Validation of novel wheeze phenotypes using longitudinal airway function and atopic sensitisation data in the first 6 years of life: Evidence from the Southampton Women's Survey.

Samuel A Collins[1,2], Katharine C Pike[1,2], Hazel M Inskip[3,4], Keith M Godfrey[2.3,4,5], Graham Roberts[1,2,3], John W Holloway[1,3], Jane SA Lucas[1,2] and the Southampton Women's Survey Study Group[4].

[1]Clinical and Experimental Sciences, University of Southampton Faculty of Medicine, Southampton, UK; [2]NIHR Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, UK. [3]Human Development and Health, University of Southampton Faculty of Medicine, Southampton, UK; [4]Southampton Medical Research Council Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK;

Abbreviated Title: Wheeze phenotypes and atopy/lung function to 6 years of age

ABSTRACT

Background

In 1995 the Tucson Children's Respiratory Study (TCRS) identified clinically distinct phenotypes amongst early wheezers; the Avon Longitudinal Study of Parents And Children (ALSPAC) has recently re-examined these.

Objectives

To validate statistically derived ALSPAC phenotypes in the Southampton Women's Survey (SWS) using infant and 6 year lung function, and allergic sensitisation at 1, 3 and 6 years, comparing these with TCRS phenotypes.

Methods

Complete 6 year follow-up data were available for 926 children, selected from 1973 infants born to 12,579 women characterised pre-conception. 95 children had V'maxFRC and $FEV_{0.4}$ measured age 5-14 weeks using rapid compression/raised volume techniques. At 6 years we performed spirometry (n=791), fractional exhaled nitric oxide (FeNO, n=589) and methacholine challenge (n=234). Skin prick testing was performed at 12m, 3 and 6 years (n=1494, 1255, 699, respectively). Using wheeze status questionnaire data at 6m, 12m, 2, 3 and 6 years we classified children into TCRS (never, transient early, persistent, late-onset) and ALSPAC based groups (never, early, transient, intermediate-onset, late-onset, persistent).

Results

Amongst ALSPAC groups, persistent and late-onset wheeze were associated with atopy at 3 and 6 years, whilst intermediate-onset wheeze showed earlier atopic association at 1 year; all three were associated with FeNO at 6 years. Persistent wheezers had lower infant (V'maxFRC $p<0.05$) and 6

year lung function (FEV_1, FEV_1/FVC and FEF_{25-75}, $p<0.05$), whilst late and intermediate-onset wheezers showed no lung function deficits. Transient wheezers were non-atopic but showed persistent lung function deficits (V'maxFRC in infancy, FEV_1 and FEF_{25-75} at 6 years, all $p<0.05$). Those who wheezed only in the first year (early phenotype) showed no lung function deficits. No associations were seen with 6 years bronchial hyper-responsiveness or infancy $FEV_{0.4}$.

Conclusion

SWS cohort data validates the statistically derived ALSPAC 6-class model. In particular, lung function and atopy successfully differentiate persistent, late-onset and intermediate-onset wheeze, whilst the Tucson 'transient early' wheeze phenotype can be sub-classified into groups that reflect early lung function. Since the 4-class model fails to adequately differentiate phenotypes based on lung function and atopy, we propose that strong consideration be given to using the 6-class paradigm for longitudinal outcome work in wheezing with onset in early life.

[354 words]

Key Words

Wheeze, asthma, phenotype, lung function, cohort, atopy

Abbreviations

SWS – Southampton Women's Survey, TCRS – Tucson Children's Respiratory Study, ALSPAC – Avon Longitudinal Study of Parents and Children, PIAMA - Prevention of Infant Asthma and Mite Allergy, $FEV_{0.4}$ – Forced Expiratory Volume in 0.4 second, FEV_1 – Forced Expiratory Volume in 1 second, FEF_{25-75} – Forced Expiratory Flow between 25% and 75% of Forced Vital Capacity , FVC – Forced Vital Capacity, V'maxFRC – Maximal Forced Expiratory Flow at Functional Residual Capacity, FeNO – Fractional exhaled Nitric Oxide, BHR – Bronchial Hyper-responsiveness, COPD – Chronic Obstructive Pulmonary Disease, CCCEH - Columbia Children's Centre for Environmental Health, LLCA – Longitudinal Latent Class Analysis, LCGA – Latent Class Growth Analysis

INTRODUCTION

Wheezing in infancy is common, however only a small proportion of these children will continue to wheeze into later childhood and beyond. A number of studies have attempted to classify wheezing pre-school children into different phenotypes in order to assist investigation of the pathways of asthma development. The Tucson Children's Respiratory Study (TCRS) published the first classification according to wheezing status up to 6 years of age[1]. TCRS characterised children into 4 groups, namely never wheezed, transient early wheeze, late wheeze and persistent wheeze; persistent and late-onset wheeze were associated with atopy, however transient early wheeze was not, as confirmed by further studies using this classification [2–5]. TCRS found persisting lung function deficits in those with transient wheeze, suggesting this was a phenomenon of small airways, with resolution of wheeze once airway calibre improves with growth, albeit without returning to normal[1]. Whilst TCRS found that persistent wheezers did not have lung function deficits at birth, the Copenhagen Prospective Studies on Asthma and Childhood (COPSAC) cohort of at-risk children did find diminished lung function shortly after birth in those with asthma at 7 years[6] and the authors hypothesized that perhaps TCRS had been underpowered to find this statistical difference. Likewise, Turner *et al* found lung function deficits that preceded the development of asthma [7]. A cohort from Perth, Australia performed longitudinal lung function testing, comparing VmaxFRC at birth and FEF_{25-75} at 4-6 years and 11 years. They found that transient wheezers (wheeze at 0-3 years) had the best lung function of all groups at birth, but this diminished over time with reference to other groups, supporting the idea that transient wheeze is associated with ongoing lung function deficits beyond the symptomatic period. Development of late and persistent wheeze has been associated with reduced airway function interacting with atopic sensitisation, immune dysregulation and airway remodelling, in line with the classical view of asthma pathogenesis[8–11]. It is possible that persistent wheezers represent an overlap between these theories of transient wheeze and more typical asthma pathogenesis with later resolution of wheeze by puberty in those who are not atopic[4].

The Tucson phenotype classification does not, however, fully describe the heterogeneity amongst wheezing children and attempts have been made to reclassify these phenotypes. The Avon Longitudinal Study of Parents And Children (ALSPAC) used longitudinal latent class analysis (LLCA) to redefine the wheezing phenotypes purely on statistical grounds, independent from any preconceived bias of the clinician. Amongst 6265 children followed-up to 6 years of age they identified six classes of pre-school wheeze; never/infrequent, transient early, prolonged early, intermediate onset, late onset and persistent [12]. They then replicated the process with 2810 children from the Prevention of Infant Asthma and Mite Allergy (PIAMA) cohort and 5760 from ALSPAC up to 8 years of age[13]. The replication identified 5 classes of wheezing children; never, transient early, intermediate onset, late onset and persistent. The intermediate onset, late onset and persistent phenotypes were strongly associated with atopy, with the strongest association seen amongst the intermediate-onset phenotype. All wheezing groups showed poorer lung function at 8 years, including transient early wheezers who were, by definition, no longer wheezing by this time. The transient early wheezers did not, however, show greater bronchial hyper-responsiveness (BHR) compared to those who never wheezed. These findings suggest there is a distinct intermediate-onset phenotype not captured in the TCRS classification and reinforces the suggestion that transient early wheezers have ongoing diminished lung function without persisting symptoms of reactive airways. It is this latter group that may be at increased risk of chronic obstructive pulmonary disease (COPD) in later life owing to continued poor lung function[14 15]. A recent application of latent class growth analysis, which differs slightly from LLCA, in the Columbia Children's Centre for Environmental Health (CCEH) cohort of African-American and Latino children identified 4 classes that were similar to those in the TCRS study[16]. Spycher *et al* also used LLCA in 1650 UK children, identifying 3 phenotypes of wheeze and 2 phenotypes of cough[17].

We hypothesised that the statistically derived ALSPAC 6-class model could be validated using longitudinal lung function and atopic sensitisation data from the first 6 years of life.

METHODS

Participants were mother-child pairs from the Southampton Women's Survey; a cohort study with the objective, among others, of studying early life environmental influences on child growth and development, including respiratory disease and asthma in childhood[18]. During the period 1998-2002, 12,579 women aged 20-34 were recruited through their GP surgeries prior to conception. There were 1973 births before the end of 2003. Those born before 35 weeks and/or twins were excluded from the study and only one child per SWS mother was included. As previously described[19] a subset of 150 infants born to the SWS women had lung function measured at 5-14 weeks postnatally, with 95 of these having a complete set of data to 6 years. Child follow-ups were conducted at 6 months (n=1896), 12 months (n=1840), 2 years (n=1735), 3 years (n=1640) and 6 years (n=940) through home visits or attendance at clinic. The 6-year follow-up was performed during 2006-2010 and of the 1523 babies born between February 2000 and June 2003, 940 had questionnaire data from a home-visit and 791 had spirometry performed. Figure 1shows the numbers at each follow-up stage and the numbers who had lung function/skin prick testing. Parental consent was obtained and ethics approval was granted by the Southampton and South West Hampshire Local Research Ethics Committee (LREC Number 276/97, 307/97, 089/99, 125/98 and 06/Q1702/104).

Atopy

Atopy was defined as any allergen response ≥ 3 mm against cat, dog, house dust mite (*Dermatophagoides pteronyssinus*), grass pollens, egg and milk allergens (Hollister-Stier, Spokane, WA) at ages 1 (n=1494) and 3 years (n=1255). At 6 year follow-up (n=699), tree pollen was added (ALK Abelló Hørsholm, Denmark). Readings were considered valid only in the presence of appropriate positive and negative control responses.

Airway inflammation

Exhaled nitric oxide (FeNO) was measured with a NIOX® chemiluminescence analyser (Aerocrine, Sweden) at a controlled expiration of 50ml/sec, by trained research nurses. The technique used was in line with the ERS/ATS recommendations[20] and a mean value was calculated from three readings where possible. FeNO data were normalised using an inverse square root transformation then standardised as a z-score. The sign of the values was reversed so that high untransformed FeNO values gave rise to high standardised scores.

Allocation to wheeze phenotype groups

The ISAAC core questionnaire wheezing module was delivered by research nurses[21]. Mothers were asked at each visit whether their child had experienced 'episodes of chestiness associated with wheezing or whistling in his/her chest in the last 12 months'. Using wheeze data collected in the first year, at 2/3 years, and at 6 years old, the children were grouped into both the Tucson[1]; never, transient early, late, persistent groups, and ALSPAC based phenotypes[13]; never, early, transient, intermediate-onset, late-onset, and persistent wheeze. Our 'early' and 'transient' groups were so named in order to differentiate them from the Tucson 'transient early' group and be roughly equivalent to the ALSPAC 'transient early' and 'prolonged transient' groups[12]. Table 1 shows how the children were classified into the wheezing phenotypes using the questionnaire data from 6m, 12m, 2 years, 3 years and 6 years, and how our naming of groups corresponds to the ALSPAC phenotypes. Children who wheezed in the first year and at 6 years but not in between were classified as persistent wheezers, in line with the ALSPAC/PIAMA LLCA allocations [13], however we acknowledge that these could be children who are a combination of the early and late-onset groups.

Lung function

In Infancy: As previously described[19] infant lung function measurements were obtained during quiet sleep augmented by oral chloral hydrate (75-100mg/kg). V'maxFRC was measured using rapid thoracic compression (RTC) during tidal breathing using an inflatable jacket and a leak-free facemask with Fleisch pneumatachograph (Dynasciences, Blue Bell, CA). RTC from a raised volume manoeuvre was used to calculate $FEV_{0.4.}$ Data were collected in RASP software (Physiologic Ltd, Newbury, Berks, UK), with SQUEEZE software (Paul Dixon, London, UK) used to analyse the flow-volume curves and calculate $FEV_{0.4}$. V'maxFRC and $FEV_{0.4}$ were corrected for age at test but not for size[19].

At age 6 years: Spirometry was performed according to ATS/ERS guidelines[20] but without noseclips. Experienced research nurses used a portable Koko spirometer and incentive software (KoKo version 4; PDS Instrumentation; Louisville, USA) to record flow-volume loops. FEV_1, FEV_1/FVC and FEF_{25-75} were corrected for height and gender, and expressed as percent predicted[22].

Bronchial hyper-responsiveness (BHR) testing was performed according to ATS/ERS guidelines[20] using dosimeter administered methacholine (Koko; PDS Instrumentation; Louisville, USA) via an air-driven nebuliser (Sidestream®; Respironics, UK). Methacholine doses ranged from 0.06 mg/ml to 16 mg/ml with termination at either the upper concentration or a 20% fall in FEV_1. Data were transformed using the formula Log.slope=100/[regression slope of FEV_1 drop and $\log_{10}$(cumulative methacholine dose) + 10] in order to remove negative values and produce a normal distribution of the variable, with a lower value indicating greater responsiveness.

Statistical Analysis

Using STATA/SE 11.0, Poisson regression with robust variance was performed for atopic sensitisation outcomes[23] giving the relative risk of atopy for each wheeze group at 1,3 and 6 years of

age compared to those who never wheezed.. Linear regression was used for lung function outcomes, within which the following were assessed for potential confounding and included in the analysis if significantly correlated with the outcome and exposure; maternal asthma, smoking in pregnancy, smoke exposure in 1st year and 6th year of life, gestation, birth weight, birth weight z score (adjusted for gestation), sex, parity, maternal atopy, pets in the home, breastfeeding duration, social class, maternal education and month of birth. This is in line with recent published recommendations for confounders in studies of childhood asthma[24]. The prevalence of the potential confounders for each of the ALSPAC and TCRS groups can be found in supplementary table 5 and 6. Infant lung function, BHR and FeNO required log transformation to normalise and therefore they are presented as ratios of geometric means, which provides consistency with the presentation of the ALSPAC data[13].

RESULTS

Study population

A total of 1840 (92.6%) children were seen at 1 year, 1735 (87.3%) at 2 years, 1640 (82.5%) at 3 years and 940 (47.3%) at 6 year follow up (Figure 1). Children were more likely to have complete follow-up to 6 years if they were higher social class, had more highly educated parents, or were exposed to lower rates of smoking, as shown in supplementary table 1. There were no important differences in participants with spirometry in the 6-year follow-up versus those without and those with infant lung function versus those without (supplementary tables 2 and 3). The differences between those with follow-up and those without were included in our analysis as potential confounders (tables E4, E5 and E6). Table 2 shows the number of children in each phenotype who contributed spirometry, BHR, FeNO and skin test results data. There were small numbers with infant lung function data in the intermediate-onset (n=4), late-onset (n=1) and persistent groups (n=5). This was also true for BHR testing; intermediate n=17, late n=4 and persistent n=17.

Wheeze phenotypes in SWS

Using the Tucson and ALSPAC phenotypes, this SWS cohort had 417 TCRS early transient wheezers (161 ALSPAC early and 256 ALSPAC transient) and 116 TCRS persistent wheezers (57 ALSPAC intermediate-onset and 59 ALSPAC persistent) with 20 late-onset wheezers (same definition in ALSPAC and TCRS) (Figure 2).

Lung function in infancy and childhood

Lower infant lung function (V'maxFRC) was seen in ALSPAC and TCRS transient groups ($p<0.05$) and in the ALSPAC persistent ($p<0.05$) but not the TCRS persistent wheeze groups (Table 3). No association was seen between infant $FEV_{0.4}$ and any phenotype.

The ALSPAC transient group showed statistically lower lung function persisting to 6 years with a mean FEF_{25-75} of 95.1% predicted and FEV_1 of 100.3% predicted (both $p<0.05$) versus 101.0% and 103.1% respectively for the never wheezed group (table 3 and figures 3 and 4) . The ALSPAC persistent group showed significantly lower 6 year FEF_{25-75} (mean 84.9%, $p<0.001$), FEV_1 (97.3%, $p<0.05$) and FEV_1/FVC (95.5%, $p<0.05$) whilst the TCRS persistent group also showed significantly lower in FEF_{25-75} (89.5%, $P<0.001$), FEV_1 (99.0%) and FEV_1/FVC (97.1%, all $p<0.05$). The TCRS transient early group (including both ALSPAC early and transient groups) showed only reduced FEF_{25-75} at 6 years (96.7%, $p<0.05$). Intermediate-onset and late-onset groups had similar lung function in infancy and at 6 years to the non-wheeze group.

No associations were seen between BHR and any of the wheeze phenotypes. Infant and 6 year lung function parameters were converted to z scores to enable tracking over time (figures 3a and 3b). When compared to others in our cohort, persistent wheezers' z scores for our lung function parameters remain below all other groups. Although the transient wheeze group are below the other groups (except persistent) in infancy, there is some improvement by age 6 relative to other wheezing groups. They do, however remain below intermediate and late-onset groups despite the fact these two groups are now symptomatic and transient wheezers are not.

Atopy in infancy and childhood

None of the groups that had ceased wheezing by 6 years of age showed an increased risk of atopy at 1, 3 or 6 years of age (table 4, figure 3). Intermediate-onset wheezers showed an increased risk for atopy at all ages with relative risks of 3.3, 3.0 and 2.9 at 1, 3 and 6 years (all $p<0.001$), however late-onset and persistent wheezers did not show an increased risk of atopy until 3 years of age (late - RR 2.5 at 3 and 6 years, $p<0.05$, persistent - RR 2.4 and 2.0 at 3 and 6 years respectively, $p<0.05$). These RR are shown across the three ages in figure 3c. TCRS groups show a different pattern (table

4). TCRS persistent wheeze showed an increased risk for atopy at all ages (RR 2.6 at 1yr, $p<0.05$, 3.2 at 3yr and 2.6 at 6yrs, both $p<0.001$). It should be noted that the TCRS persistent group would include the ALSPAC intermediate-onset wheezers that showed atopic sensitisation at all ages.

Exhaled nitric oxide showed the same pattern of association as atopy at 6 years, being significantly associated with intermediate, late and persistent wheeze (all $p<0.001$) and significantly associated with atopy at 6 years in all children ($p<0.001$) (table 4).

DISCUSSION

The ALSPAC phenotypes [12] which were statistically derived based on symptoms, have successfully been validated using longitudinal lung function and atopic sensitisation data from the SWS cohort. This study, which included measurements not available within ALSPAC including infant lung function, FeNO at 6 years and 1 year atopy data, not only confirms that these phenotypes are real, but also provide insight into the underlying pathophysiology of each phenotype. In particular, among those who are transient early wheezers, there appear to be physiologic differences between those who wheeze only in the first year and those who wheeze beyond the first year but have stopped wheezing by age 6. . Atopy was manifested quite early (by age 1) in subjects with intermediate onset wheeze, and by age 3 in those with late onset and persistent disease. This lends further weight to the existence of separate late and intermediate-onset phenotypes and supports the hypothesis that persistent wheeze is an interplay between diminished lung function from birth and development of atopic sensitisation[4 25].

ALSPAC did not have FeNO data[12], and our data have been able to show that wheeze at 6 years is not only associated with skin sensitisation but also with FeNO which is an indirect estimate of lower airway eosinophilic inflammation. We have also shown that those who wheeze only in the first year of life appear distinct from other 'transient' wheezing groups, in that they show no lung function deficits in infancy or childhood and are perhaps similar to children in the never/infrequent wheeze group.

In keeping with previous studies[1 6] we found that, when compared to children who have never wheezed, children with transient wheeze outside the first year are more likely to have persistently lower lung function even when symptoms have resolved. In contrast to the ALSPAC/PIAMA [13] and TCRS studies [1], the intermediate onset and late onset groups did not show significantly diminished

lung function at 6 years compared to those who never wheeze. It may be that greater numbers in these previous studies gave the power to detect small differences.

It is widely accepted that transient early wheeze may be related to small airways [1], with improvement in wheeze as the child grows; however the pathogenesis is clearly more complex than this. By separating the TCRS 'transient early' wheeze into ALSPAC 'early' (wheeze only in the first year) and 'transient' wheeze, differing associations were seen. Early wheeze was not associated with diminished lung function or atopy, whilst the remainder of the transient wheezers showed diminished infant lung function (V'maxFRC) with persistence of these deficits up to 6 years (FEF_{25-75} and FEV_1), at which time they were, by definition, asymptomatic. This is in keeping with the theory that transient wheeze is a product of reduced airway calibre that improves with age, such that children are no longer symptomatic but still have minor deficits in lung function. Since persistent wheeze was not significantly associated with atopy at 1 year, this group may reflect a larger group of those with diminished lung function at birth that either cease to wheeze with growth (transient wheeze), have such diminished airway function that they fail to grow out of wheeze by 6 years (persistent non-atopic wheeze) or develop atopic sensitisation that leads to persistent wheezing at 6 years (classical asthma). It is possible that the 'early' wheezers have normal airways but an altered immune response to early viral infections that manifests as wheeze in the first year of life but improves as the immune system matures. Since there was no significant difference in month of birth between the early group and the never wheezers or transient wheezers (data not shown), this does not simply reflect a seasonal effect of increased respiratory tract infections. ALSPAC had classified children into a never/infrequent group [12] and our 'early' phenotype may correspond more closely to this group, differentiating them from those who wheeze as a result of poor lung function and possibly reflecting a difference between bronchiolitis and viral-induced wheeze. It is possible that the PIAMA LLCA analysis, which found a 5-class model to be optimal, had included these children

within the never/infrequent group [13] and that our infant lung function data allowed identification of this extra class. The existence of this early group suggests that many of those who wheeze only in the first year of life do not do so as a result of significantly altered lung function. This is in contrast to the theory suggested by Young *et* al who found that those who wheezed only in the first year of life had impaired lung function that resolved by 12m of age, whereas wheezing through the first 2 years or in year 2 only was associated with persisting lung function deficits [11]

Our findings differ from ALSPAC/PIAMA as they found that all wheeze groups had significantly lower FEV_1 at 8 years, whereas our intermediate and late groups did not show any significant difference from those who never wheezed. This is most likely a reflection of the greater power in their studies and may also be why we did not show lung function deficits in the intermediate/late groups prior to the onset of wheeze, as previously suggested by the Aberdeen cohort [7] and COPSAC [6]. Like the Perth cohort[26], we found significantly reduced lung function in persistent wheezers both shortly after birth and at 6 years, but, unlike them, we found transient wheezers had diminished lung function shortly after birth as well. We had the benefit of greater numbers in this wheeze group and, in line with the original TCRS infant lung function findings, our results likely reflect a significant difference in lung function at birth in transient wheezers. Our findings that wheeze at school age was related to atopic sensitisation reinforces the findings of the German Multicentre Allergy Study (MAS), a birth cohort of 1314 healthy children, who found that persistence of wheezing through school years was related to development of atopy[4].

Strengths of this study are its infant lung function data, exhaled nitric oxide measurements and longitudinal atopic sensitisation data. In particular, other cohorts have not included 1 year allergic sensitisation data. Together, these data allow tracking of lung function and onset of atopy in relation to different wheeze phenotypes. A particular limitation of our study is the small numbers of children

who had infant lung function and were wheezing at 6 years of age. This lack of power may explain the absence of association between wheeze phenotypes and $FEV_{0.4}$ in infancy (figure 3). Similarly, there was a surprising lack of significant association with bronchial hyper-responsiveness at 6 years in children who were wheezing at 6 years. Again, lack of power is likely to be an issue as there were few children with BHR data in the intermediate (n=17), late (n=4) and persistent groups (n=17). A planned meta-analysis of cohort data may overcome this problem. As with most cohort studies of this type, there was a tendency for children of higher social class to attend follow-up; however we controlled for associated confounders (maternal smoking in pregnancy and during childhood, education and social class) in our analysis. There were also differences between SWS and ALSPAC in wheezing time points used to classify the phenotypes with ALSPAC having data from 6m, 12m, 18m 30m, 42m, 54m, 69m and 81m. Rather than map our follow-up time points to these, we used the phenotype tracking figure that arose from their analysis and fitted our data to this graph[12]. Also, the 6 year lung function outcomes showed lung function that was statistically significantly lower in transient and persistent wheezers, however when expressed as percent predicted they are still within the expected normal range. This means that the differences are potentially important on a population level but may not be clinically significant in the individuals.

Many studies have attempted to identify risk factors for asthma/wheeze including fetal/postnatal growth parameters[27 28], genetics[29–33] and infant lung function[7 26 34], with varying results. It is only by understanding the wheeze phenotypes that these relationships can be better elucidated and the diluting effects of grouping wheeze together in a small number of categories can be overcome. Table 3 shows how some significant associations are lost by analysing the children according to the broader Tucson phenotypes including failure to delineate the intermediate-onset group, failure to show infant lung function deficits in the persistent group and lack of diminished FEV_1 at 6 years in the transient wheezers. There is now good evidence that COPD and all-cause mortality in later life is

related to airway function in childhood[14 15 35 36] and this may be related to wheeze phenotype[37]. Since infant lung function is impractical in most wheezing children, it is wheeze phenotype, particularly our transient wheeze group, that may provide information about later risk of asthma/COPD and risk of mortality from a wide range of causes.

In conclusion, using longitudinal lung function and atopic sensitisation data, the SWS cohort has not only validated the statistically derived ALSPAC phenotypes of childhood wheeze, but has also provided insights to the underlying pathophysiology. Our findings confirm the existence of an 'intermediate-onset' phenotype that is similar to late-onset wheeze but has earlier atopic sensitisation. The SWS cohort demonstrates the utility of separating the 'transient early' wheeze group, by considering those who wheeze in their first year only as a separate group.. We suggest that future research should focus on a 6-class model of early childhood wheeze. Further work is needed to investigate whether the group of children who only wheeze in the first year of life reflect a separate phenotype or can be considered alongside those who never wheeze and thus a 5-class model may be appropriate class model of early childhood wheeze.

REFERENCES

1 Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995;**332**:133–8.

2 Sandin A, Björkstén B, Bråbäck L. Development of atopy and wheezing symptoms in relation to heredity and early pet keeping in a Swedish birth cohort. *Pediatr Allergy Immunol* 2004;**15**:316–22.

3 Kurukulaaratchy RJ, Fenn M, Twiselton R, Matthews S, Arshad SH. The prevalence of asthma and wheezing illnesses amongst 10-year-old schoolchildren. *Respir Med* 2002;**96**:163–9.

4 Illi S, Von Mutius E, Lau S, Niggemann B, Grüber C, Wahn U. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* 2006;**368**:763–70.

5 Brussee JE, Smit H a, Van Strien RT, Corver K, Kerkhof M, Wijga AH, Aalberse RC, Postma D, Gerritsen J, Grobbee DE, *et al.* Allergen exposure in infancy and the development of sensitization, wheeze, and asthma at 4 years. *J Allergy Clin Immunol* 2005;**115**:946–52.

6 Bisgaard H, Jensen SM, Bønnelykke K. Interaction between asthma and lung function growth in early life. *Am J Respir Crit Care Med* 2012;**185**:1183–9.

7 Turner SW, Young S, Goldblatt J, Landau LI, Le Souëf PN. Childhood asthma and increased airway responsiveness: a relationship that begins in infancy. *Am J Respir Crit Care Med* 2009;**179**:98–104.

8 Wilson NM, Lamprill JR, Mak JCW, Clarke JR, Bush A, Silverman M. Symptoms, lung function, and beta2-adrenoceptor polymorphisms in a birth cohort followed for 10 years. *Pediatr Pulmonol* 2004;**38**:75–81.

9 Lowe LA, Simpson A, Woodcock A, Morris J, Murray CS, Custovic A. Wheeze phenotypes and lung function in preschool children. *Am J Respir Crit Care Med* 2005;**171**:231–7.

10 Brussee JE, Smit HA, Koopman LP, Wijga AH, Kerkhof M, Corver K, Vos APH, Gerritsen J, Grobbee DE, Brunekreef B, *et al.* Interrupter Resistance and Wheezing Phenotypes at 4 Years of Age. *Am J Respir Crit Care Med* 2004;**169**:209–13.

11 Young S, Arnott J, O'Keeffe PT, Le Souef PN, Landau LI. The association between early life lung function and wheezing during the first 2 yrs of life. *Eur Respir J* 2000;**15**:151–7.

12 Henderson J, Granell R, Heron J, Sherriff A, Simpson A, Woodcock A, Strachan DP, Shaheen SO, Sterne JAC. Associations of wheezing phenotypes in the first 6 years of life with atopy, lung function and airway responsiveness in mid-childhood. *Thorax* 2008;**63**:974–80.

13 Savenije OE, Granell R, Caudri D, Koppelman GH, Smit H a, Wijga A, De Jongste JC, Brunekreef B, Sterne J a, Postma DS, *et al.* Comparison of childhood wheezing phenotypes in 2 birth cohorts: ALSPAC and PIAMA. *J Allergy Clin Immunol* 2011;**127**:1505–12.e14.

14 Postma DS, De Vries K, Koëter GH, Sluiter HJ. Independent influence of reversibility of air-flow obstruction and nonspecific hyperreactivity on the long-term course of lung function in chronic air-flow obstruction. *Am Rev Respir Dis* 1986;**134**:276–80.

15 Martinez FD. The origins of asthma and chronic obstructive pulmonary disease in early life. *Proc Am Thorac Soc* 2009;**6**:272–7.

16 Chen Q, Just AC, Miller RL, Perzanowski MS, Goldstein IF, Perera FP, Whyatt RM. Using latent class growth analysis to identify childhood wheeze phenotypes in an urban birth cohort. *Ann Allergy Asthma Immunol* 2012;**108**:311–315.e1.

17 Spycher BD, Silverman M, Brooke a M, Minder CE, Kuehni CE. Distinguishing phenotypes of childhood wheeze and cough using latent class analysis. *Eur Respir J* 2008;**31**:974–81.

18 Inskip HM, Godfrey KM, Robinson SM, Law CM, Barker DJP, Cooper C. Cohort profile: The Southampton Women's Survey. *Int J Epidemiol* 2006;**35**:42–8.

19 Lucas JS, Inskip HM, Godfrey KM, Foreman CT, Warner JO, Gregson RK, Clough JB. Small size at birth and greater postnatal weight gain: relationships to diminished infant lung function. *Am J Respir Crit Care Med* 2004;**170**:534–40.

20 Beydon N, Davis SD, Lombardi E, Allen JL, Arets HGM, Aurora P, Bisgaard H, Davis GM, Ducharme FM, Eigen H, *et al.* An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. *Am J Respir Crit Care Med* 2007;**175**:1304–45.

21 Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;**8**:483–91.

22 Stanojevic S, Wade A, Stocks J, Hankinson J, Coates AL, Pan H, Rosenthal M, Corey M, Lebecque P, Cole TJ. Reference ranges for spirometry across all ages: a new approach. *Am J Respir Crit Care Med* 2008;**177**:253–60.

23 Zou G. A Modified Poisson Regression Approach to Prospective Studies with Binary Data. *Am J Epidemiol* 2004;**159**:702–6.

24 Nurmatov U, Nwaru BI, Devereux G, Sheikh A. Confounding and effect modification in studies of diet and childhood asthma and allergies. *Allergy* 2012;**67**:1041–59.

25 Nelson HS, Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST. Airway remodeling in asthma: New insights. *Journal of Allergy and Clinical Immunology* 2003;**111**:215–25.

26 Turner SW, Palmer LJ, Rye PJ, Gibson N a, Judge PK, Cox M, Young S, Goldblatt J, Landau LI, Le Souëf PN. The relationship between infant airway function, childhood airway responsiveness, and asthma. *Am J Respir Crit Care Med* 2004;**169**:921–7.

27 Pike KC, Crozier SR, Lucas JS a, Inskip HM, Robinson S, Roberts G, Godfrey KM. Patterns of fetal and infant growth are related to atopy and wheezing disorders at age 3 years. *Thorax* 2010;**65**:1099–106.

28 Sonnenschein-van der Voort AMM, Jaddoe VW V, Raat H, Moll H a, Hofman A, De Jongste JC, Duijts L. Fetal and Infant Growth and Asthma Symptoms in Preschool Children. The Generation R Study. *Am J Respir Crit Care Med* 2012;**185**:731–7.

29 Simpson A, Maniatis N, Jury F, Cakebread J a, Lowe L a, Holgate ST, Woodcock A, Ollier WER, Collins A, Custovic A, *et al.* Polymorphisms in a disintegrin and metalloprotease 33 (ADAM33) predict impaired early-life lung function. *Am J Respir Crit Care Med* 2005;**172**:55–60.

30 Koppelman GH, Meyers DA, Howard TD, Zheng SL, Hawkins GA, Ampleford EJ, Xu J, Koning H, Bruinenberg M, Nolte IM, *et al.* Identification of PCDH1 as a novel susceptibility gene for bronchial hyperresponsiveness. *Am J Respir Crit Care Med* 2009;**180**:929–35.

31 Sadeghnejad A, Karmaus W, Arshad SH, Kurukulaaratchy R, Huebner M, Ewart S. IL13 gene polymorphisms modify the effect of exposure to tobacco smoke on persistent wheeze and asthma in childhood, a longitudinal study. *Respir Res* 2008;**9**:2.

32 Melén E, Umerkajeff S, Nyberg F, Zucchelli M, Lindstedt A, Gullstén H, Wickman M, Pershagen G, Kere J. Interaction between variants in the interleukin-4 receptor alpha and interleukin-9 receptor genes in childhood wheezing: evidence from a birth cohort study. *Clin Exp Allergy* 2006;**36**:1391–8.

33 Holloway JW, Arshad SH, Holgate ST. Using genetics to predict the natural history of asthma? *J Allergy Clin Immunol* 2010;**126**:200–9.

34 Pike KC, Rose-Zerilli MJ, Osvald EC, Inskip HM, Godfrey KM, Crozier SR, Roberts G, Clough JB, Holloway JW, Lucas JS, *et al.* The relationship between infant lung function and the risk of wheeze in the preschool years. *Pediatr Pulmonol* 2011;**46**:75–82.

35 Postma DS, Kerkhof M, Boezen HM, Koppelman GH. Asthma and chronic obstructive pulmonary disease: common genes, common environments? *Am J Respir Crit Care Med* 2011;**183**:1588–94.

36 Stern DA, Morgan WJ, Wright AL, Guerra S, Martinez FDC-P, Fernando D. Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. *Lancet* 2007;**370**:758–64.

37 Sherrill DL, Guerra S, Wright AL, Morgan WJ, Martinez FD. Relation of early childhood growth and wheezing phenotypes to adult lung function. *Pediatr Pulmonol* 2011;**46**:956–63.

Figure Legends

Figure 1. Diagram showing numbers in follow-up groups at each age and number who had skin prick testing and lung function measurements.

Figure 2. Venn diagram showing distribution of children amongst Tucson groups (black circle) and ALSPAC groups (grey circles)

Figure 3. Graph showing tracking of internal z scores for each ALSPAC phenotype for a) maximal flow at functional residual capacity (V'maxFRC) in infancy and forced expiratory flow at 25-75% vital capacity (FEF_{25-75}) at 6 years of age, b) forced expiratory volume in 0.4 seconds ($FEV_{0.4}$) in infancy and forced expiratory volume in 1 second (FEV_1) at 6 years of age and c) tracking of relative risk (RR) of atopy at 1, 3 and 6 years.