bp1	adam33fp1	adam33stp7	adam33vm3	adam33v4	Hap-Freq <sup>a</sup>	Hap-Score <sup>b</sup>	Uncorrected P-value <sup>c</sup>	Corrected P-value <sup>d</sup>
Respiratory rate (n= 103)	G	G	C	T	G	0.02351	-2.13291	0.0329	0.43
Crs (n = 82)	G	A	C	T	C	0.0137	2.8227	0.0048	0.06
V' max <sub>FRC</sub> (n= 105)	A	G	C	T	G	0.07069	-1.85387	0.0638	
Atopy (n= 82)	A	A	T	T	C	0.03186	1.65231	0.0985	
Atopic wheeze (n= 57)	G	A	T	C	G	0.04798	2.40585	0.0161	0.24
	A	G	C	T	G	0.0539	1.89707	0.0578	

<sup>a</sup>Only haplotypes with a greater than 1% frequency presented. <sup>b</sup>Positive z-score means the haplotype associates with increased measurement (continuous variable) or presence of a trait (binomial). <sup>c</sup>P values <0.1 are highlighted in bold. <sup>d</sup>Significant 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<sub>0.4</sub> measurements (log transformed). (n= 76)**

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

<sup>a</sup>Positive z-score means the haplotype associates with increased measurement (continuous variable) or presence of a trait (binomial). <sup>b</sup>P values <0.1 are highlighted in bold. <sup>c</sup>Significant 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 5 ADAM33 SNP haplotypes and Crs measurement (log transformed). (n = 82)

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

<sup>a</sup>Positive z-score means the haplotype associates with increased measurement (continuous variable) or presence of a trait (binomial). <sup>b</sup>P values <0.1 are highlighted in bold. <sup>c</sup>Significant 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 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 <sup>a</sup>	P-value <sup>b</sup>	P-value <sup>c</sup>
G	G	C	C	C	0.36409	-0.25286	0.80038	N/A
G	G	C	T	C	0.18429	1.30254	0.19273	N/A
G	A	C	C	C	0.11114	-0.08324	0.93366	N/A
A	G	C	T	G	0.07049	-0.19078	0.8487	N/A
G	A	T	C	G	0.05388	1.40582	0.15978	N/A
A	A	C	C	C	0.0511	-1.05761	0.29024	N/A
G	A	T	C	C	0.04914	0.12155	0.90325	N/A
A	A	T	T	C	0.0388	-0.49199	0.62273	N/A
A	G	C	T	C	0.02461	-0.22106	0.82504	N/A
G	G	C	T	G	0.02351	-2.13291	<b>0.03293</b>	0.43
A	A	T	T	G	0.00746	-0.96629	0.3339	N/A
A	G	C	C	C	0.00657	0.15884	0.87379	N/A
G	G	T	T	C	0.00605	-0.6583	0.51034	N/A

<sup>a</sup>Positive z-score means the haplotype associates with increased measurement (continuous variable) or presence of a trait (binomial). <sup>b</sup>P values <0.1 are highlighted in bold. <sup>c</sup>Significant 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 7 ADAM33 Haplotyping and V'max<sub>FRC</sub> (log transformed). (n= 105)**

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

<sup>a</sup>Positive z-score means the haplotype associates with increased measurement (continuous variable) or presence of a trait (binomial). <sup>b</sup>P values <0.1 are highlighted in bold. <sup>c</sup>Significant 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 8 ADAM33 Haplotyping and atopy. (n= 82)

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

<sup>a</sup>Positive z-score means the haplotype associates with increased measurement (continuous variable) or presence of a trait (binomial). <sup>b</sup>P values <0.1 are highlighted in bold. <sup>c</sup>Significant 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 9 ADAM33 Haplotyping and wheeze in first year of life. (n= 105)**

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

<sup>a</sup>Positive z-score means the haplotype associates with increased measurement (continuous variable) or presence of a trait (binomial). <sup>b</sup>P values <0.1 are highlighted in bold. <sup>c</sup>Significant 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 10\_ADAM33 Haplotyping and wheeze in the third year of life. (n= 101)**

ADAM33	ADAM33	ADAM33	ADAM33	ADAM33	Hap	Hap-	Uncorrected	Corrected
Bp1	Fp1	STp7	Vm3	V4	Freq	Score <sup>a</sup>	P-value <sup>b</sup>	P-value <sup>c</sup>
G	G	C	C	C	0.37754	0.2363	0.8132	N/A
G	G	C	T	C	0.17981	-0.47127	0.63745	N/A
G	A	C	C	C	0.10778	0.57664	0.56418	N/A
A	G	C	T	G	0.06289	-0.03682	0.97063	N/A
G	A	T	C	G	0.05556	0.16215	0.87119	N/A
G	A	T	C	C	0.04453	1.42632	0.15378	N/A
A	A	C	C	C	0.04166	1.19777	0.23101	N/A
A	A	T	T	C	0.03834	-0.96884	0.33263	N/A
A	G	C	T	C	0.027	-0.82586	0.40889	N/A
G	G	C	T	G	0.02338	-1.32961	0.18365	N/A
G	A	T	T	C	0.01231	-0.57808	0.60513	N/A
G	A	C	T	C	0.00997	-0.12154	0.90327	N/A
A	A	T	T	G	0.00669	0.02954	0.97644	N/A
A	G	C	C	C	0.00659	-0.51704	0.56321	N/A
G	G	T	T	C	0.00595	-0.61456	0.53885	N/A

<sup>a</sup>Positive z-score means the haplotype associates with increased measurement (continuous variable) or presence of a trait (binomial). <sup>b</sup>P values <0.1 are highlighted in bold. <sup>c</sup>Significant 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 11\_ADAM33 Haplotyping and atopic wheeze in the third year of life. (n= 57)**

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

<sup>a</sup>Positive z-score means the haplotype associates with increased measurement (continuous variable) or presence of a trait (binomial). <sup>b</sup>P values <0.1 are highlighted in bold. <sup>c</sup>Significant 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=67)**

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

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

## 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.

For Peer Review



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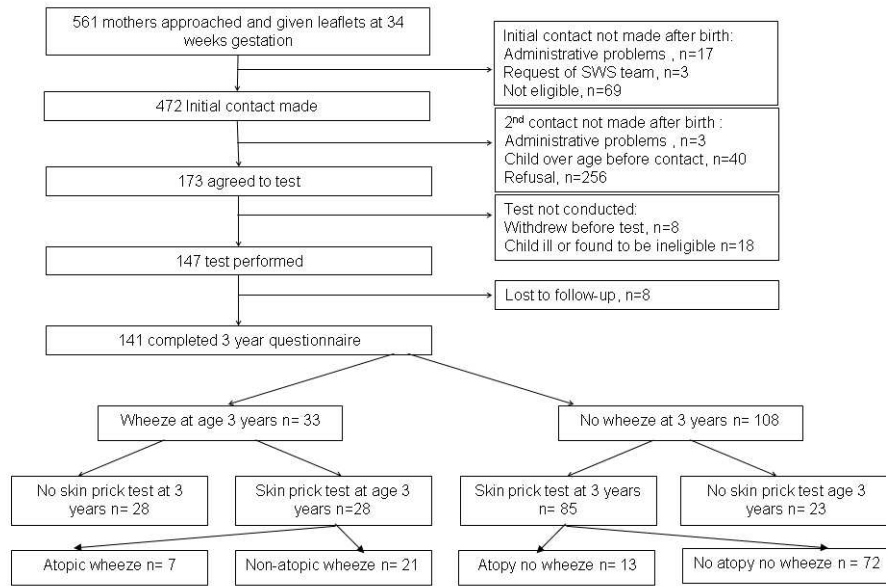


Figure 1 Flow diagram of recruitment process and follow up  
275x190mm (96 x 96 DPI)

Review

**Table 1 Characteristics of those children who performed the lung function test compared with those who were eligible but did not participate**

	Performed lung function test (n=147)	Did not perform lung function test (n=327)	P- 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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Degree	35 (23.8)	34 (10.4)	<0.001
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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 those who wheezed % (95% CI)	No wheeze		Wheeze		Reduction/increase in those who wheezed % (95% CI)
	Mean (95% CI)	n	Mean (95% CI)	n		Mean (95% CI)	n	Mean (95% CI)	n	
$V'_{\max\text{FRC}}^*$	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%)
$\text{FEV}_{0.4}^{**}$	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%)
$\text{CRs}^{***}$	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 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		Mean (95% CI)	n	Mean (95% CI)	n	
V <sub>maxFRC</sub> *	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 <sub>0.4</sub> **	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		No wheeze but atopic		Reduction/increase in those who did not wheeze but 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)	Mean (95% CI)	n	Mean (95% CI)
V <sub>maxFRC</sub> *	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 <sub>0.4</sub> *	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 (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%)	45.1 (40.5 to 50.2)	13	0.9% (-11.0% to 11.5%)			

\* 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		No wheeze but atopic		Reduction/increase in those who did not wheeze but 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)	n	Mean (95% CI)	
$V'_{\max\text{FRC}}^*$	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%)			
$\text{FEV}_{0.4}^*$	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%)			
$\text{CrS}^{**}$	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 (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%)	45.1 (40.5 to 50.2)	13	0.9% (-11.0% to 11.5%)			

\* 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