Associations of wheezing phenotypes in the first six years of life with atopy, lung function and airway responsiveness in mid childhood.

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ABSTRACT

Background
Patterns of wheezing during early childhood may indicate differences in etiology and prognosis of respiratory illnesses. Improved characterization of wheezing phenotypes could lead to the identification of environmental influences on the development of asthma and airway diseases in predisposed individuals.

Methods
Data on 6,265 children from a longitudinal birth cohort (the ALSPAC study) with data collected on wheezing at seven time points from birth to seven years were analysed. Latent class analysis was used to assign phenotypes based on patterns of wheezing. Measures of atopy, airway function (FEV1, FEF25-75) and bronchial responsiveness were made at 7-9 years of age.

Results
Six phenotypes were identified. The strongest associations with atopy and airway responsiveness were found for intermediate onset (18 months) wheezing (OR for atopy 8.36, 95% CI 5.2-13.4; mean difference dose response to methacholine 1.76, 95% CI 1.41-2.12 %FEV1 per μmol, compared with infrequent/never wheeze phenotype). Late onset wheezing (after 42 months) was also associated with atopy (OR 6.6, 95% CI 4.7-9.4) and airway responsiveness (mean difference 1.61, 95% CI 1.37-1.85 %FEV1 per μmol). Transient and prolonged early wheeze were not associated with atopy but were weakly associated with increased airway responsiveness and persistent wheeze had intermediate associations with these outcomes.

Conclusions
The wheezing phenotypes most strongly associated with atopy and airway responsiveness were characterised by onset after age 18 months. This has potential implications for the timing of environmental influences on the initiation of atopic wheezing in early childhood.

Key words: Respiratory sounds (wheeze); Asthma; Hypersensitivity; Respiratory function tests; Latent Class Analysis; Phenotype
INTRODUCTION

Asthma is a complex, heterogeneous disease comprising a number of discrete phenotypes, such that the term “asthma” has recently been called into question. Cohort studies followed to adulthood have reported that more severe childhood wheezing phenotypes are less likely to remit in later life. Pulmonary function abnormalities associated with persistent wheezing become established during early childhood and track to adult life, suggesting that early life exposures are critical in determining the onset and natural history of wheezing illnesses. Therefore, an improved understanding of these phenotypes is of fundamental importance to studies of risk factors for asthma and wheezing illnesses in children.

In a seminal report based on the Tucson Children’s Respiratory Study, Martinez and colleagues proposed three patterns of wheezing during the first six years of life leading to the concepts of transient early wheezing in the first three years, non-atopic wheezing in the preschool years and IgE-mediated wheeze or asthma. Although these have served as useful models of wheezing phenotypes in early childhood, there is evidence that phenotypes diverge earlier than three years and a recent report described variations in immune responses within seemingly homogeneous phenotypes of IgE-mediated asthma. We used a novel, symptom-driven approach to define wheezing phenotypes using repeat measurements of wheeze during the first 7 years of childhood in a large, population-based birth cohort study, the Avon Longitudinal Study of Parents and Children (ALSPAC). We investigated associations of these phenotypes with physician-diagnosed asthma and objectively measured atopy and airway function at age 7-9 years.
METHODS

Participants
The Avon Longitudinal Study of Parents and Children (ALSPAC) is a longitudinal, population-based birth cohort study that recruited 14,541 pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992. There were 14,062 liveborn children. The study protocol has been described previously10;11 and further details are on the ALSPAC website: http://www.alspac.bris.ac.uk. Ethical approval for all aspects of data collection was obtained from the ALSPAC Law and Ethics Committee (IRB 00003312).

Data Collection
At 6, 18, 30, 42, 54, 69 and 81 months after birth, study mothers were sent a self-completion questionnaire about the health of their child. They were asked to report the occurrence of 15 common symptoms, including wheezing, in the previous 12 months (6 months for the initial questionnaire) and, if present, whether they consulted a doctor. In a separate section, they were asked whether in the past 12 months (6 months in the first questionnaire) their child had, “Wheezing with whistling on the chest when (s)he breathed?” At 91 months of age mothers were asked in a separate questionnaire to report if a physician had ever told them that their child had asthma.

Children’s atopic status was determined at age 7-8 years by skin prick test responses to a panel of up to 12 common allergens including house dust mite (D. pteronyssinus), mixed grasses and cat (ALK Abelló, Hoersholm, Denmark). Sensitisation to one of these three allergens has been shown to identify 95% of all sensitised children in this population12. A positive response was defined as a mean weal diameter of >2mm with an absent response to negative control solution and atopy was defined as a positive response to one or more of house dust mite, cat or grass pollen. Mothers were asked to report a personal history of asthma or allergy in a questionnaire administered during pregnancy.

Between 8-9 years of age, lung function was measured by spirometry (Vitalograph 2120, Maids Moreton, England) according to American Thoracic Society criteria13. Flow-volume curves were reviewed by one respiratory physician (JH) to ensure adherence to standards, resulting in the rejection of 338 (4.6%) measurements and the correction of 883 (11.5%) where the automated programme had selected an inappropriate curve. Each variable (FEV1, FVC & FEF25-75) was converted to gender, age and height-adjusted standard deviation units14. Airway responsiveness to methacholine was measured using the method of Yan et al.15 and expressed for each subject as the dose-response slope of FEV1 (%decline from baseline) per micromole methacholine.

Statistical Methods
Wheeze was defined as present if the response to either question about wheezing was, “Yes,” and absent if the response to both was, “No.” All other combinations were classed as missing (1.3%). As there were two levels of response to questions about wheeze at seven time points, there were \(2^7 = 128\) different patterns of wheezing possible. Therefore, to derive phenotypes with similar wheezing patterns over time, we used latent class analysis. This is a statistical method for finding subtypes of related cases (latent classes) from multivariable categorical data (in this case responses to wheezing questions across seven time points). Briefly, individuals were clustered into a number of discrete latent classes (phenotypes) on the basis of the pattern of responses to each of the wheezing questions. The latent class model aims to determine the minimum number of latent classes that describe the observed patterns of responses in the data\(^{16}\). For a full description of the latent class analysis used in this study and methods used to evaluate the best fitting model, please refer to the online supplement. The posterior probability of each individual belonging to a particular phenotype was estimated and, from these data, the estimated prevalence of wheeze at each time point was calculated for each phenotype.

Children with complete reports of wheezing at all seven time points were included in the analyses. Logistic and linear regression was used to estimate associations of phenotype membership with physician diagnosed asthma and objective measurements of atopy, lung function and bronchial responsiveness in mid childhood and with maternal self-reported asthma and allergy. As latent class analysis is robust to missing data and misclassification of data items, such as faulty recall of wheezing episodes, we repeated all analyses in children who returned questionnaires at two or more time points.

All analyses were done using MPlus 4.1 software\(^{17}\).
RESULTS

Of 11,678 children with reports of wheezing on at least two occasions, 6,265 (54%) had complete data. The characteristics of the study population are shown in Table 1. Children with complete data were less likely to come from socially deprived backgrounds and had lower prevalence of reported wheezing in early childhood than children with missing data.

Table 1. Characteristics of the study population with complete data on wheezing from birth to 81 months (n=6265) compared with those with missing data.

| Demographic data                          | Children with complete data on wheezing (N=6265) | Children with 2-6 observations (N=5413) | Children with 0-1 observations (N=2384) |
|------------------------------------------|-----------------------------------------------|----------------------------------------|----------------------------------------|
|                                          | N/total | % | N/total | % | N/total | % |
| Females                                  | 3029/6265 | 48% | 2623/5413 | 48% | 1138/2382 | 48% |
| Rented house                             | 973/6143 | 16% | 1583/5116 | 31% | 943/1483 | 51% |
| Mother not married                       | 1041/6202 | 17% | 1427/5154 | 28% | 758/1864 | 41% |
| Overcrowding                             | 218/6088 | 4% | 404/5019 | 8% | 260/1771 | 15% |
| One or more siblings                     | 3289/6125 | 54% | 2862/5069 | 56% | 1043/1813 | 58% |
| Low maternal education*                  | 3496/6183 | 57% | 3531/4975 | 71% | 1038/1309 | 79% |
| Teenage mother                           | 89/6265 | 1% | 303/5413 | 6% | 263/2384 | 11% |
| Mother manual occupation                 | 793/5380 | 15% | 933/3851 | 24% | 284/868 | 33% |
| Partner manual occupation                | 2158/5728 | 38% | 2112/4285 | 49% | 583/985 | 59% |

Prevalence of wheeze

| 6 months | 1506/6265 | 24% | 1338/4644 | 29% | 165/507 | 33% |
Comparison of Bayesian Information Criteria (BIC) (see online supplement) suggested that a model with six phenotypes provided the best fit (BIC from models with 3, 4, 5, 6 and 7 phenotypes were 34709, 34357, 34304, 34275 and 34285 respectively). Bootstrap likelihood ratio tests (BLRT) suggested a further improvement in fit comparing models with seven and six phenotypes in cases with complete data only; analyses of data from children with at least two measures of wheezing from 6-81 months suggested, based on both BIC and BLRT, that a six-phenotype model provided the best fit. We therefore selected the more parsimonious solution and based further analyses on six-phenotype models for both datasets. The estimated prevalence of wheezing at each time point in the six phenotypes is displayed in Figure 1. Hereafter, we describe the phenotypes as follows: 1. Never/infrequent wheeze (59.3% of children) had approximately 10% prevalence of wheezing at 6 months, with declining prevalence of sporadic wheeze thereafter and included subjects (76.5% of this category) who never reported wheeze; 2. Transient early wheeze (16.3%) had 50-60% prevalence up to 18 months, declining to low prevalence from 42 months; 3. Prolonged early wheeze (8.9%) had a peak prevalence of around 65%, at 30 months, declining to low prevalence from 69 months; 4. Intermediate onset wheeze (2.7%) had a low prevalence up to 18 months, rising rapidly to high prevalence from age 42 months; 5. Late onset wheeze (6.0%) had approximately 20% prevalence up to 42 months, rising to 50% or higher prevalence thereafter; 6. Persistent wheeze (6.9%) had 65% prevalence at 6 months with approximately 90% prevalence thereafter. Patterns of wheezing were similar in 11,678 children with missing data (see online supplement Figure E1).

**Association of wheezing phenotypes with atopy and parental asthma/allergy**

Table 2 shows associations of wheezing phenotypes with skin test responses at age 7-8 years. Intermediate onset wheeze, late onset wheeze and persistent wheeze were strongly associated with atopy. Neither of the early wheezing phenotypes was associated with atopy or specific allergen sensitisation. Intermediate onset wheezing showed the strongest associations with atopy and with sensitisation to cat and house dust mite (*D.*
pteronyssinus) allergens. Late onset wheezing was also strongly associated with cat and house dust sensitisation and had the strongest association with grass pollen sensitisation.

Table 2. Associations of wheezing phenotype with asthma and atopy in 5,397 children with complete data on wheezing and asthma at 7½ years and 4,331 children with skin prick test data at 7-8 years

| Phenotype        | Physician diagnosed asthma | Atopy (any skin prick sensitivity) | Skin prick sensitivity to D.pteronyssinus* | Skin prick sensitivity to cat* | Skin prick sensitivity to grass* |
|------------------|----------------------------|-----------------------------------|------------------------------------------|-------------------------------|---------------------------------|
|                  | N/total (%)                | OR (95% CI)                       | N/total (%)                              | OR (95% CI)                   | N/total (%)                     | OR (95% CI)                     |
| Transient early  | 79/931 (8.5%)              | 2.46 (1.48, 4.09)                 | 95 / 700 (13.6%)                         | 0.8 (0.55,1.17)               | 52 / 707 (7.4%)                 | 0.84 (0.51,1.38)                |
|                  |                            |                                   |                                         |                               | 22 / 702 (3.1%)                | 0.86 (0.42,1.77)                |
|                  |                            |                                   |                                         |                               | 52 / 706 (7.4%)                | 0.8                             |
| Prolonged early  | 183/509 (36.0%)            | 14.87 (10.68,20.71)               | 57 / 383 (14.9%)                         | 0.89 (0.58,1.38)              | 32 / 387 (8.3%)                 | 0.99 (0.56,1.72)                |
|                  |                            |                                   |                                         |                               | 11 / 384 (2.9%)                | 0.77 (0.27,2.2)                 |
|                  |                            |                                   |                                         |                               | 25 / 386 (6.5%)                | 0.69 (0.36,1.35)                |
| Intermediate     | 141/152 (92.8%)            | 325.75 (137.78, 770.14)           | 71 / 114 (62.3%)                         | 8.36 (5.24,13.36)             | 59 / 116 (50.9%)                | 11.12 (7.06,17.53)              |
|                  |                            |                                   |                                         |                               | 42 / 115 (36.5%)               | 15.51 (9.45,25.46)              |
|                  |                            |                                   |                                         |                               | 37 / 115 (32.2%)               | 4.83 (2.98,7.82)                |
| Late             | 260/341 (76.2%)            | 84.6 (56, 127.8)                  | 145 / 257 (56.4%)                        | 6.62 (4.67,9.39)              | 93 / 259 (35.9%)                | 5.97 (4.13,8.63)                |
|                  |                            |                                   |                                         |                               | 69 / 257 (26.8%)               | 9.73 (6.32,14.98)               |
|                  |                            |                                   |                                         |                               | 101 / 259 (39.0%)              | 6.54 (4.57,9.35)                |
| Persistent       | 362/393 (92.1%)            | 307.93 (185.86, 510.18)           | 123 / 296 (41.6%)                        | 3.64 (2.76,4.81)              | 91 / 299 (30.4%)                | 4.66 (3.42,6.36)                |
|                  |                            |                                   |                                         |                               | 66 / 296 (22.3%)               | 7.54 (5.17,11)                  |
|                  |                            |                                   |                                         |                               | 73 / 298 (24.5%)               | 3.27 (2.37,4.53)                |
| Never/infrequent | 126/3397 (3.7%)            | 1 (reference)                     | 419 / 2554 (16.4%)                       | 1 (reference)                 | 219 /2580 (8.5%)                | 1 (reference)                  |
|                  |                            |                                   |                                         |                               | 92 / 2560 (3.6%)               | 1 (reference)                  |
|                  |                            |                                   |                                         |                               | 232 / 2575 (9.0%)              | 1 (reference)                  |
*Mean weal diameter $\geq 2$mm
Maternal self-reported asthma and allergy were positively associated with all wheezing phenotypes compared with infrequent wheeze (Table 3). The strongest association with both maternal phenotypes was seen with persistent wheeze.

Table 3. Associations of wheezing phenotypes with maternal self reported asthma and allergy in 6133 children with complete data on wheezing and maternal history of asthma/allergy

| Wheezing phenotype | Maternal asthma Odds ratio (95% CI) | Maternal allergy Odds ratio (95% CI) |
|--------------------|------------------------------------|------------------------------------|
| Transient early    | 2.26 (1.62,3.15)                   | 1.37 (1.11,1.71)                   |
| Prolonged early    | 2.68 (1.89,3.78)                   | 1.79 (1.4,2.29)                    |
| Intermediate       | 3.54 (2.21,5.67)                   | 1.53 (1.05,2.22)                   |
| Late               | 2.37 (1.58,3.57)                   | 1.41 (1.06,1.88)                   |
| Persistent         | 4.17 (3.12,5.56)                   | 2.09 (1.67,2.62)                   |
| Never/infrequent   | 1 (reference)                     | 1 (reference)                     |

Association of wheezing phenotypes with asthma and lung function

All wheezing phenotypes were associated with physician-diagnosed asthma by age 91 months, compared with the never/infrequent wheeze phenotype. The proportion of subjects with physician-diagnosed asthma and odds ratios (95% CI) were as follows: transient early wheeze, 8.5%; OR 2.5 (1.5-4.1), prolonged early wheeze, 36%; OR 14.9 (10.7-20.7), intermediate onset wheeze, 92.8%; OR 326 (138-770), late onset wheeze, 76.2%; OR 85 (56-128), and persistent wheeze, 92.1%; OR 308 (186-510).

Compared with the late onset phenotype, the intermediate onset phenotype was associated with a higher prevalence of doctor-diagnosed asthma (OR 3.9, 95% CI 1.1 to 13.2). Similarly, compared with the transient early phenotype, the prolonged early phenotype had an odds ratio of asthma at 91 months of 6.0 (95% CI 3.5 to 10.3) reflecting the marked difference in prevalence of doctor-diagnosed asthma (9% and 36% respectively) in the two groups.

Table 4 shows associations of wheezing phenotypes with lung function and airway responsiveness at 8-9 years of age. All phenotypes were associated with decrements of FEV1 and FEF25-75 and increased airway responsiveness compared with never/infrequent wheeze. The greatest decrements were associated with prolonged early, intermediate onset and persistent wheezing. Airway responsiveness was highest in the intermediate and late onset phenotypes.

Compared with late onset wheezing, the intermediate onset phenotype was associated with decrements of FEV1 and FEF25-75 of 0.33 (95% CI 0.10 to 0.55) and 0.28 (95% CI 0.05 to 0.52) standard deviations respectively. There was also a decrement in mid-expiratory flow (mean difference for FEF25-75 -0.22 SD units (95% CI -0.34 to -0.11) in the prolonged early compared with the transient early wheezing group.
Table 4. Associations of wheezing phenotype with lung function in 4,448 children with complete data on wheezing and lung function measurements at 8-9 years and 2,957 with airway responsiveness measurements at 8-9 years

| Phenotype          | FEV₁ (L) | FEF₂₅-₇₅ (L/s) | Airway responsiveness* |
|--------------------|----------|-----------------|------------------------|
|                    | Total    | Mean (sd)       | Mean difference (95% CI)| Total | Mean (sd)       | Mean difference (95% CI) | Total | Mean (sd)       | Mean difference (95% CI) |
|                    |          | Mean difference |                        |        | Mean difference |                        |        | Mean difference |                        |
| Transient early    | 724      | -0.16 (0.91)    | -0.29 (-0.37, -0.21)    | 735    | -0.12 (0.96)    | -0.31 (-0.39, -0.24)    | 481    | 0.03 (1.5)     | 0.29 (0.13, 0.44)         |
| Prolonged early    | 396      | -0.17 (1.11)    | -0.3 (-0.41, -0.2)      | 402    | -0.34 (0.95)    | -0.54 (-0.64, -0.44)    | 263    | 0.01 (1.5)     | 0.27 (0.07, 0.47)         |
| Intermediate       | 118      | -0.4 (1.18)     | -0.53 (-0.71, -0.35)    | 120    | -0.49 (1.17)    | -0.69 (-0.87, -0.51)    | 79     | 1.51 (1.7)     | 1.76 (1.41, 2.12)         |
| Late               | 265      | -0.08 (0.98)    | -0.21 (-0.33, -0.08)    | 269    | -0.21 (1.07)    | -0.4 (-0.53, -0.28)     | 176    | 1.36 (1.7)     | 1.61 (1.37, 1.85)         |
| Persistent         | 306      | -0.27 (1.05)    | -0.4 (-0.52, -0.28)     | 310    | -0.49 (1.12)    | -0.68 (-0.8, -0.57)     | 203    | 0.94 (1.8)     | 1.19 (0.96, 1.42)         |
| Never/infrequent   | 2639     | 0.13 (0.98)     | 0 (reference)            | 2679   | 0.2 (0.97)      | 0 (reference)            | 1755   | -0.26 (1.6)    | 0 (reference)             |

Mean of least squares dose-response slope (% decline in FEV₁ per μmol methacoline)
Never wheeze versus infrequent wheeze
As 2979/3896 (76.5%) of the subjects assigned to the never/infrequent wheeze phenotype had never reported wheeze, the associations with objective outcomes of these children were compared with the 917 subjects assigned to this group that reported at least one episode of wheeze. The never wheeze group had higher FEV$_1$ (mean difference (95% CI) 0.14 SD units (0.05-0.22)) and FEF$_{25-75}$ (mean difference 0.18 SD units (0.10-0.27)) and lower airway responsiveness (-0.30 %FEV$_1$ per μmol methacholine (-0.13, -0.47)) than those with at least one reported episode of wheeze. There were no differences in the prevalence of atopy or individual skin prick test responses between these two groups.

Associations of wheezing phenotypes with other outcomes in children with missing data
The associations of wheezing phenotypes with maternal asthma and with later childhood outcomes in 11,678 children who returned at least two questionnaires on wheezing are shown in the online supplement (Tables E3-E5). These gave very similar results to the analyses based on children with complete data on wheezing between 6 and 81 months of age.
DISCUSSION

Using data on reported wheezing collected at frequent intervals during the first 7 years in a large, population-based birth cohort, we have identified six childhood wheezing phenotypes and quantified their associations with objective measures of atopy and lung function in mid-childhood. Two of these phenotypes have not been described previously. Prolonged early wheeze (around 9% of children) was characterised by wheezing from age 6 to 54 months with low prevalence from age 69 months onwards. It was not associated with aeroallergen sensitisation but was associated with increased airway responsiveness and lower lung function at ages 8-9 years, compared with the never/infrequent wheeze phenotype. Intermediate onset wheeze (around 2.5% of children) had onset between ages 18 and 42 months. This phenotype was characterised by the strongest association with atopy (particularly skin prick sensitivity to *D. pteronyssinus* and cat allergen), lower lung function and higher levels of airway responsiveness compared with the never/infrequent wheeze phenotype. Such associations (represented figuratively in Table 5) may reveal differing aetiological or environmental influences on the inception of asthma in young children.

Although the phenotypes identified here have similarities to previously reported patterns of early childhood wheezing, there were differences in their associations with objective outcomes. In the Tucson study, children with persistent and late onset wheeze had the strongest associations with atopy and those with persistent and transient early wheeze had the greatest decrements of lung function at age 6th and 11 years, with only persistent wheeze being positively associated with increased airway responsiveness at 11 years. Our results challenge these paradigms in that intermediate onset wheezing was most strongly associated with atopy and airway responsiveness in our study, although it should be noted that this pattern would have been included in the persistent wheeze phenotype as defined by the Tucson group. Persistent wheeze in the present study was less strongly associated with atopy than intermediate or late onset wheeze but was associated with similar lung function deficits to intermediate onset wheeze, suggesting that persistent wheeze may represent a mixture of structural airway abnormalities associated with early onset wheezing and atopic wheeze that develops during early childhood.

In order to interpret the relevance of these findings to the heterogeneity of early childhood wheezing, it is necessary to appreciate the advantages and limitations of the latent class method used to identify the phenotypes in this study. As the term “latent classes” implies, these are not directly observed phenomena but were constructed post hoc on the basis of the pattern of responses to wheezing over a fixed number of observation periods. Therefore, these methods are not applicable to predicting natural history of wheezing in individual subjects. Each child was assigned a probability of membership of each class, based on their overall wheezing history. As shown in Table E1, some children had a high probability of membership of a single class, while the assignment of others was less certain. This becomes increasingly evident in analyses including children with some missing observations of wheeze. Children with clear patterns of reported wheezing, such as those who always or never wheezed, had the highest probability of belonging to a single phenotype and therefore contributed the greatest weight to analyses of associations of these phenotypes with other outcomes. The advantage of the latent class approach is that the phenotypes were not constrained by pre-specified notions of their number or nature: these were determined by the
patterns observed in the data. Clear and interpretable associations of the different phenotypes with physician diagnosed asthma and with objective measures of atopy and lung function at ages 7 to 9 years confirmed the utility of the approach. However, we acknowledge that the question of whether these phenotypes represent discrete pathophysiological entities cannot be resolved by the present analysis. For instance, it is conceivable that prolonged and transient early wheezing represent different severities of the same broad phenotype, with the more severe phenotype being associated with longer duration of wheeze and poorer prognosis. Future analyses of associations of these derived phenotypes with early life exposures that may contribute to their aetiology will help to address some of these issues.

An advantage of our study compared with previous cohort studies of the natural history of wheezing is its larger size, which allows investigation of associations of wheezing phenotypes with different measures of atopy and lung function, despite the fact that some phenotypes represented relatively small proportions of children. For comparison, the Tuscon Study reported on 826 children during the first 6 years\(^6\), the Dunedin study on 613 subjects with complete respiratory data from 9-26 years\(^3\), the Perth study of infant lung function reported outcomes at 11 years in 183 infants\(^19\), and a cohort of 2860 infants in Perth reported on asthma to age 6 years\(^20\).

There were also a number of limitations of our data. In common with most cohort studies\(^21\), loss to follow up was greater in children from more socially deprived backgrounds. Given known associations of social deprivation with early childhood wheezing\(^22\), it is likely that children excluded because of missing data had a higher proportion of transient early wheezing than those included. We addressed this problem more comprehensively than previous cohort studies, because we found similar results when we repeated latent class analyses using 11,678 children with two or more observations of wheeze.

The wheeze questionnaire that was devised for the ALSPAC study in 1991 contains similar questions to the ISAAC questionnaire now in common use\(^23\). Care was taken to resolve discrepancies in responses to different questions about wheezing in the present study. However it has been reported that parental reported wheezing in early life is imprecise\(^24\) and correlates poorly with objective observations\(^25\) or with wheeze assessed by health professionals\(^26\). Reassuringly, we found extremely strong associations (odds ratios up to 326) between wheezing phenotypes and physician-diagnosed asthma reported at age 91 months. Because we had up to seven observations of wheeze, and because the latent class approach allows for misclassification, lack of reliability of parental reporting of wheeze appears not to have been a problem in this study.

We did not have reliable data on treatment for wheeze in early life, although recent studies have suggested that treatment with inhaled corticosteroids in infancy does not alter the natural history of wheezing illnesses in children\(^27,28\). However, it is conceivable that treatment suppressed symptoms of wheeze completely in some subjects, which may have biased reporting towards those with more severe symptoms. Alternatively, suppression of symptoms by treatment with inhaled steroids may have contributed to misclassification of phenotypes that were based on parental reported wheeze. As this is likely to have affected those phenotypes with the strongest associations with doctor-diagnosed asthma, we would have expected such an effect to
attenuate differences between these and other phenotypic groups rather than to lead to spurious associations with objective outcomes. We plan to investigate markers of severity within phenotypes in future studies.

Our finding that the intermediate and late onset phenotypes had the strongest associations with atopy is consistent with a critical window of immunological responses during which environmental influences, such as allergens or viral respiratory infections, interact with genetic variants in immune responsiveness to influence the risk of developing asthma and allergy. The association of late onset wheezing with grass pollen sensitization may also represent a complex interplay of environmental exposures and genetic predisposition with the later onset of symptoms related to seasonal as opposed to ubiquitous allergen exposure.

Transient early wheezing has been associated with reduced lung function soon after birth, and there is evidence from several studies that such deficits are likely to improve partially in later childhood, although may continue to track below normal values. Early postnatal measurements were not available in our study, but based on this literature it seems plausible that early decrements of lung function were associated with the three early-onset wheezing phenotypes, which were less strongly associated with atopy and airway responsiveness than later onset wheezing. Reduced lung function soon after birth is associated with asthma and airway responsiveness in later childhood. Our finding of mid-childhood lung function decrements in children with prolonged early and persistent wheezing could reflect persistence of developmental airway abnormalities, but is also consistent with allergenic or non-allergenic postnatal exposures aggravating existing structural airway abnormalities in subgroups of early onset wheeze. The importance of such decrements in mid-childhood is that, once established, they are likely to persist to adulthood.

In summary, the childhood wheezing phenotypes most strongly associated with atopy and airway responsiveness in our study were characterised by onset of wheezing after age 18 months. Wheezing onset soon after birth was not associated with atopy or airway responsiveness except when it persisted to later childhood. Persistent wheeze may represent a complex phenotype comprising different pathophysiological components encompassing early structural or functional airway changes modified by inflammatory processes during early childhood. Environmental influences on the initiation of atopic wheezing or which modify existing wheezing phenotypes are likely to have a major influence during the first years after birth. The search for modifiable factors that account for the rise in asthma and allergic diseases in industrialised countries should focus on interactions between genes and environment during this critical period. The availability of early environmental data in the ALSPAC cohort will enable these associations to be examined in relation to the phenotypes described here.
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Competing interests: None

Figure 1: Estimated prevalence of wheezing at each time point from birth to 81 months for each of the six wheezing phenotypes identified by latent class analysis in 6,265 children with complete data.

Figure E1: Estimated prevalence of wheezing at each time point from birth to 81 months for each of the six wheezing phenotypes identified by latent class analysis in 11,678 children with two or more responses to wheeze questionnaires.
References

(1) A plea to abandon asthma as a disease concept. Lancet 2006; 368(9537):705.

(2) Phelan PD, Robertson CF, Olinsky A. The Melbourne Asthma Study: 1964-1999. J Allergy Clin Immunol 2002; 109(2):189-194.

(3) Sears MR, Greene JM, Willan AR, Wieck EM, Taylor DR, Flannery EM et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med 2003; 349(15):1414-1422.

(4) Jenkins MA, Hopper JL, Bowes G, Carlin JB, Flander LB, Giles GG. Factors in childhood as predictors of asthma in adult life. Br Med J 1994; 309(6947):90-93.

(5) Morgan WJ, Stern DA, Sherrill DL, Guerra S, Holberg CJ, Guilbert TW et al. Outcome of asthma and wheezing in the first 6 years of life: follow-up through adolescence. Am J Respir Crit Care Med 2005; 172(10):1253-1258.

(6) 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; 332:133-138.

(7) Stein RT, Martinez FD. Asthma phenotypes in childhood: lessons from an epidemiological approach. Paediatr Respir Rev 2004; 5(2):155-161.

(8) Sherriff A, Peters TJ, Henderson J, Strachan D, ALSPAC Study Team. Avon Longitudinal Study of Parents and Children. Risk factor associations with wheezing patterns in children followed longitudinally from birth to 3(1/2) years. Int J Epidemiol 2001; 30(6):1473-1484.

(9) Heaton T, Rowe J, Turner S, Aalberse RC, de KN, Suriyaarachchi D et al. An immunoepidemiological approach to asthma: identification of in-vitro T-cell response patterns associated with different wheezing phenotypes in children. Lancet 2005; 365(9454):142-149.

(10) Golding J, Pembrey M, Jones R. ALSPAC—the Avon Longitudinal Study of Parents and Children. I. Study methodology. Paediatr Perinat Epidemiol 2001; 15(1):74-87.

(11) Pembrey M. The Avon Longitudinal Study of Parents and Children (ALSPAC): a resource for genetic epidemiology. Eur J Endocrinol 2004; 151 Suppl 3:U125-U129.

(12) Roberts G, Peckitt C, Northstone K, Strachan D, Lack G, Henderson J et al. Relationship between aeroallergen and food allergen sensitization in childhood. Clin Exp Allergy 2005; 35(7):933-940.

(13) Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med 1995; 152(3):1107-1136.
(14) Chinn S, Rona RJ. Height and age adjustment for cross sectional studies of lung function in children aged 6-11 years. Thorax 1992; 47:707-714.

(15) Yan K, Salome C, Woolcock AJ. Rapid method for measurement of bronchial responsiveness. Thorax 1983; 38(10):760-765.

(16) Rabe-Hesketh S, Skrondal A. Classical latent variable models for medical research. Stat Methods Med Res 2007.

(17) Muthén L, Muthén B. MPlus Users' Guide. Fourth ed. Los Angeles: Muthén & Muthén; 2006.

(18) Stein RT, Holberg CJ, Morgan WJ, Wright AL, Lombardi E, Taussig L et al. Peak flow variability, methacholine responsiveness and atopy as markers for detecting different wheezing phenotypes in childhood. Thorax 1997; 52(11):946-952.

(19) Turner SW, Palmer LJ, Rye PJ, Gibson NA, Judge PK, Young S et al. Infants with flow limitation at 4 weeks: outcome at 6 and 11 years. Am J Respir Crit Care Med 2002; 165(9):1294-1298.

(20) Oddy WH, Holt PG, Sly PD, Read AW, Landau LI, Stanley FJ et al. Association between breast feeding and asthma in 6 year old children: findings of a prospective birth cohort study. Br Med J 1999; 319(7213):815-819.

(21) Young AF, Powers JR, Bell SL. Attrition in longitudinal studies: who do you lose? Aust N Z J Public Health 2006; 30(4):353-361.

(22) Baker D, Henderson J. Differences between infants and adults in the social aetiology of wheeze. The ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. J Epidemiol Community Health 1999; 53(10):636-642.

(23) Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995; 8:483-491.

(24) Elphick HE, Sherlock P, Foxall G, Simpson EJ, Shiell NA, Primhak RA et al. Survey of respiratory sounds in infants. Arch Dis Child 2001; 84(1):35-39.

(25) Elphick HE, Ritson S, Rodgers H, Everard ML. When a "wheeze" is not a wheeze: acoustic analysis of breath sounds in infants. Eur Respir J 2000; 16(4):593-597.

(26) 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(3):231-237.

(27) Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, Szeffler SJ et al. Long-term inhaled corticosteroids in preschool children at high risk for asthma. N Engl J Med 2006; 354(19):1985-1997.
(28) Murray CS, Woodcock A, Langley SJ, Morris J, Custovic A. Secondary prevention of asthma by the use of Inhaled Fluticasone propionate in Wheezy INfants (IFWIN): double-blind, randomised, controlled study. Lancet 2006; 368(9537):754-762.

(29) van der Velden V, Laan MP, Baert MR, de Waal MR, Neijens HJ, Savelkoul HF. Selective development of a strong Th2 cytokine profile in high-risk children who develop atopy: risk factors and regulatory role of IFN-gamma, IL-4 and IL-10. Clin Exp Allergy 2001; 31(7):997-1006.

(30) Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. N Engl J Med 1990; 323:502-507.

(31) Brussee JE, Smit HA, van Strien RT, Corver K, Kerkhof M, Wijga AH et al. Allergen exposure in infancy and the development of sensitization, wheeze, and asthma at 4 years. J Allergy Clin Immunol 2005; 115(5):946-952.

(32) Singh AM, Moore PE, Gern JE, Lemanske RF, Jr., Hartert TV. Bronchiolitis to asthma: a review and call for studies of gene-virus interactions in asthma causation. Am J Respir Crit Care Med 2007; 175(2):108-119.

(33) Hoffjan S, Nicolae D, Ostrovnaya I, Roberg K, Evans M, Mirel DB et al. Gene-environment interaction effects on the development of immune responses in the 1st year of life. Am J Hum Genet 2005; 76(4):696-704.

(34) Lau S, Illi S, Sommerfeld C, Niggemann B, Volkel K, Madloch C et al. Transient early wheeze is not associated with impaired lung function in 7-yr-old children. Eur Respir J 2003; 21(5):834-841.

(35) Haland G, Carlsen KC, Sandvik L, Devulapalli CS, Munthe-Kaas MC, Pettersen M et al. Reduced lung function at birth and the risk of asthma at 10 years of age. N Engl J Med 2006; 355(16):1682-1689.

(36) Turner SW, Palmer LJ, Rye PJ, Gibson NA, Judge PK, Cox M et al. The relationship between infant airway function, childhood airway responsiveness, and asthma. Am J Respir Crit Care Med 2004; 169(8):921-927.

(37) The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet 1998; 351(9111):1225-1232.
## Table 5. Strength and direction of associations between derived phenotypes and clinical outcomes

| Phenotype       | Asthma | Atopy | FEV$_1$ | FEF$_{25-75}$ | AHR |
|-----------------|--------|-------|---------|---------------|-----|
| Transient early | ✓      | ✗     | ✗       | ✗             | ✓   |
| Prolonged early | ✓✓     | ✗     | ✗       | ✗             | ✓   |
| Intermediate onset | ✓✓✓✓ | ✓✓ | ✗       | ✗             | ✓   |
| Late onset      | ✓✓✓✓   | ✓✓    | ✗       | ✗             | ✓   |
| Persistent      | ✓✓✓✓   | ✓     | ✗       | ✗             | ✓   |

The strength of association of each wheezing phenotype with each outcome is represented by the number of symbols (✓, ✗ or ✗) with a cross (✗) representing absence of association with that outcome.
Associations of wheezing phenotypes in the first six years of life with atopy, lung function and airway responsiveness in mid childhood.

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Online Supplement
METHODS

Classification of wheezing

Because there were two questions about wheezing at each time point, inconsistent responses (yes to one question and no to the other) were possible. We investigated the prevalence of subsequent wheezing to such inconsistent responses and found this to be similar to the prevalence of subsequent wheezing in respondents who answered yes to both questions. Therefore, we defined wheezing as present if the response to either question was ‘Yes’ at a given time point, and absent if the response to both questions was ‘No’. All other combinations were classed as missing (1.3%).

Latent class analysis was used to define wheezing phenotypes in children (n=6,265) whose parents returned questionnaires on wheezing at all seven time points from 6-81 months. A further 5413 children had returned between 2 and 6 questionnaires. These children were included in a secondary analysis of all 11,678 children whose parents had returned at least two questionnaires on wheezing.

Latent class analysis

Latent class analysis is based on the assumption that there exist within the population a number of subpopulations that are characterised by related responses to multivariate categorical data. It is useful for the identification of disease subtypes, for example based on the presence or absence of a set of symptoms or for classifying diseases based on the trajectory of symptoms over time\(^1\), and for comparison of diagnostic tests when no gold standard exists on which to base case definition\(^2,3\). In the case of the present study, there were two categories of wheeze (present/absent) at seven time points, which resulted in \(2^7\) (128) possible combinations of responses. In the case of subjects with missing questionnaires,
when three responses were possible at each point (present/absent/missing), the number of possible combinations grew to $3^7=2187$. Therefore, it was neither feasible nor meaningful to examine the association of every observed pattern of wheezing with objective outcomes. Further, some misclassification of true wheezing status is inevitable, through misinterpretation of wheeze, faulty recall or errors in transcription of questionnaire data. Therefore, latent class analysis was used to identify subpopulations of children with similar patterns of wheezing.

Latent class analysis uses the observed data to estimate two sets of model parameters: (1) The prevalence of each of $C$ latent classes and (2) The conditional probability of response (wheezing at each time point) given membership of each class. The approach aims to identify the smallest number of latent classes that accounts for the associations between wheezing variables at different time points\(^4\). Once the optimal number of latent classes has been identified, the posterior probability of each child’s membership of each class can be calculated. From these, we calculated the prevalence of wheezing at each time point for each class.

A number of methods to measure the goodness of fit of the latent class model have been proposed. For these analyses, we used the Bayesian Information Criterion (BIC), which penalises the log likelihood for model complexity\(^5\); the optimal number of clusters occurs when the BIC is lowest. Starting with a model assuming 3 phenotypes, we compared models with increasing numbers of phenotypes using BIC. We also used bootstrap likelihood ratio tests (BLRT) to compare models with increasing numbers of phenotypes\(^6\). The analyses were initially applied to children with complete data at all seven time points and repeated for children whose parents had returned at least two wheezing questionnaires.
Associations of phenotype membership with maternal self-reported asthma and allergy, physician diagnosed asthma in the child and objective measures of atopy, lung function and airway responsiveness were estimated using logistic regression for binary outcomes and linear regression for numerical (continuous) outcomes.

All analyses were carried out using Mplus 4.1 software (http://www.statmodel.com/).
RESULTS

Table E1 shows estimated probabilities that children belonged to the different phenotypes, according to their recorded wheezing at each time point. The seven digits represent, in order, wheezing at ages 6, 18, 30, 42, 54, 69 and 81 months, with 1=yes, 0=no, and the ten most frequent patterns for each phenotype are displayed. The next most likely phenotype, together with the probability of belonging to this phenotype, is also displayed for each pattern. For some patterns, there was a high probability of membership of a particular phenotype; for example the 2979 children who never wheezed had a 96% chance of belonging to the never/infrequent wheeze phenotype and 135 children who wheezed at each time had a 100% chance of belonging to the persistent wheeze phenotype. On the other hand, some patterns are consistent with belonging to more than one phenotype; for example 322 children who wheezed only at 18 months had a 60% chance of belonging to the never/infrequent wheeze phenotype and a 35% chance of belonging to the transient early wheeze phenotype. Table E2 shows corresponding results when 11,678 children with two or more measures of wheeze were included in the latent class analyses.

Tables E3-E5 show the associations of wheezing phenotypes derived from subjects with at least two returned questionnaires (n=11,678) with atopy, maternal asthma and lung function.
Reference List

(1) Croudace TJ, Jarvelin MR, Wadsworth ME, Jones PB. Developmental typology of trajectories to nighttime bladder control: epidemiologic application of longitudinal latent class analysis. Am J Epidemiol 2003; 157(9):834-842.

(2) Szatmari P, Volkmar F, Walter S. Evaluation of diagnostic criteria for autism using latent class models. J Am Acad Child Adolesc Psychiatry 1995; 34(2):216-222.

(3) Goetghebeur E, Liinev J, Boelaert M, Van der SP. Diagnostic test analyses in search of their gold standard: latent class analyses with random effects. Stat Methods Med Res 2000; 9(3):231-248.

(4) Rabe-Hesketh S, Skrondal A. Classical latent variable models for medical research. Stat Methods Med Res 2007.

(5) Burnham KP, Anderson DR. Model selection and inference: a practical information-theoretic approach. New York: Springer-Verlag; 1998.

(6) Langeheine R, Pannekoek J, VandePol F. Bootstrapping goodness-of-fit measures in categorical data analysis. Sociological Methods & Research 1996; 24(4):492-516.
Table E1. Most frequently occurring patterns of wheeze from age 6 to 81 months in 6,265 children with complete data

| Most likely phenotype | N. | Pattern of wheeze* | p† | Next class‡ | Most likely phenotype | N. | Pattern of wheeze* | p† | Next class‡ | Most likely phenotype | N. | Pattern of wheeze* | p† | Next class‡ |
|-----------------------|----|--------------------|----|-------------|-----------------------|----|--------------------|----|-------------|-----------------------|----|--------------------|----|-------------|
| Transient Early (TE)  | 70 | 10100000 0.66 PE 0.17 | 34 | 11101000 0.80 TE 0.17 | Intermediate (IO) | 9  | 00110101 0.79 L 0.16 |
|                       | 25 | 11001000 0.50 PE 0.45 | 34 | 00011000 0.58 IO 0.16 |                       | 8  | 00111101 0.80 P 0.08 |
|                       | 21 | 01000001 0.57 L 0.25  | 31 | 10010000 0.39 I 0.32  |                       | 6  | 10011111 0.51 P 0.29 |
|                       | 18 | 1000001 0.46 I 0.35   | 24 | 00110000 0.78 I 0.12  |                       | 2  | 10011011 0.34 PE 0.28 |
|                       | 9  | 11000001 0.86 L 0.07  | 24 | 01001000 0.41 TE 0.35 |                       | 24 | 11100010 0.93 P 0.06 |
|                       | 6  | 10101000 0.62 PE 0.17 | 24 | 01110000 0.93 I 0.09  |                       | 2979| 00000000 0.96 TE 0.04 |
| Late (LO)             | 44 | 00001111 0.91 IO 0.09 | 135| 11111111 1.00 L 0.00 |                       | 2979| 00000000 0.96 TE 0.04 |
|                       | 38 | 00000111 0.97 I 0.03  | 79 | 01111111 0.98 IO 0.02 |                       | 420| 10000000 0.67 TE 0.31 |
|                       | 26 | 00011110 0.89 I 0.06  | 29 | 11111111 0.93 PE 0.07 |                       | 173| 00100000 0.67 TE 0.24 |
|                       | 16 | 10000100 0.42 I 0.38  | 23 | 11111101 0.92 PE 0.08 |                       | 96 | 00001000 0.80 L 0.08  |
|                       | 13 | 00100100 0.62 I 0.20  | 22 | 01111110 0.84 PE 0.13 | Never/infrequent (NI)| 82 | 00000100 0.64 L 0.33  |
|                       | 12 | 01001111 0.91 P 0.08  | 21 | 10111111 0.80 IO 0.18 |                       | 78 | 00100000 0.81 PE 0.11 |
|                       | 11 | 01000111 0.97 TE 0.01 | 17 | 11011111 0.97 L 0.02  |                       | 68 | 00000001 0.75 L 0.16  |
|                       | 10 | 00001011 0.79 I 0.12  | 13 | 01011111 0.75 L 0.16  |                       | 18 | 10010001 0.37 TE 0.31 |
|                       | 10 | 00110111 0.65 IO 0.28 | 13 | 11101111 0.96 L 0.04  |                       | 11 | 01101111 0.74 L 0.24  |

* The seven digits represent, in order, wheezing at ages 6, 18, 30, 42, 56, 69 and 81 months; 1=yes, 0=no.
† Probability that a child with this pattern of wheezing belongs to this phenotype
‡ Next most probable phenotype, and the probability that a child with this pattern of wheezing belongs to this phenotype
### Table E2. Most frequently occurring patterns of wheeze from age 6 to 81 months in 11,678 children with at least two measurements of wheeze.

| Most likely phenotype | Pattern of wheeze* | N   | P† | Next class‡ | Pattern of wheeze* | N   | P‡ | Most likely phenotype | Pattern of wheeze* | N   | P† | Next class‡ | Pattern of wheeze* | N   | P‡ |
|-----------------------|--------------------|-----|----|------------|--------------------|-----|----|-----------------------|--------------------|-----|----|------------|--------------------|-----|----|
| Transient Early (TE)  |                    |     |    |            |                    |     |    |                       |                    |     |    |            |                    |     |    |
| 238 11000000          | 0.83               | 238 | NW | 0.10      | 42 01110000        | 0.94 | TE | 0.04                  | 69 0011111         | 0.87 | P  | 0.10      | 103 1110000         | 0.77 | PE | 0.22      |
| 103 11100000          | 0.77               | 103 | PE | 0.31      | 38 11110000        | 0.91 | TE | 0.08                  | 20 0001110         | 0.54 | L  | 0.42      | 98 0110000          | 0.59 | PE | 0.31      |
| 98 01100000           | 0.59               | 98  | PE | 0.31      | 34 01010000        | 0.69 | NW | 0.17                  | 10 00*1111         | 0.86 | L  | 0.09      | 70 1010000          | 0.54 | NW | 0.23      |
| 70 10100000           | 0.54               | 70  | NW | 0.23      | 34 11100000        | 0.72 | TE | 0.25                  | 10 0011110         | 0.72 | L  | 0.11      | 62 11*****          | 0.41 | P  | 0.33      |
| 62 11*****           | 0.41               | 62  | P  | 0.33      | 34 11101000        | 0.72 | TE | 0.25                  | 10 0011110         | 0.72 | L  | 0.11      | 25 1100100          | 0.54 | PE | 0.40      |
| 25 11001000          | 0.54               | 25  | PE | 0.40      | 33 00011000        | 0.63 | NW | 0.16                  | 9 0001101          | 0.63 | L  | 0.30      | 22 11*0000         | 0.81 | PE | 0.12      |
| 22 11*0000          | 0.81               | 22  | PE | 0.12      | 31 10010000        | 0.49 | NW | 0.38                  | 8 001111**         | 0.69 | PE | 0.18      | 38 0001100         | 0.54 | PE | 0.40      |
| 38 00011000         | 0.54               | 38  | PE | 0.40      | 24 01001000        | 0.40 | TE | 0.29                  | 7 000111*          | 0.77 | L  | 0.21      | 21 110****          | 0.63 | PE | 0.17      |
| 21 110****          | 0.63               | 21  | PE | 0.17      | 24 01001100        | 0.40 | TE | 0.29                  | 7 0001111          | 0.77 | L  | 0.21      | 20 110000*          | 0.83 | NW | 0.10      |
| 20 110000*          | 0.83               | 20  | NW | 0.10      | 24 01111000        | 0.90 | P  | 0.09                  | 7 0011***          | 0.51 | PE | 0.32      | 44 0000111         | 0.95 | I  | 0.04      |
| 44 0000111         | 0.95               | 44  | I  | 0.04      | 135 11111111       | 1.00 | L  | 0.00                  | 2979 0000000       | 0.98 | TE | 0.02      | 38 0000011         | 0.97 | NW | 0.03      |
| 38 0000011         | 0.97               | 38  | NW | 0.03      | 79 0111111         | 0.99 | L  | 0.01                  | 420 1000000        | 0.79 | TE | 0.19      | 26 0000110         | 0.92 | NW | 0.05      |
| 26 0000110         | 0.92               | 26  | NW | 0.05      | 35 111****         | 0.51 | PE | 0.24                  | 322 0100000        | 0.60 | TE | 0.35      | 16 1000010         | 0.42 | NW | 0.37      |
| 16 1000010         | 0.42               | 16  | NW | 0.37      | 29 1111111         | 0.96 | PE | 0.03                  | 252 00****         | 0.84 | L  | 0.06      | 13 0010010         | 0.59 | NW | 0.24      |
| 13 0010010         | 0.59               | 13  | NW | 0.24      | 23 1111101         | 0.94 | PE | 0.06                  | 220 000000*        | 0.97 | TE | 0.02      | 12 0100111         | 0.90 | P  | 0.10      |
| 12 0100111         | 0.90               | 12  | P  | 0.10      | 22 0111110         | 0.91 | PE | 0.06                  | 188 0000**         | 0.95 | L  | 0.03      | 11 000010*         | 0.51 | NW | 0.47      |
| 11 000010*         | 0.51               | 11  | NW | 0.47      | 21 1011111         | 0.63 | I  | 0.34                  | 176 00000*0        | 0.97 | TE | 0.02      | 10 0100011         | 0.95 | TE | 0.03      |
| 10 0100011         | 0.95               | 10  | TE | 0.03      | 19 0111***         | 0.61 | PE | 0.37                  | 173 0010000        | 0.76 | TE | 0.13      | 11 0101011         | 0.76 | I  | 0.18      |
| 11 0101011         | 0.76               | 11  | I  | 0.18      | 17 1101111         | 0.96 | L  | 0.04                  | 158 0000**         | 0.96 | TE | 0.02      | 10 0010111         | 0.81 | NW | 0.13      |
| 10 0010111         | 0.81               | 10  | NW | 0.13      | 18 1111111         | 0.75 | PE | 0.23                  | 164 00*0000        | 0.96 | TE | 0.02      | 10 0010111         | 0.76 | I  | 0.18      |

* The seven digits represent, in order, wheezing at ages 6, 18, 30, 42, 56, 69 and 81 months; 1=yes, 0=no, *=missing.
† Probability that a child with this pattern of wheezing belongs to this phenotype
‡ Next most probable phenotype, and the probability that a child with this pattern of wheezing belongs to this phenotype.
Table E3. Associations of wheezing phenotype with atopy among 6,332 children with at least two measurements of wheezing and skin prick test data at 7-8 years

| Wheezing phenotype | Atopy (any skin prick sensitivity) | Skin prick sensitivity to *D. pteronyssinus* | Skin prick sensitivity to cat | Skin prick sensitivity to grass* |
|--------------------|-----------------------------------|---------------------------------------------|-------------------------------|---------------------------------|
|                    | N/total*  OR (95% CI)              | N/total*  OR (95% CI)                       | N/total*  OR (95% CI)         | N/total*  OR (95% CI)           |
| Transient early     | 47 / 810  0.77 (0.46,1.31)        | 18 / 819  0.83 (0.37,1.86)                  | 12 / 813  0.85 (0.3,2.38)    | 24 / 817  0.62 (0.29,1.34)     |
| Prolonged early     | 40 / 618  0.87 (0.51,1.49)        | 12 / 625  0.77 (0.31,1.89)                  | 6 / 620   0.56 (0.12,2.59)   | 33 / 623  1.15 (0.65,2.04)     |
| Intermediate        | 59 / 150  8.16 (5.4,12.33)        | 35 / 152  11.1 (6.84,18.03)                 | 43 / 151  22.87 (13.98,37.42)| 8 / 151   1.22 (0.48,3.09)     |
| Late               | 132 / 402 6.13 (4.47,8.42)       | 56 / 406  5.91 (3.78,9.22)                  | 65 / 403  11.03 (6.95,17.52) | 61 / 405  3.64 (2.43,5.47)     |
| Persistent         | 115 / 476 3.99 (3.04,5.24)       | 60 / 481  5.29 (3.65,7.66)                  | 57 / 478  7.7 (5.1,11.63)    | 39 / 480  1.84 (1.23,2.75)     |
| Never/infrequent    | 287 / 3876 1 (reference)         | 102 / 3918 1 (reference)                   | 66 / 3890 1 (reference)      | 180 / 3907 1 (reference)       |
Table E4. Associations of wheezing phenotypes with maternal self reported asthma and allergy in 11031 children with at least two measurements of wheezing and maternal history of asthma/allergy

| Wheezing phenotype | Maternal asthma Odds ratio (95% CI) | Maternal allergy Odds ratio (95% CI) |
|--------------------|-------------------------------------|--------------------------------------|
| Transient early    | 1.98 (1.49, 2.69)                   | 1.34 (1.11, 1.62)                    |
| Prolonged early    | 2.4 (1.83, 3.14)                    | 1.55 (1.28, 1.89)                    |
| Intermediate       | 2.67 (1.78, 3.99)                   | 1.71 (1.24, 2.36)                    |
| Late               | 1.73 (1.21, 2.46)                   | 1.33 (1.05, 1.68)                    |
| Persistent         | 3.94 (3.2, 4.85)                    | 2.0 (1.68, 2.37)                     |
| Never/infrequent   | 1 (reference)                      | 1 (reference)                        |
Table E5. Associations of wheezing phenotype with lung function among 6,402 children with at least two measurements of wheezing and lung function measurements at 8-9 years and 4,245 with airway responsiveness measurements at 8-9 years.

| Wheezing phenotype | Total | FEV<sub>1</sub> (SD units) | FEF<sub>25-75</sub> (SD units) | Airway responsiveness* |
|-------------------|-------|-----------------------------|-------------------------------|------------------------|
|                   |       | Mean (sd)                   | Mean difference (95% CI)      | Total | Mean (sd) | Mean difference (95% CI) |
| Transient early   | 819   | -0.14 (0.95)                | -0.26 (-0.33, -0.19)          | 832   | -0.14 (0.97) | -0.32 (-0.39, -0.25) | 543   | -0.03 (1.54) | 0.24 (0.09,0.38) |
| Prolonged early   | 625   | -0.24 (1.05)                | -0.35 (-0.44, -0.27)          | 634   | -0.4 (0.91)  | -0.58 (-0.66, -0.5)  | 414   | 0.17 (1.58)  | 0.43 (0.27,0.59)  |
| Intermediate      | 152   | -0.24 (1.16)                | -0.36 (-0.52, -0.2)           | 154   | -0.46 (1.14) | -0.64 (-0.79, -0.48) | 101   | 1.51 (1.65)  | 1.78 (1.47,2.08)  |
| Late              | 406   | -0.06 (1)                   | -0.18 (-0.28, -0.08)          | 412   | -0.2 (1.01)  | -0.38 (-0.48, -0.28) | 269   | 1.22 (1.78)  | 1.48 (1.28,1.68)  |
| Persistent        | 481   | -0.27 (1.05)                | -0.39 (-0.48, -0.3)           | 489   | -0.44 (1.11) | -0.62 (-0.71, -0.53) | 319   | 0.86 (1.81)  | 1.12 (0.94,1.31)  |
| Never/infrequent  | 3919  | 0.12 (0.97)                 | 0 (reference)                 | 3978  | 0.18 (0.96)  | 0 (reference)        | 2599  | -0.26 (1.54) | 0 (reference)     |
