

Wheeze Phenotypes and Lung Function in Preschool Children

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Distinct phenotypes can be identified in childhood wheezing illness. Within the context of a birth cohort study, we investigated the association between preschool lung function and phenotypes of wheeze. From parentally reported history of wheeze (interviewer-administered questionnaire, age 3 and 5 years), children were classified as never wheezers, transient early wheezers, late-onset wheezers, or persistent wheezers. Lung function (specific airway resistance [sRaw]; kPa/second) was assessed at age 3 ($n = 463$) and 5 years ($n = 690$). Persistent wheezers had markedly poorer lung function compared with other groups. In children who had wheezed by age 3, the risk of persistent wheeze increased with increased sRaw (odds ratio [OR] 5.2, 95% confidence interval [CI] 1.3–22.0; $p = 0.02$). In a multivariate model, increasing sRaw (OR 5.5, 95% CI 1.2–25.9; $p = 0.03$) and the child's sensitization (OR 2.8, 95% CI 1.3–5.8; $p = 0.008$) were significant independent predictors of persistent wheezing. We found no association between lung function at age 3 and late-onset wheeze in children who had not wheezed previously (OR 0.6, 95% CI 0.07–5.3; $p = 0.64$). In conclusion, poor lung function at age 3 predicted the subsequent persistence of symptoms in children who had wheezed within the first 3 years, but was not associated with the onset of wheeze after age 3 in children who had not wheezed previously.

Keywords: dry air challenge; lung function; specific airway resistance; wheeze phenotypes

Evidence suggests that early life events can determine respiratory health throughout life (1, 2). Most cases of persistent wheezing and asthma begin in early childhood (3), often in association with reduced infant lung function (4, 5) and, in some studies, with increased airway responsiveness in infancy (4–6). In recent years, our understanding of the nature of childhood wheezing illness has been augmented by the characterization of distinct wheeze phenotypes. The Tucson Children's Respiratory Study assigned children according to their history of wheezing as never wheezers, transient early wheezers, late-onset wheezers, and persistent wheezers (7). It has so far been difficult to predict which preschool children will have only transient early-life symptoms and to distinguish them from those whose symptoms will persist and who may go on to subsequently develop asthma (8).

Asthma is at least partially defined by abnormalities in lung function, including variable airway obstruction and increased airway reactivity (9). Young preschool children cannot always reliably perform forced maneuvers, and in this age group, this

process is difficult and time-consuming. Several studies suggested that dynamic lung volumes can be measured in preschool children using conventional spirometry (10–13). However, although most of these studies included subjects aged between 3 and 6 years, few participants were from the lower spectrum of the age range, and these children were also less likely able to perform the test adequately (13). There is a growing interest in developing objective measures of lung function that can be applied in young preschool children to elucidate the end-organ factors involved in asthma development and allow more accurate identification of children likely to be at risk of persistent symptoms. The measurement of specific airway resistance (sRaw) is a potentially useful method to assess lung function in young children (14–16). We have recently reported that sRaw measurement can be successfully performed in approximately 65% of children within 4 weeks of their third birthday (17).

The aims of this study were to determine the following: (1) the association between lung function (plethysmographic measure of sRaw) at ages 3 and 5 years and distinct phenotypes of childhood wheezing, and (2) whether lung function measured at age 3 years predicts the subsequent persistence or development of wheeze.

METHODS

The Manchester Asthma and Allergy Study is a population-based birth cohort (17–19). A detailed description of the study population and methods is presented in the online supplement. Participants were recruited prenatally (18) and attended review clinics at age 3 and 5 years (± 4 weeks). The study was approved by the local research ethics committee, and informed consent was obtained from all parents.

Outcomes

Symptoms. A standard respiratory questionnaire was interviewer-administered at age 3 and 5 years to collect the information on symptoms, physician-diagnosed illnesses, and treatments received. According to parentally reported history of wheeze at two follow-up evaluations, children were assigned to the following categories (7):

No wheezing: no wheeze during the first 3 years of life, no wheezing ever by age 5

Transient early wheezing: wheezing during the first 3 years, no wheezing in the previous 12 months at age 5

Late-onset wheezing: no wheeze during the first 3 years, wheezing in the previous 12 months at age 5

Persistent wheezing: wheezing during the first 3 years, wheezing in the previous 12 months at age 5.

sRaw was measured at age 3 and 5 years using a whole-body plethysmograph (Jaeger, Würzburg, Germany) as previously described (15–17). The sRaw was calculated from a simple algebraic manipulation of the known formulas for airway resistance and total gas volume (TGV) (14). This step precludes the need for shutter occlusion, because sRaw is derived directly from the relationship between plethysmographic volume and measurements of respiratory flow (14, 15). The diagnostic value of the single-step technique is equivalent to the conventional method of incorporating measurements of Raw and TGV (20).

Three measurements of sRaw were performed, and each was calculated from the medians of five consecutively measured technically acceptable loops (each child performed at least 15 loops) (17). The mean of these

(Received in original form June 1, 2004; accepted in final form October 10, 2004)

Supported by grants from Asthma UK (Grant No. 01/012) and the Moulton Charitable Trust.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 171, pp 231–237, 2005

Originally Published in Press as DOI: 10.1164/rccm.200406-695OC on October 22, 2004

Internet address: www.atsjournals.org

three measurements of *effective* sRaw was used in the analysis. Children were asymptomatic at the time of assessment of lung function.

Airway reactivity was assessed at age 5 by eucapnic voluntary hyperventilation (EVH) challenge (21, 22). Subjects hyperventilated gas containing 21% O₂, 5% CO₂, remainder N₂ with a water content of less than 10 mg/L for 6 minutes at a ventilation rate of 75% of maximum voluntary ventilation. The highest sRaw value measured at 2, 5, or 10 minutes after challenge was recorded, and the response was expressed as percentage change in sRaw.

Postbronchodilator lung function was measured 15 minutes after administration of 400 µg of albuterol via a spacer device. Among children who underwent EVH challenge, only those in whom sRaw had returned to within 5% of the baseline value were included.

Allergic sensitization (mite, cat, dog, grasses, milk, egg) was ascertained at age 3 and 5 by skin-prick testing; sensitization was defined as a wheal at least 3 mm greater than the negative control.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows, version 11.0 (SPSS, Chicago, IL). The sRaw measurements followed a log-normal distribution, and therefore were subject to a log_e transformation before analysis. Results are reported as geometric means (GM). One-

way analysis of variance (ANOVA) was used to compare differences between the four wheeze phenotypes. Variables found to be significantly associated with any of the outcomes in the univariate analyses were included in multivariate analyses.

RESULTS

The study profile is shown in Figure 1. We reviewed 996 children at age 3 years, of whom 122 were prenatally randomized to an environmental control regime (23, 24) and were excluded from this analysis. Of the remaining 874 children, 530 (60.6%) successfully performed sRaw measurement. A total of 496 (93.6%) of these 530 children attended the 5-year follow-up (248 never wheezed, 115 had transient early wheeze, 22 developed late-onset wheezing, and 78 had persistent wheezing). A further 33 children could not fit into this classification (e.g., no wheeze, but receiving asthma medication at age 5). A total of 730 children successfully performed sRaw measurement at age 5 years (384 never wheezed, 162 transient early wheeze, 40 late-onset wheeze, 104 persistent wheeze, and 40 impossible to classify). Of 690 children who

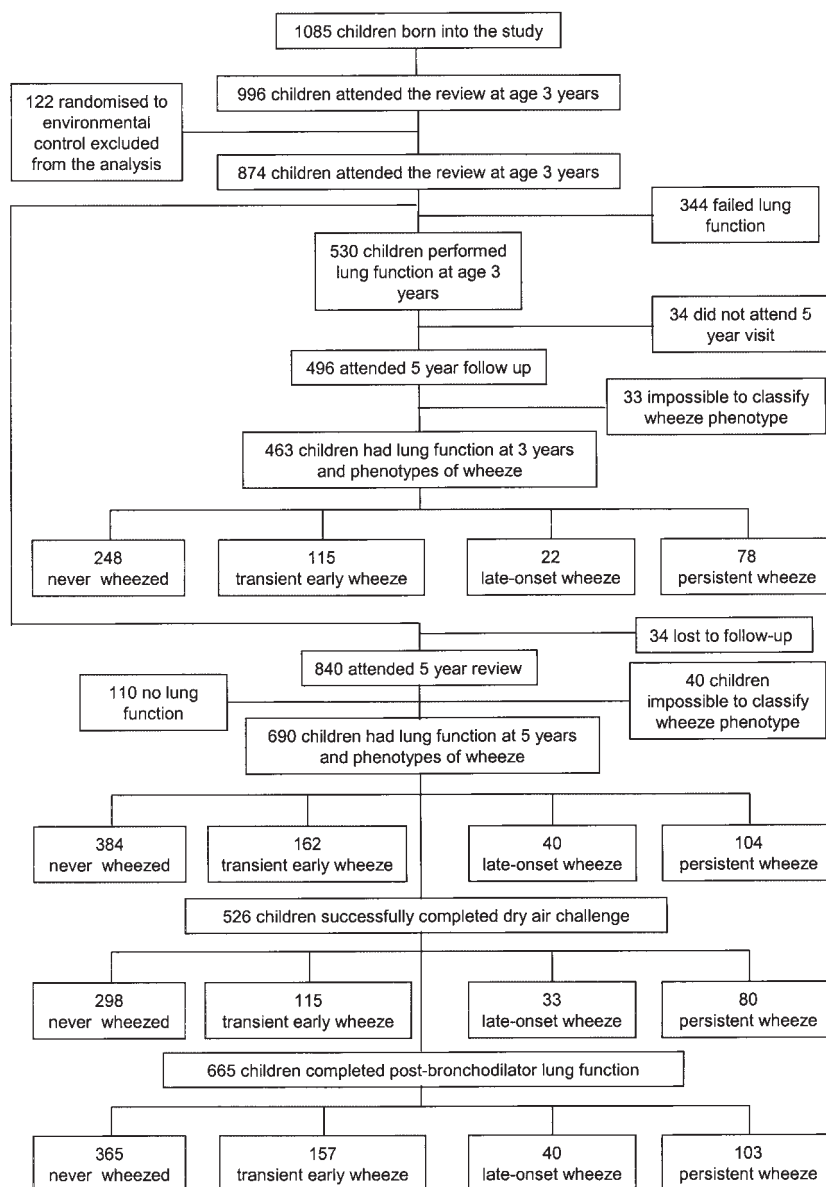


Figure 1. Schematic representation of trial profile.

could be classified into four phenotypes of wheeze, 526 completed EVH challenge (in 6 children, sRaw had not returned to within 5% of the baseline), and 665 had postbronchodilator lung function (19 parents refused permission for the bronchodilator to be administered). Atopic status at age 3 and 5 years was highly associated ($p < 0.0001$; McNemar test), and sensitization at age 3 was a very strong predictor of sensitization at age 5 (odds ratio [OR] 38.0, 95% confidence interval [CI] 22.0–65.6; $p < 0.0001$).

Baseline Lung Function and Phenotypes of Wheeze

We found a highly significant association between sRaw at age 3 and 5 years and wheeze phenotypes (both $p < 0.0001$, ANOVA; Figure 2). Multiple comparison test (Scheffe) revealed that at age 3 years there was no significant difference in sRaw between children who had never wheezed (kPa/second; GM 1.06, 95% CI 1.04–1.09) and those with transient early wheeze (GM 1.11, 95% CI 1.07–1.15; $p = 0.34$) or late-onset wheeze (GM 1.04, 95% CI 0.95–1.14; $p = 1.0$; Figure 2a). The sRaw was significantly higher in persistent wheezers (GM 1.19, 95% CI 1.13–1.25) compared with late-onset wheezers ($p = 0.04$) and never wheezers ($p < 0.001$).

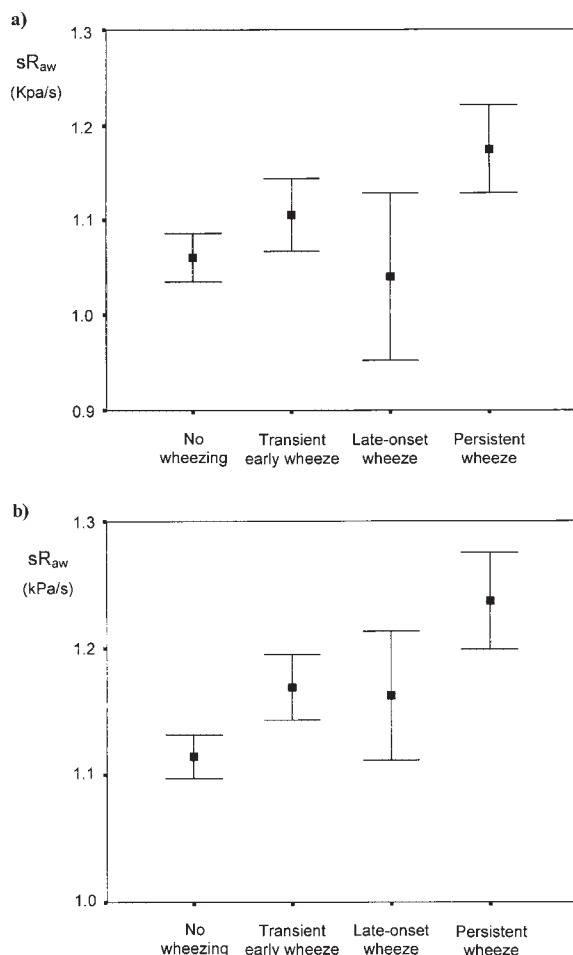


Figure 2. The sRaw (kPa/second, GM, and 95% CIs) in different wheeze phenotypes. (a) Age 3 years (248 children had never wheezed, 115 had transient early wheeze, 22 developed late-onset wheezing, and 78 had persistent wheezing). (b) Age 5 years (384 children had never wheezed, 162 had transient early wheeze, 40 developed late-onset wheezing, and 104 had persistent wheezing).

At age 5 years, sRaw in persistent wheezers (GM 1.27, 95% CI 1.22–1.32) was significantly higher compared with transient early wheezers (GM 1.18, 95% CI 1.15–1.22; $p = 0.02$) and children who had never wheezed (GM 1.12, 95% CI 1.10–1.14; $p < 0.001$; Figure 2b). Lung function was significantly poorer in transient early compared with never wheezers ($p = 0.01$), but there was no significant difference between never wheezers and late-onset wheezers (GM 1.18, 95% CI 1.12–1.24; $p = 0.43$).

Multivariate analysis. Multiple factor ANOVA modeling was performed including variables for which a significant association with sRaw was found in the univariate analyses (child's sensitization status, age 3 or 5; maternal asthma; maternal smoking during pregnancy; parental atopic status). In these models, the association between wheeze phenotypes and sRaw remained significant ($p = 0.02$ at age 3, $p = 0.004$ at age 5); furthermore, numeric differences in sRaw between different wheeze phenotypes appeared more marked (estimated marginal means [i.e., means adjusted for the previously described confounders] for sRaw in multiple ANOVA models are shown in Table 1). There were no material differences in the results of multiple comparison tests (Scheffe) in the univariate and multivariate analyses between the four phenotypes of wheeze, both for age 3 and age 5 years.

Lung Function at Age 3 Years and Prediction of Wheeze

To test the hypothesis that lung function at age 3 predicts the persistence of wheeze by age 5, we compared children with transient early wheezing to those with persistent wheeze. Among the subgroup of children who had wheezed by age 3, the risk of persistent wheeze increased markedly with increasing sRaw values (for the log_e sRaw data: OR 5.2, 95% CI 1.3–22.0; $p = 0.025$; e.g., increase in sRaw from 1.1 to 1.2 kPa/second increases the odds for persistence of wheeze by $\sim 18\%$). Figure 3a demonstrates the fitted predicted probability curve for persistent wheezing by age 5 years in relation to sRaw at age 3 derived from the logistic regression analysis.

To test the hypothesis that lung function at age 3 predicts the development of wheeze in children who have not wheezed previously, we compared late-onset wheezers with children who had never wheezed. In a marked contrast to the finding of a significant association between lung function and the persistence of symptoms in children who have wheezed within the first

TABLE 1. ESTIMATED MARGINAL MEANS (MEANS ADJUSTED FOR CHILD'S SENSITIZATION STATUS, AGE 3 OR 5; MATERNAL ASTHMA; MATERNAL SMOKING DURING PREGNANCY; PARENTAL ATOPIC STATUS) AND 95% CONFIDENCE INTERVALS OF THE sRAW LEVELS IN RELATION TO WHEEZE PHENOTYPE IN A MULTIPLE ANOVA MODEL

	sRaw (kPa/s) (GM, 95% CI)	
	Age 3 yr*	Age 5 yr†
Never wheezed	1.10 (1.05, 1.16)	1.14 (1.10–1.19)
Transient early wheeze	1.08 (1.01, 1.16)	1.20 (1.15–1.26)
Late-onset wheeze	1.10 (0.95, 1.27)	1.16 (1.08–1.24)
Persistent wheeze	1.25 (1.16, 1.36)	1.30 (1.24–1.37)

Definition of abbreviations: CI = confidence interval; GM = geometric mean.

* Persistent wheeze vs. never wheeze ($p < 0.001$); persistent wheeze vs. transient early wheeze ($p = 0.10$); persistent wheeze vs. late-onset wheeze ($p = 0.03$). Transient early wheeze vs. never wheeze ($p = 0.3$); transient wheeze vs. late-onset wheeze ($p = 0.5$). Never wheeze vs. late-onset wheeze ($p = 0.9$).

† Persistent wheeze vs. never wheeze ($p < 0.001$); persistent wheeze vs. transient early wheeze ($p = 0.001$); persistent wheeze vs. late-onset wheeze ($p = 0.03$). Transient early wheeze vs. never wheeze ($p = 0.03$); transient early wheeze vs. late-onset wheeze ($p = 1.0$). Never wheeze vs. late-onset wheeze ($p = 0.6$).

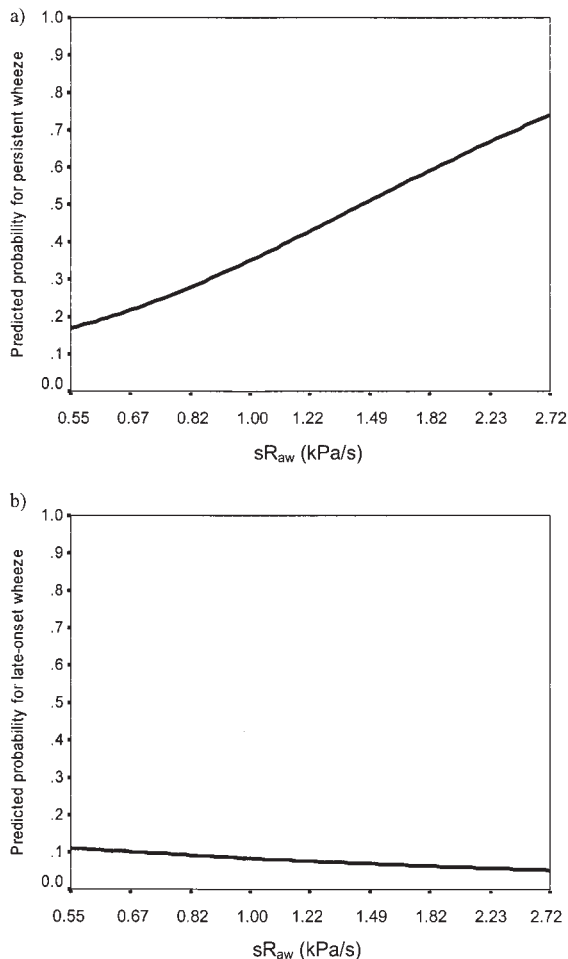


Figure 3. (a) Fitted predicted probability curve for persistent wheezing by age 5 years in relation to sRaw at age 3 years derived from the logistic regression analysis. (b) Fitted predicted probability for late-onset wheezing by age 5 years in relation to sRaw at age 3 years derived from the logistic regression analysis.

3 years of life, we found no association between sRaw at age 3 and development of wheeze in children who had not wheezed by age 3 (OR 0.6, 95% CI 0.07–5.3; $p = 0.64$; Figure 3b). There was no difference in the frequency of allergic sensitization at age 3 between never wheezers and children with late-onset wheeze (19.2% vs. 18.4%, respectively; $p = 1.0$).

Multivariate analysis. A multiple logistic regression model that included children with transient early wheeze and persistent wheeze was used to identify risk factors associated with persistent symptoms. The model included variables for which a significant association with persistent wheezing was found in the univariate analysis (sRaw and child's sensitization at age 3, maternal asthma, maternal smoking during pregnancy, and parental atopic status). Increasing sRaw value (OR 5.5, 95% CI 1.2–25.9; $p = 0.03$) and child's sensitization (OR 2.8, 95% CI 1.3–5.8; $p = 0.008$) remained significant and independent associates and substantially and significantly increased the risk of persistent wheezing.

Airway Reactivity and Phenotypes of Wheeze

We found a significant association between the magnitude of response to dry air challenge (expressed as a percentage of increase in sRaw) and wheeze phenotypes ($p = 0.019$, ANOVA;

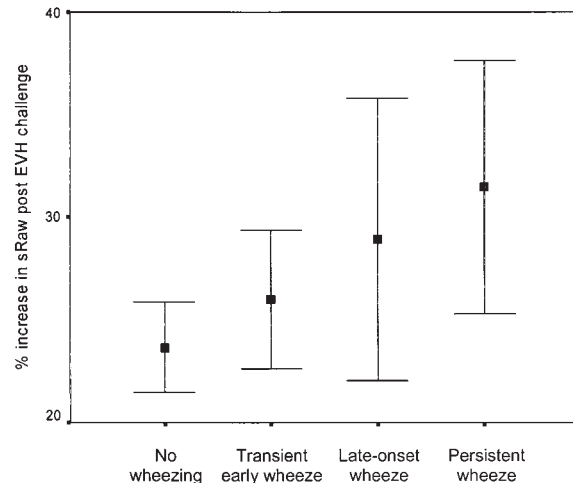


Figure 4. Airway reactivity (percentage of change in sRaw post EVH challenge: GM and 95% CIs) in different wheeze phenotypes (298 children had never wheezed, 115 had transient early wheeze, 33 developed late-onset wheezing, and 80 had persistent wheezing).

Figure 4). A multiple comparison test revealed that children with persistent wheeze had a significantly larger increase in sRaw following EVH challenge ($n = 80$, mean percent increase 31.5, 95% CI 26.9–36.0) compared with those who had never wheezed ($n = 298$, mean percent increase 23.75, 95% CI 21.3–26.0; $p = 0.017$). There was no significant difference between never wheezers and late-onset wheezers ($n = 33$, mean percent increase 28.9, 95% CI 21.9–36.0; $p = 0.66$) or transient early wheezers ($n = 115$, mean percent increase 26.0, 95% CI 22.2–29.8; $p = 0.89$). Adjusting the analysis for the prechallenge sRaw value (analysis of covariance) did not materially change the results.

When the results of dry air challenge were expressed as a change in sRaw following EVH challenge, children with persistent symptoms had more reactive airways (kPa/second, mean change 0.42, 95% CI 0.33–0.50) compared with children with transient early wheeze (mean change 0.30, 95% CI 0.26–0.35; $p = 0.03$) and those who had never wheezed (mean change 0.27, 95% CI 0.24–0.29; $p < 0.001$). There was no significant difference between persistent and late-onset wheezers (mean change 0.34, 95% CI 0.25–0.44; $p = 0.61$).

Using the data in children with no history of wheeze, we defined the normal response to EVH challenge as a mean percentage of change plus or minus 2 SDs (i.e., 95% reference range), and estimated that an increase in sRaw of more than 60% should be considered a positive response. The proportion of children with positive EVH challenge was significantly higher among persistent wheezers (12.5%) and late-onset wheezers (12.1%) compared with never wheezers (5.4%) and transient wheezers (2.6%; $p = 0.01$).

Postbronchodilator Lung Function and Phenotypes of Wheeze

We found a significant difference in postbronchodilator sRaw between different wheeze phenotypes ($p = 0.04$, ANOVA; Figure 5). After bronchodilator administration, there remained a strong trend for children with persistent wheeze to have a higher sRaw ($n = 103$; kPa/second, GM 1.01, and 95% CI 0.98–1.05) than children who had never wheezed ($n = 365$; GM 0.96, 95% CI 0.95–0.98; $p = 0.07$). There was, however, no difference in the postbronchodilator sRaw between persistent wheezers and those with transient early wheeze ($n = 157$; GM 0.99, 95% CI 0.96–1.01;

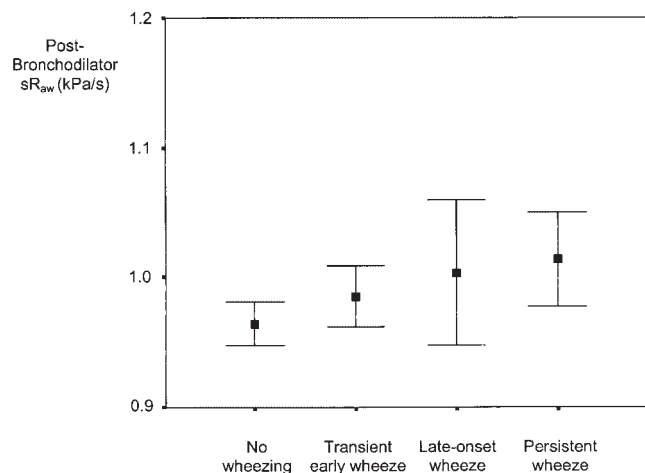


Figure 5. Postbronchodilator lung function (kPa/second, GM, and 95% CIs) in different wheeze phenotypes (365 children had never wheezed, 157 had transient early wheeze, 40 developed late-onset wheezing, and 103 had persistent wheezing).

$p = 0.61$) or late-onset wheeze ($n = 40$; GM 1.00, 95% CI 0.95–1.06; $p = 0.56$).

Relationship between Lung Function at Age 3 and 5 Years

There was a significant positive correlation between sRaw at 3 and 5 years (Pearson correlation, $r = 0.43$; $p < 0.001$). In the multivariate linear regression analysis controlling for the effect of other risk factors shown to be associated with lung function (maternal and paternal asthma, maternal smoking, child's sensitization, child's history of wheeze, and eczema), sRaw measured at age 3 remained the only significant and independent predictor of sRaw at 5 years ($\beta = 0.45$, $t = 10.02$; $p < 0.001$). In 430 children who successfully performed lung function measurements at both time points, the mean ratio between sRaw measured at age 3 and 5 years was 1.07 (95% limits of agreement 0.70–1.61), suggesting a trend for sRaw to increase with increasing age (by an average of 7% between age 3 and 5 years).

DISCUSSION

Our results show that among children with a history of parentally reported wheeze within the first 3 years of life, lung function was reduced in those who subsequently continued with wheezing until age 5 years (persistent wheezers) compared with children who then stopped wheezing (transient early wheezers). However, we found no difference in lung function at age 3 years between children who had not wheezed by age 5 years compared with those who developed wheeze after age 3 (late-onset wheezers). Thus, reduced lung function at age 3 predicted the subsequent persistence of symptoms in children who had wheezed within the first 3 years of life, but this factor was not associated with the onset of wheeze after age 3 in children who had not wheezed previously. With an increased understanding of the underlying physiology, measuring lung function in symptomatic, young preschool children may enable targeting children who are most likely to benefit from treatment interventions and monitoring.

One potential limitation of the study was that lung function at age 3 was available in a subset of subjects. Thus, depending on whether the analyses pertain to sRaw measurements at 3 years or at 5 years, different cohorts of children were included. This raises the question of whether the children who were unable

to provide sRaw at 3 years were a biased subgroup. However, this is unlikely to be the case, because we found no difference between children who completed lung function measurement at age 3 compared with those who did not complete this measurement in the prevalence of sensitization, reported symptoms, maternal smoking, and maternal asthma.

How do our data compare with the other cohort studies? In the Tucson study, children with persistent wheezing had significantly reduced lung function at age 6 years; however, their lung function in the first year of life appeared similar to children who had never wheezed (7). Transient early wheezers tended to have reduced lung function in infancy and at age 6 years when compared with children with no history of wheeze (7). In contrast, a recent Australian study suggested that individuals with transient wheeze have normal lung function at the age of 1 month, and that reduced lung function in infancy was associated with persistent wheeze at age 11 years, independently of increased airway responsiveness and atopic sensitization in childhood (25). It is difficult to explain the differences between these two studies, because the techniques used to measure infant lung function were not identical (although definitions of wheeze phenotypes were not identical). Delacourt and colleagues (26) measured maximal flow at functional residual capacity (V'_{max} FRC) at age 17 months and reported that persistent, but not transient, wheeze was associated with reduced lung function. Our study fills in the gap with the data on lung function in early preschool age, which was largely unavailable previously. Lung function at age 3 and 5 years was reduced in children with persistent wheeze compared with transient early wheezers and nonwheezing children, with transient wheezers falling between nonwheezing children and persistent wheezers. The pattern of lung function between different wheeze phenotypes appeared very similar at age 3 and 5 years. However, although the difference in sRaw between children who had never wheezed and those categorized as transient early wheezers failed to reach statistical significance at age 3 years, it was highly significant at age 5 (probably because of a higher proportion of children who successfully completed lung function measurement). It is worth noting that sRaw value increased significantly with age (by $\sim 7\%$) in all groups, and this observation warrants further investigation.

Brussee and colleagues (27) used an alternative technique to measure lung function in 4-year-old children enrolled in the Dutch Prevention and Incidence of Asthma and Mite Allergy study. They found that resistance measured by the interrupter technique was higher in children with persistent wheeze than in children who had never wheezed and those with transient early wheeze.

Our data suggest that both transient and persistent wheezers have reduced lung function compared with nonwheezing children, but the deficit is considerably greater in persistent wheezers. It is possible that the deficits in lung function in persistent and transient wheezers may have already been present at a much younger age. We do not have sufficient data on lung function in infancy to make any firm conclusion about this point. However, it is worth noting that we have measured V'_{max} FRC at age 4 weeks in a subsample of their population (34 children had data on lung function in infancy and at age 3 years, and 57 successfully completed infant and 5-year lung function testing) (28). Reduced V'_{max} FRC in infancy was associated with increased sRaw at both ages 3 and 5 years (data not shown). Dezateux and colleagues (29) reported that increased plethysmographic airway resistance during the first few months of life (before any respiratory illness) preceded and predicted subsequent wheezing illness throughout the first year of life. Furthermore, these changes in airway resistance persisted to at least the age of 1 year (30). Thus, a pattern emerges in which poor

lung function in the first few years of life (and perhaps infancy) is associated with persistent wheezing independently of atopic sensitization. Children with comparatively smaller deficits in lung function may develop only transient wheezing. In individuals with a history of wheeze in early life and deficit in lung function, IgE-mediated sensitization at the age of 3 years further increases the risk of persistence of symptoms, at least by the age of 5 years. The monitoring of lung function and atopic sensitization in symptomatic children from an early age may enable physicians to identify those children at risk of persistent disease and may be an important factor in modifying outcomes in later life.

Late-onset wheezers had lung function that was not significantly different from children who had never wheezed, but because of the relatively smaller size of this group, these data must be interpreted with caution. However, airway hyperresponsiveness in this group appeared to be similar to persistent wheezers and more common compared with transient wheezers and children who had not wheezed.

Longitudinal data provide evidence for the relationship between deficits in lung function and the clinical expression of asthma, suggesting that subjects with persistent asthma in childhood continue to have impairments in lung function that track to adulthood (31, 32). In the Melbourne Asthma Study, subjects with asthma had persistent airflow obstruction throughout childhood and into adult life (31). The magnitude of the difference in lung function between different asthma severity groups did not increase over time, suggesting that the deficits had occurred in early childhood and did not progress. The early identification of children with persistent symptoms and consequent early intervention aimed at the improvement in lung function may reduce or delay irreversible structural changes and improve outcomes later in life.

Further evidence of the relationship between childhood events and respiratory health in adult life comes from a study of 1,037 children in New Zealand who were followed from the age of 9 to 26 years (32). Postbronchodilator FEV₁/VC ratio was used as a marker of airway remodeling. Subjects with a low ratio at age 26 years already had a disordered lung function at the age of 9 years, with a slow progressive loss of reversibility, indicating that airway remodeling may begin in early childhood. Alternatively, impaired lung function tracking from childhood into adult life may represent a primary airway abnormality, which increases the risk of the subsequent respiratory disease (i.e., the airways may be "premodeled" for later asthma development) (17).

Our results show that children who experience wheeze symptoms during the first 5 years of life already have some evidence of irreversible airway obstruction, as assessed by the postbronchodilator lung function. In particular, this appears to be the feature of children with persistent symptoms. This finding may suggest that not all airway narrowing in these children is caused by smooth muscle hypertrophy and hyperplasia and that structural change may exist in very young children.

Our data also demonstrate that children with persistent symptoms have increased airway reactivity at age 5 when compared with those with transient early wheeze or those who have never wheezed. It is worth emphasizing that the hyperventilation challenge was performed with dry rather than cold air as has been used in previous studies. In our experience, dry air appears more acceptable to preschool children than the cold air challenge. Our data are in agreement with Kurukulaaratchy and colleagues (33), who reported that persistent wheezing in early childhood was associated with increased airway reactivity at age 10 years compared with nonwheezers or those with transient early symptoms only. Turner and coworkers (25) demonstrated that poor lung

function at birth and increased airway reactivity at age 11 years were associated with persistent wheeze.

In summary, our data demonstrate that reduced lung function and IgE-mediated sensitization at age 3 may predict the subsequent persistence of symptoms in children who have wheezed within the first 3 years of life. However, in children who have not wheezed previously, the onset of wheeze after age 3 is not associated with reduced lung function at age 3. We emphasize that although there was a highly significant relationship between lung function at 3 years of age and subsequent symptoms within this large birth cohort study, the predictive value of such testing for individual children is likely to be relatively low because of an overlap between the various wheezing phenotypes.

This study suggests the possibility that in addition to the systemic immune response manifested by the IgE-mediated sensitization, a primary lung determinant reflected by the increase in sRaw in early life underlies the clinical manifestation of persistent wheeze. It is therefore essential to elucidate genetic and environmental factors responsible for diminished lung function in early life to complement the studies investigating the causes of the increase in allergic sensitization.

Conflict of Interest Statement: L.A.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; A.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; A.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; J.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; C.S.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; A.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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WHEEZE PHENOTYPES AND LUNG FUNCTION IN PRE-SCHOOL CHILDREN

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ONLINE SUPPLEMENT

METHODS

The Manchester Asthma and Allergy Study is a prospective study which follows the development of asthma and other atopic disorders in a cohort of infants. The setting is the maternity catchment area of Wythenshawe and Stepping Hill Hospitals, comprising of 50 square miles of South Manchester and Cheshire, UK, a stable mixed urban-rural population. Study was approved by the Local Research Ethics Committee. Informed consent was obtained from all parents.

Screening & Recruitment

All pregnant women were screened for eligibility at 'Booking' antenatal visits (8th-10th week of pregnancy). The study was explained to the parents, and informed consent for initial questionnaires and skin prick testing was obtained. Both parents completed a questionnaire about their and their partner's history of asthma and allergic diseases and smoking habits. The two hospitals organised antenatal "booking in" clinics differently. Therefore, in close consultation with the staff at both sites, two different organisational strategies were used to allow the greatest access to patients whilst causing the least disturbance to the normal running of antenatal clinics.

At Wythenshawe Hospital, dedicated "booking in" antenatal clinics were organised (three sessions per week). In these clinics, the study information and study questionnaires were given to all mothers attending the clinic by the reception staff before going for an ultra sound scan. This was followed up by an interview after the scan, whilst ladies were waiting for the midwife or doctor. When informed consent had been obtained, skin prick testing was carried out in a screened area of the waiting room.

In Stepping Hill Hospital, “booking” patients were reviewed along with other antenatal patients during eight sessions per week. In order to ensure that equal numbers of participants were recruited from the two study sites, subjects for the study were recruited on six out of the eight sessions. Again, the reception staff gave out study information and questionnaires to all suitable attendees (i.e., those in early stages of pregnancy - <10 weeks pregnant). When consent was obtained pregnant ladies and their partners were skin prick tested in one of the vacant consulting rooms.

If the pregnant woman’s partner was not present at the antenatal clinic visit, an invitation was sent for him to attend an open-access evening clinic at Wythenshawe Hospital for skin prick testing and questionnaire. Once both parents had completed questionnaires and skin prick testing, a full explanation of the proposed future follow-up for the child was given. Of the 1499 couples who met the inclusion criteria (≤ 10 weeks of pregnancy, maternal age ≥ 18 years, questionnaire and skin test data available for both parents), 288 declined to take part in the study. Of 1211 couples who initially agreed to take part, 1085 had a successful full-term pregnancy (>36 weeks gestation) and gave consent to a further follow-up. Of those, 128 were prenatally randomized into an environmental control group (see below), and 957 were followed as an observational arm of the cohort.

Allocation to study groups

Children were assigned to risk groups according to parental sensitisation status. Nested within the cohort is a primary prevention study in which a subgroup of children at high risk (both parents atopic) without pets in home was randomised to either stringent environmental control (allergen impermeable covers for the parental and infant bed, hot washing of bedding weekly, HEPA vacuum cleaner, hard floor for the nursery) or a

normal regime. Children randomized to the environmental control group were excluded from this analysis.

Follow-up

The children have been followed prospectively, and attended review clinics at age 3 and 5 years (± 4 weeks). We completed assessment at age 5 years in April 2003. The overall follow-up rate during the first five years of life in the observational part of the cohort was exceptionally high (874/957 [91.3%] age 3 years; 840/957 [87.8%] age 5 years).

Outcomes

Symptoms

A standard respiratory questionnaire was interviewer-administered at age 3 and 5 years to collect the information on symptoms, physician-diagnosed illnesses and treatments received.

Specific airway resistance

At age 3 and 5 years we carried out measurements of specific airway resistance (sR_{aw}) to assess airway function in all children who were willing to cooperate. Measurements of sR_{aw} were made using a constant volume whole body plethysmograph (Masterscreen Body 4.34; Jaeger, Würzburg, Germany). Flow and volume were measured with a heated differential pressure screen-type pneumotachograph with a resistance of $0.036 \text{ kPa}^{-1}\text{s}$ and a dead space of 160mls. Pressure measurements were made with a pressure transducer (Nr.660.99007; Hube Control AG, Wuerenlos, Switzerland) with an input range of $\pm 100 \text{ Pa}$, a resolution of 0.05 Pa and a linear response up to 10 Hz . The plethysmograph was calibrated daily. Sensors in an ambient unit supplied with the plethysmograph recorded ambient data on temperature, humidity and barometric pressure. The pneumotachograph

was volume calibrated according to the American Thoracic Society recommendations using a 2 L syringe at flow rates of 0 – 1.5, 1.5 – 5 and >5 l/s. The half value period was calibrated to ensure a specific leakage in the box of 4-7 seconds.

The pressure transducer was calibrated using a 50 mL motor driven piston pump to generate sinusoidal variations of plethysmographic pressure. Electronic body temperature, pressure, and saturation (BTPS) compensation was applied throughout, using a time-shift of 60 ms.

sR_{aw} is measured by a single-step procedure from the simultaneously measured changes of respiratory flow and changes of plethysmographic pressure, omitting the measurement of TGV^{E1} . Measurements were carried out during tidal breathing using a facemask, which was adapted by fitting a standard paediatric facemask with a non-compressible mouthpiece made from silicone tubing. The end of the tubing was made rigid with an aluminium splint. The purpose of this was to maintain stable airway opening, prevent nose breathing and support the cheeks. The procedure was explained to the accompanying adult and the use of the facemask demonstrated to the child. The children were encouraged to sit in the plethysmograph alone but if they refused, the accompanying adult, usually a parent, accompanied the child in the plethysmograph cabinet with the child seated on their knee. The door of the plethysmograph was closed and the subject asked to breathe through the facemask.

Children were encouraged to breathe at a rate of 30-45 breaths per minute. If a parent accompanied the child, the adult was asked to inhale and hold their breath for approximately 20 seconds. sR_{aw} measurements were made once a stable breathing pattern had been re-established. Once a stable breathing pattern was established, at least

three measurements of sR_{aw} were performed, and each was calculated from the medians of 5 consecutively measured technically acceptable loops^{E2} (each child performed at least 15 loops). The mean of these 3 measurements of *effective* sR_{aw} was used in the analysis. The measured values of sR_{aw} were corrected for the influence of the pneumotachograph screen (R_{screen}) and for the volume displacement caused by the subject (or subject + parent).

Children were asymptomatic at the time of assessment of lung function. A total of 530 children successfully performed lung function measurement at age 3 years, and 730 had sR_{aw} measured at age 5 years.

Airway reactivity

At the 5-year follow-up, airway reactivity was assessed by means of a Eucapnic Voluntary Hyperventilation (EVH) challenge. We constructed a breathing circuit consisting of a precision flow meter which directs gas from a cylinder into a large capacity reservoir bag. The subject hyperventilated, via a demand valve and large bore tubing, from a cylinder of certified medical gas containing 21% O₂, 5% CO₂, remainder N₂ with a water content <10mg per litre for 6 minutes at a ventilation rate of 75% of maximum voluntary ventilation (MVV) which was estimated pre-challenge as $FEV_1 \times 22.5$. Some children were unable to perform forced manoeuvres satisfactorily and FEV_1 could therefore not be measured. In these cases the predicted FEV_1 was used to calculate MVV.

EVH challenge was performed by a single-step procedure using a protocol established by Zach et al^{E3}. Parents were asked to withhold from giving the children short acting bronchodilators for 6 hours and long acting bronchodilators for 12 hours prior to testing.

Children inhaled through a modified facemask as described previously. They were encouraged to match their ventilation to the reservoir balloon with the aim of keeping it partially inflated. This ensured that the correct level of ventilation was maintained. Specific airway resistance was measured at baseline and 2, 5 and 10 minutes post challenge to assess the degree of airway reactivity. The highest sR_{aw} value measured at 2, 5 or 10 minutes post challenge was recorded, and the response was expressed as percent change in sR_{aw} . A bronchodilator (400 micrograms Salbutamol via a large volume spacer device) was given if the child became symptomatic or sR_{aw} increased by $>100\%$ for therapeutic purposes ($n=6$). Lung function was reassessed after 15 minutes to ascertain whether it had returned to baseline values.

Post-bronchodilator lung function

All children were invited to complete a test of response to bronchodilator (19 parents refused). Bronchodilator response was measured after administration of 400 µg of Salbutamol via a large volume spacer device (Volumatic). Specific airway resistance was measured before and 15 minutes after bronchodilator administration. In order to minimize the possible confounding effect of the reduction in lung function following the dry air challenge test on postbronchodilator lung function, amongst children who have undergone EVH challenge, only those in whom sR_{aw} had returned to within 5% of the baseline value have been included in the analysis.

Allergic sensitization

We ascertained allergic sensitisation at age 3 and 5 by skin prick testing (mite, cat, dog, grasses, milk, egg. Sensitization was defined as wheal at least 3 mm greater than the negative control.

Allocation to the phenotypes of wheeze

The study population comprised children who successfully completed lung function measurement at either age 3 or age 5 years, and could be allocated into one of four phenotypes of wheeze based on the questionnaire data. According to parentally-reported history of wheeze at the follow-ups at age 3 and 5 years, children were assigned to the following categories using criteria proposed by Martinez et al^{E4}:

- i) No wheezing: no recorded wheeze during the first three years of life, no wheezing at age 5 years (248 age 3 years; 384 age 5 years)
- ii) Transient early wheeze: at least one episode of wheezing during the first three years of life, no wheezing at age 5 years (115 age 3 years; 162 age 5 years)
- iii) Late-onset wheeze: no recorded wheeze during the first three years of life, but reported wheezing in the previous 12 months at age 5 years (22 age 3 years, 40 age 5 years)
- iv) Persistent wheeze: at least one episode of wheezing during the first three years of life, reported wheezing in the previous 12 months at age 5 years (78 age 3 years, 104 age 5 years)

Some children could not fit into this classification (e.g. no reported wheeze, but receiving asthma medication at age 5 years; 33 age 3 years, 40 age 5 years).

Statistical analysis

Statistical analysis was carried out using SPSS for Windows, version 11.0. sR_{qW} measurements followed a log-normal distribution, hence were subject to a log e-transformation prior to analysis. Results are reported as geometric means (GM). One-way Analysis of variance (ANOVA) was used to compare differences between the four

wheeze phenotypes. Specific differences between phenotypes were assessed using Scheffe's multiple comparison tests. Variables found to be significantly associated with any of the outcomes in the univariate analyses were included in multivariate analyses. Multiple factor ANOVAs were used to assess the relationship between sRaw and wheeze phenotype after adjusting for confounding factors such as child's sensitisation status and maternal asthma. The risks of persistent wheeze and late-onset wheeze were evaluated using logistic regression analysis. Significant risk factors associated with persistent symptoms were identified by means of multiple logistic regression analysis. We used Pearson correlation to investigate the relationship between sR_{aw} at 3 and 5 years. Significant predictors of sR_{aw} at 5 years were ascertained using multiple linear regression analysis.

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