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Wheezing phenotypes in the first 7 years of life, lung function and fractional exhaled nitric oxide in adolescence: a longitudinal birth-cohort study.

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Wheezing phenotypes in the first 7 years of life, lung function and

fractional exhaled nitric oxide in adolescence: a longitudinal birth-cohort study.

Short title: Wheezing phenotypes, lung function, and FeNO

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What is the key question?

Do longitudinal wheezing patterns during early childhood indicate differences in long-term

prognosis of respiratory illnesses?

What is the bottom line?

Early onset wheezing phenotypes persisting after 18 months of age showed the strongest

associations with adverse respiratory outcomes in adolescence.

Why read on?

The current study suggests evidence for associations of specific wheezing phenotypes

during early childhood with asthma, low lung function and increased allergic airway

inflammation in adolescence, and additionally, lung function worsening from mid-childhood

until adolescence.

ABSTRACT

Background Longitudinal wheezing patterns during early childhood may indicate differences in long-term prognosis of respiratory illnesses. We examined the associations of different childhood wheezing phenotypes with asthma, lung function and fractional exhaled nitric oxide (FeNO) in adolescence.

Methods In a population-based, prospective cohort study of 6,841 children, we used latent class analysis to identify wheezing phenotypes (never/infrequent; transient early; prolonged early; intermediate-onset; late-onset; persistent) from birth to 7 years. Main outcomes were assessed at age 14-15 years and included information about physician-diagnosed asthma, lung function (forced expiratory volume in 1 s (FEV_1), ratio FEV_1/forced vital capacity (FVC), forced mid-expiratory flow (FEF_{25-75}), and FeNO**.**

Results Compared with never/infrequent wheeze, intermediate-onset and persistent wheeze were consistently strongest associated with higher odds of asthma (odds ratio [95% CI]: 52.7 [31.4, 88.2]; 38.9 [29.1, 51.9], respectively), lower FEV_1/FVC ratio (mean difference standard deviation-units [MD-SDU] [95% CI]: -0.36 [-0.56, -0.16]; -0.50 [-0.62, -0.38], respectively), lower FEF_{25-75} (MD-SDU: -0.30 [-0.51, -0.10]; -0.42 [-0.54, -0.29], respectively), and increased FEV_1 bronchodilator reversibility (MD-SDU: 0.11 [0.01, 0.22]; 0.18 [0.06, 0.30], respectively) at age 14-15 years. Prolonged early and persistent wheeze were associated with a decline in FEV_1/FVC ratio and FEF_{25-75} between 8-9 and 14-15 years. Intermediate-onset, late-onset and persistent wheeze were associated with higher FeNO ratios (ratio [95% CI]: 1.93 [1.59, 2.34]; 1.60 [1.42, 1.81]; 1.37 [1.22, 1.54], respectively, compared with never/infrequent wheeze).

Conclusions Early onset wheezing phenotypes persisting after 18 months of age show the strongest associations with asthma, lower lung function and higher FeNO levels in adolescence.

INTRODUCTION

Asthma is a complex heterogeneous disease comprising a number of discrete phenotypes.

Childhood wheezing is the most common clinical manifestation of asthma onset. Severe and

persistent childhood wheezing patterns are more likely to lead to asthma and abnormal

pulmonary function in later life, than mild and transient childhood wheezing patterns (1-4).

Recently, a novel symptom-driven approach to define different wheezing patterns was

introduced using repeated 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) (5). This led to the identification of six different phenotypes including

never/infrequent wheezing, transient early wheezing, prolonged early wheezing,

intermediate-onset wheezing, late-onset wheezing, and persistent wheezing. We found that

intermediate-onset wheezing showed the strongest associations with atopy, airway

hyperresponssiveness and lower lung function in mid-childhood. Thus far, it is not known

whether the higher risk of asthma and abnormal pulmonary function in mid-childhood

persists or worsen into adolescence, or whether different wheezing phenotypes in early

childhood are related to eosinophilic airway inflammation, which can be assessed using the

fractional concentration of nitric oxide in exhaled air (FeNO).

Therefore, we examined the associations of previously reported wheezing phenotypes

during the first 7 years of childhood with asthma, lung function and FeNO in adolescence in

the ALSPAC birth cohort study. We also examined whether any associations were modified

by tobacco smoke exposure during pregnancy or early childhood, active smoking and

reported use of asthma medications.

METHODS

Design and setting This study was embedded in ALSPAC, a population-based birth cohort

study of pregnant women resident in Avon, UK, and their children (6). The cohort has been

followed up with annual questionnaires and, since the age of 7 years, with objective

measures in research clinics. The study protocol has been published previously (6) and further details can be found at http://www.bris.ac.uk/alspac.

Wheezing phenotypes At 6, 18, 30, 42, 54, 69 and 81 months after birth, mothers were sent a self-completion questionnaire about the health of their child. In 2 separate sections, they reported the occurrence of wheezing in the previous 12 months (6 months for the initial questionnaire) and, if present, whether they consulted a physician. Wheeze was defined as present if the response to any of the questions about wheezing was ''yes'' and absent if the response to both was ''no''. All other combinations were classified as missing (1.3%).

Asthma Information on asthma was obtained by a parent-completed questionnaire at a mean age of 14 (SD 0.16) years. Parents reported whether a physician ever diagnosed asthma or eczema in their child (no; yes, asthma; yes, eczema; yes, asthma and eczema) or whether their child had asthma during the past 12 months (no; yes, did not see a physician; yes, did see a physician). Based on the first obtained question, we grouped children into 'ever physician-diagnosed asthma' (no; yes, asthma or asthma and eczema). Reported asthma during the past 12 months was used to reclassify 'ever physician-diagnosed asthma' (no; yes) where appropriate.

Lung function Children visited research clinics at mean ages of 8.6 (SD 0.25) and 15.4 (SD 0.30) years. Lung function was measured by spirometry (Vitalograph 2120, Maids Moreton, UK) according to American Thoracic Society criteria (7). Each variable (forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and mid forced expiratory flow (FEF_{25-75})) was converted into sex-, age- and height-adjusted standard deviation units (SDU) (8). Bronchodilator reversibility was calculated as the difference in SDU of lung function variables change before and 15 minutes after receiving 400 micrograms of salbutamol by metered aerosol and spacer. Lung function change between mid-childhood and adolescence was calculated as the difference in SDU of lung function variables between these two assessments.

FeNO Fractional exhaled nitric oxide (FeNO) was measured online at a constant flow of 50mL/s according to European Respiratory Society (ERS) and American Thoracic Society

(ATS) guidelines using a Sievers NOA-280i nitric oxide analyser (GE Analytical Instruments, Boulder, CO.). Additionally, 758 children with less stringent criteria (individual blows >5% deviation but at least 1 within acceptable rang of 10-200 ppb) were included. FeNO measurements were done before spirometry measurements. Children were requested to omit their inhaled corticosteroids if applicable 48 hours before their visit to the clinic. At the time of measurements, 14 children (0.3%) received oral steroids, 221 (5.2%) had a chest infection and/or fever with a cold in the preceding three weeks, and 39 (0.9%) and 89 (2.1%) had used short acting or long acting bronchodilators 6 or 24 hours, respectively, prior to the respiratory assessments. These children, and those with less stringent criteria of FeNO values, were included in our analyses since we observed no differences in results when they were included or excluded.

Covariates Information on maternal age, deprivation index, anxiety, smoking during pregnancy and history of asthma or allergy were obtained from self-completion questionnaires at 18 weeks or 32 weeks of gestation. Birth weight, gestational age and gender of the children were obtained from midwife and hospital registries at birth. Childhood maternal questionnaires from 3-15 months after birth provided information about breastfeeding, home overcrowding (>0.75 persons per room), pet contact and childhood smoke exposure. At 14 years of age, parents reported if their child had a chest infection in the past 12 months, ever had hay fever or eczema, and use of medication for asthma (no; yes, preventer inhaler; yes, reliever inhaler; yes, other inhaler or asthma medicine). Children themselves reported if they actively smoked (yes: sometimes but < 1 a week; usually 1-6 a week; usually > 6 a week but not every day, usually ≥ 1 every day) or not (no; only ever tried once or twice; used sometimes but never now).

Statistical analysis We used latent class analysis to derive distinct phenotypes of wheezing as described previously (5). Briefly, this identifies the minimum number of classes that describe the variation of observed responses in multicategorical data which in this case were wheeze at different time points (9). The posterior probabilities (probability of each individual of belonging to each phenotype) were estimated and used in weighted logistic and linear

regression models to estimate associations of wheezing phenotypes with physician-diagnosed asthma, lung function and FeNO levels in adolescence. Similar analyses were used with bronchodilator reversibility and longitudinal lung function change between mid-childhood and adolescence as continuous outcome variables. Confounders were included in the adjusted models based on literature or change in effect estimates of ≥ 10%. We additionally adjusted for lung function data in mid-childhood when lung function changes between mid-childhood and adolescence were used as the outcomes. Data on confounders was missing for 7% at most and included in the analyses as separate categories or means. We explored effect modification of exposure to smoke during pregnancy or early life, active smoking of the child and use of asthma medication by including these variables as interaction terms in the linear regression models with lung function and FeNO as the outcomes. Finally, to assess whether the associations of the wheezing phenotypes with FeNO levels were modified by the use of inhaled corticosteroids, we repeated these analyses with FeNO levels as the outcome in strata of reported inhaled corticosteroid use (no, yes). FeNO levels were log-transformed to obtain normality and therefore geometric means and ratios of geometric means are reported. Measures of associations with FeNO levels as the outcome are presented as back-transformed geometric means or ratios. All measures of association are presented with their 95% confidence intervals (CI). Statistical analyses were performed using Stata v11 (StataCorp, College Station, TX).

RESULTS

Wheezing phenotypes were characterized by timing of onset and subsequent decline or persistence: never/infrequent wheeze (no or low frequency wheeze < 6 months; 61%), transient early wheeze (6-18 months; 13%), prolonged early wheeze (6-69 months; 10%), intermediate-onset wheeze (>18 months; 2%), late-onset wheeze (>42 months; 6%), and persistent wheeze (>6 months; 8%) (5). Of 11.678 children with information about wheezing phenotypes during the first 7 years of life, 6,841 (59%) had data on at least one respiratory outcome. Characteristics of the study population are shown in table 1.

Wheezing phenotypes, asthma and lung function All wheezing phenotypes were associated with asthma at age 15 (Supplementary Table S1). The strongest associations for asthma were observed for the intermediate-onset (odds ratio [OR] [95% confidence interval]: 52.7 [31.4, 88.2]) and persistent wheeze phenotype (OR 38.9 [29.1, 51.9]), compared with never/infrequent wheeze.

Compared with never/infrequent wheeze in early childhood, only persistent wheeze was associated with lower levels of FEV_1 (mean difference in standard deviation unit [SDU] [95% confidence interval]: -0.15 [-0.27, -0.02]) at age 15 years (Figure 1A, Supplementary Table S2). Wheezing phenotypes were associated with lower FEV_1/FVC and, except for transient early wheeze, with lower FEF_{25-75} (Figures 1B and C, respectively, Supplementary Table S2). Compared with never/infrequent wheeze, all wheezing phenotypes, except transient early wheeze, showed evidence of increased bronchodilator reversibility of FEV_1 and FEF_{25-75} but not consistently for FEV_1/FVC (Figures 1A-C, Supplementary Table S2). Prolonged early and persistent wheeze were most consistently associated with a decline in change of FEV_1/FVC ratio (Mean difference in SDU [95%CI]: -0.25 [-0.36, -0.15] and -0.28 [-0.40, -0.15], respectively) and change of FEF_{25-75} (-0.10 [-0.20, -0.00] and -0.14 [-0.25, -0.02], respectively) between 8-9 and 14-15 years (Figure 2B and C, Supplementary Table S3). We found no strong evidence for modification lung function by prenatal smoke exposure, childhood smoke exposure, active smoking or use of asthma medication (results not reported, p-values for interaction > 0.05).

Wheezing phenotypes and FeNO Compared with never/infrequent wheeze, intermediate-onset, late-onset and persistent wheeze, were associated with higher ratios of FeNO at age 14-15 years (Ratio of geometric means [95% CI]: 1.93 [1.59, 2.34], 1.60 [1.42, 1.81], and 1.37 [1.22, 1.54], respectively) (Figure 3, Supplementary Table S4). Higher odds for FeNO were observed for these wheezing phenotypes when we used the clinical cut-off level of high FeNO of ≥ 20 ppb (OR 2.31 [1.18, 4.54], 2.03 [1.36, 3.05] and 1.45 [1.01, 2.08], respectively) (Supplementary Table S5). Among children diagnosed with asthma, use of a corticosteroid inhaler was associated with higher levels of FeNO compared with those children who did not

use a corticosteroid inhaler (54.3 ppb *vs* 26.6 ppb, p-value <0.01). Stratified analyses

showed that the associations of intermediate-onset, late-onset and persistent wheeze with

levels of FeNO were stronger in children who did not use a corticosteroid inhaler, but the

evidence for an interaction was weak (Supplementary Table S6; p-value for interaction >

0.05). Similarly, there was no strong evidence that the associations of wheezing phenotypes

with levels of FeNO were modified by smoke exposure during pregnancy, childhood smoke

exposure, or active smoking (results not reported, p-values for interaction > 0.05).

DISCUSSION

Our results suggest that previously observed associations of wheezing phenotypes during

the first 7 years of life with increased odds of asthma and lower lung function in mid-

childhood persist until adolescence. Additionally, we observed that prolonged early and

persistent wheeze were associated with a further decline in lung function between the ages

of 8-9 and 14-15 years. Also, we found evidence for increased allergic airway inflammation

at 14-15 years for the intermediate-onset, late-onset and persistent wheezing phenotypes.

Interpretation of findings We previously identified two unreported wheezing phenotypes

during the first 7 years of childhood including prolonged early and intermediate-onset

wheezing (10). The intermediate-onset wheezing phenotype was replicated (11) and

validated (12) in two independent birth cohorts and, additionally, evidence was provided that

latent class analysis derived wheezing phenotypes are clinically distinct and have different

genetic origins (13, 14). We now show that the associations of intermediate-onset, late-onset

and persistent wheezing phenotypes with asthma in adolescence are consistent with both

our previously published associations with outcomes at younger ages (5, 11), and other

studies using different categories of wheezing and asthma in late childhood and

adolescence (3, 15). Among currently wheezing children aged 10 years, asthma was

diagnosed more often for persistent than late-onset wheezing children (76.0% vs. 51.9%,

p<0.01) (15). Also, late-onset and persistent, and not transient early, wheezing children were

more likely to continue to wheeze from age 8-9 to 14-15 years (OR 3.12 [2.5, 3.9] and 3.8 [3.1, 4.7], respectively), compared with children who never wheezed (3).

Previous studies reported inconsistent results for the associations of wheezing phenotypes with pulmonary function in adolescence (3, 15-17). The differences in results might be explained by the different study designs, small number of subjects for sufficient power to detect small effects, definition of wheezing, time point, and method of lung function, such as spirometry, multiple-breath wash-out or peak flow variability, measured. Of studies that observed associations between wheezing phenotypes and lung function measured by spirometry, the strongest and most consistent effects were observed for persistent wheezing with lower levels of FEV_1 (-87 ml to -90 ml) and FEV_1/FVC ratio (-2.5% to -3.4%), compared with the never wheezing phenotype (3, 15), even into adulthood (2). Our results suggest that the two recently identified wheezing phenotypes, prolonged early and intermediate-onset, are also negatively associated with lung function into adolescence. Possible mechanisms of lower lung function in adolescence include developmental abnormalities persisting from infancy, and ongoing inflammation and repair of the airways leading to remodeling. This is supported by the evidence of increased airway inflammation levels in adolescence for our intermediate-onset, late-onset, and persistent wheezing phenotypes.

We observed associations of prolonged early and persistent wheezing with change in lung function between 8-9 years and 14-15 years, which implies that irreversible adverse changes persist and for some wheezing phenotypes further decline after early childhood. Our results are partly consistent with findings from the Tuscon study that showed associations of early transient wheeze with lower maximal expiratory flow at the ages of 2.4 months and 6, 11 and 16 years (3). Late-onset wheeze was not associated with maximal expiratory flow at any age, but persistent wheeze was associated with maximal expiratory flow at 6 years and older. However, they did not observe a further decline of lung function after the age of 6 years for the phenotypes when compared with their peers.

Our results with levels of FeNO as a respiratory morbidity outcome are not in line with previous studies, which did not show higher levels of FeNO due to a specific wheezing

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phenotype (17-20). Differences with our results could be explained by their use of other definitions of wheezing phenotype (multi-trigger vs. episodic, atopic vs. non-atopic, recurrent vs. no wheezing phenotype), partly lack of adjustment for confounders, younger age at which FeNO levels were measured, and smaller sample sized cohorts. Our observed effect estimates of the associations between wheezing phenotypes and FeNO were consistently strongest for intermediate-onset wheeze when mean levels of FeNO, ratios of continuously measured FeNO or clinical cut-off levels of FeNO were used. This might be due to different specific underlying mechanisms for specific wheezing phenotypes. We speculate that the intermediate-onset and late-onset wheezing phenotype mostly arise due to atopic mechanisms including sensitivity to common allergens (5, 21) leading to higher risk of (atopic) asthma, higher levels of FeNO and lower lung function, while persistent wheezing phenotype arise due to a mixture of growth, infectious and atopic mechanisms (22-25) leading to lower lung function only. FeNO levels were low compared with clinic-based samples. However, in the current population-based sample, which probably contains a higher proportion of mild asthmatics, there was still clear evidence of wheezing phenotype associated airway inflammation.

Strengths and methodological issues This study was embedded in a population-based, prospective cohort study with a large number of subjects being studied from early life onwards with detailed and frequently measured information about wheezing, asthma and lung function, and adjustment for a large number of confounders. Furthermore, this cohort study is the first study that measured levels of FeNO in adolescence. In common with most longitudinal cohort studies, there was loss to follow up that was not random. Of children with complete cases on wheezing until age 7 years (n = 6,265) (5), the range of missing values on respiratory assessments at age 15 years was 8.3% (asthma) to 55.8% (FeNO). Selection bias due to loss to follow-up would be present in our study if the associations of wheezing phenotypes with respiratory outcomes were different between adolescents lost to follow-up and those who remained in the study. Although this cannot be totally excluded, qualitative

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conclusions about the direction and approximate magnitude of the effect estimates will most probably not change (26). We used parental-reported wheezing symptoms which is widely accepted in epidemiological studies (27). Under- or overreporting could be present but misclassification of wheeze influencing our results seem unlikely because seven observations were used, and latent class analyses allows for misclassification. However, data on treatment with inhaled steroids was not available < 8 years of age and misclassification due to suppression of symptoms by treatment might be present. The wheeze patterns that were observed in our data were not constrained by prespecified notions of their number or nature. The strong associations of the wheezing phenotypes with asthma, lung function, and levels of FeNO until adolescence confirms the appropriate use of wheeze patterns derived by latent class analyses at least in population-based cohorts studying underlying mechanisms and prognosis of respiratory morbidity. Other phenotypes such as episodic viral and multi-trigger wheeze seem more appropriate for clinical practice (28). Whether all different classifications of wheeze identified for various purposes eventually merge remains to be studied. Although we adjusted for a large number of confounders, residual confounding due to obesity (29) or genetic variants (30-32) might be present.

In conclusion, our study suggest that associations of wheezing patterns in early childhood with increased odds of asthma and lower lung function in mid childhood persist until adolescence. Lung function from mid childhood until adolescence further declined for the prolonged early and persistent wheezing phenotypes. We found evidence for increased allergic airway inflammation in adolescence for wheezing phenotypes with early onset that persisted after 18 months of age. Further studies are needed to explore possible underlying mechanisms to develop specific intervention strategies that optimize lung development in early life and prevent additional decline by adolescence.

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Table 1. Characteristics of children and their mothers.

Values are absolute numbers (percentages), means (SD) or #medians (5-95th percentile).

	Cohort for analysis *n = 6,841*	
	N measured (%)	Mean (SD) or median (5-95th percentile)
Mother		
Age at delivery (years)	6,841 (100)	29.1 (4.6)
Maternal deprivation index#	6,669 (97.5)	12.6 (4.9-43.2)
Parity ≥ 1	6,660 (97.4)	3,485 (52.3)
Anxiety index at 32 weeks gestation#	6,351 (92.8)	4.0 (0-12)
Smoking during pregnancy (%)	6,623 (96.8)	1,278 (19.3)
History of asthma or allergy (%)	6,571 (96.1)	3,094 (47.1)
Child		
Gender (female)	6,841 (100)	3,425 (50.1)
Preterm birth (<37 weeks) (%)	6,841 (100)	331 (4.8)
Low birth weight (<2.5 kg) (%)	6,764 (98.9)	279 (4.1)
Ever breastfed in first 6 months (%)	6,553 (95.8)	5,344 (81.6)
Overcrowded at home (%)	6,592 (96.4)	1,248 (18.9)
Day care attendance during 1st year (%)	6,525 (95.4)	439 (6.7)
Pet contact during 1st year (%)	6,574 (96.1)	4,513 (68.6)
Childhood smoke exposure 1st year (%)	6,518 (95.3)	1,180 (18.1)
Age at respiratory assessments (years)	4,798 (70.1)	15.4 (0.3)
Height (cm), age 15 y	4,750 (69.4)	169.3 (8.3)
Active smoking, age 14-15 y (%)	5,902 (86.3)	1,375 (23.3)
Asthma treatment, age 14 y (%)		
'Preventer' inhaler	4,952 (72.4)	414 (8.4)
'Reliever' inhaler	5,096 (74.5)	594 (11.7)
Other inhaler or asthma medicine	4,646 (67.9)	58 (1.3)

Figure 1. Association of wheezing phenotypes with lung function in 14-15 year old adolescents.

Values are mean differences of sex-, age- and height-adjusted standard deviation units of primary (●) and bronchodilator reversibility (○) lung function measurements with their 95% confidence intervals (95% CI), compared with never/infrequent wheeze. FEV_1, forced expiratory volume in 1 second (A); FEV_1/FVC, forced expiratory volume in 1 second / forced vital capacity (B); FEF_{25-75}, mid forced expiratory flow (C). Models are adjusted for maternal age at delivery, parity, anxiety, smoking during pregnancy, history of asthma and allergy, children's sex, low birth weight, preterm birth, breastfeeding and childhood tobacco smoke exposure.

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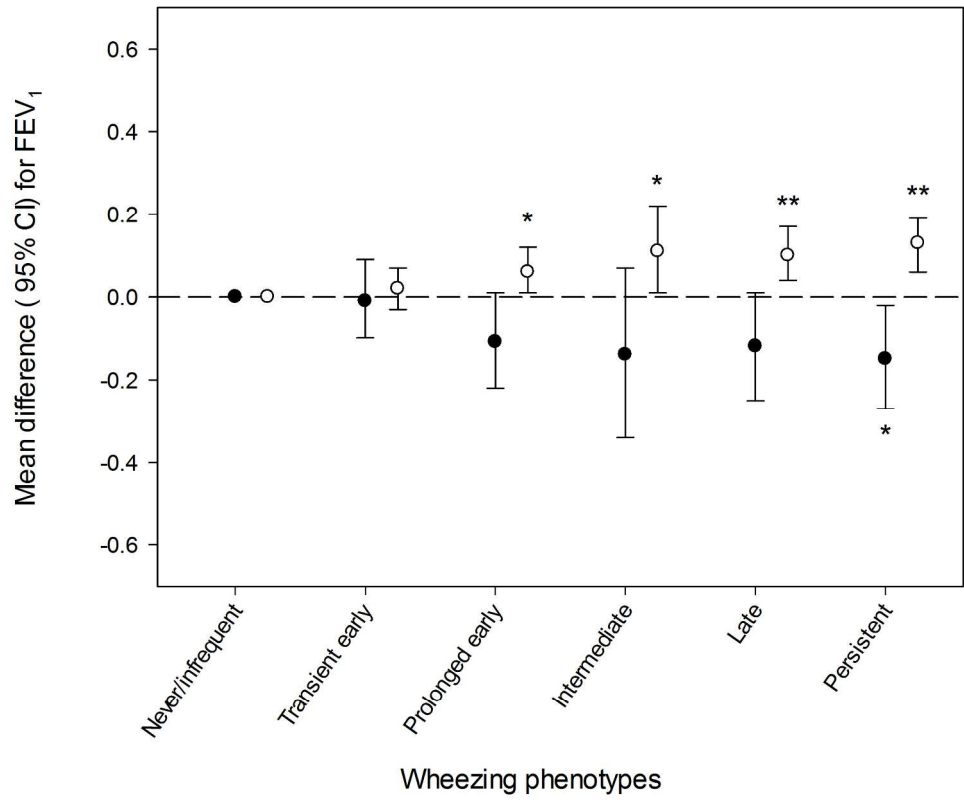41
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Figure 2. Association of wheezing phenotypes with lung function change between mid-childhood (8-9 years) and adolescence (14-15 years).

Values are mean differences of sex-, age- and height-adjusted standard deviation units with their 95% confidence intervals (95% CI), compared with never/infrequent wheeze. FEV_1, forced expiratory volume in 1 second (A); FEV_1/FVC, forced expiratory volume in 1 second / forced vital capacity (B); FEF_{25-75}, mid forced expiratory flow (C). Models are adjusted for maternal age at delivery, parity, anxiety, smoking during pregnancy, history of asthma and allergy, children's sex, low birth weight, preterm birth, breastfeeding and childhood tobacco smoke exposure, and additionally for lung function standard deviations scores in mid-childhood.

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Figure 3. Association of wheezing phenotypes with Fractional exhaled Nitric Oxide (FeNO) in 14-15 year old adolescents.

Values are ratios of geometric means (●) with their 95% confidence interval (95% CI), compared with never/infrequent wheeze, and geometric means (∆). Models are adjusted for maternal age at delivery, parity, anxiety, smoking during pregnancy, history of asthma and allergy, children's sex, low birth weight, preterm birth, breastfeeding and childhood tobacco smoke exposure.
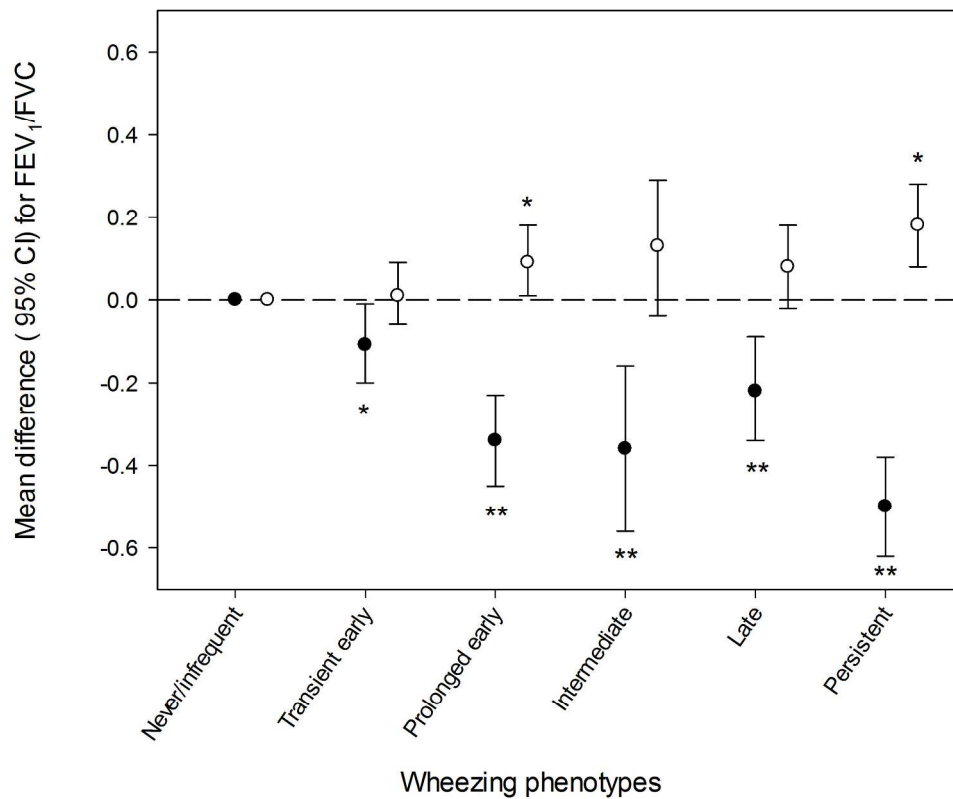
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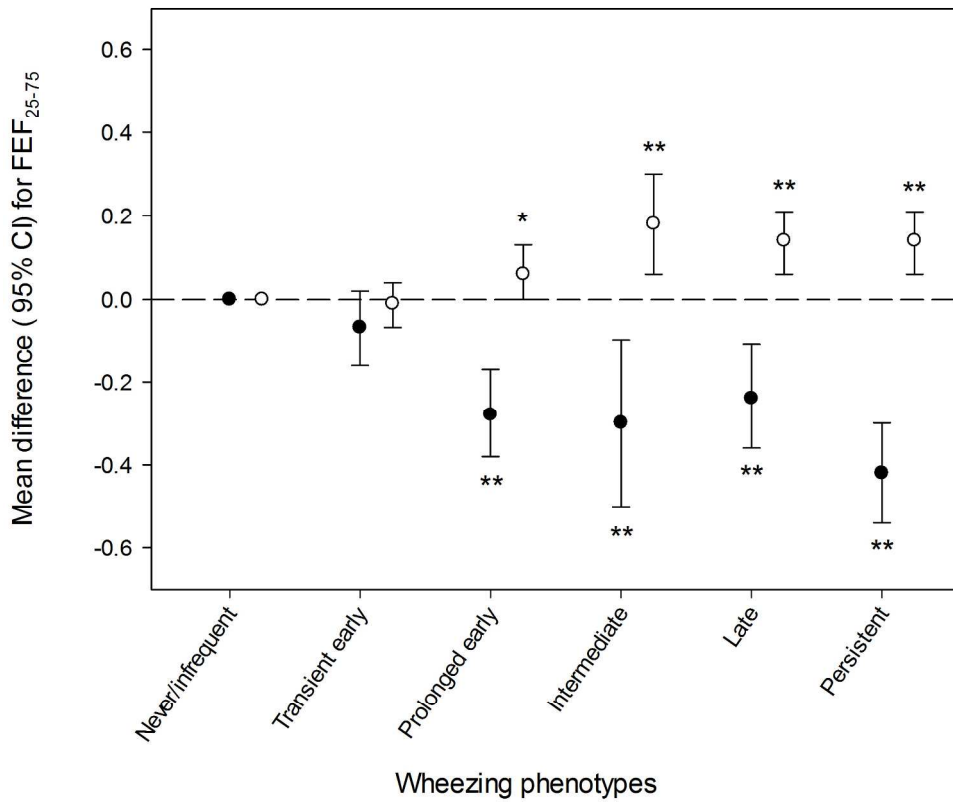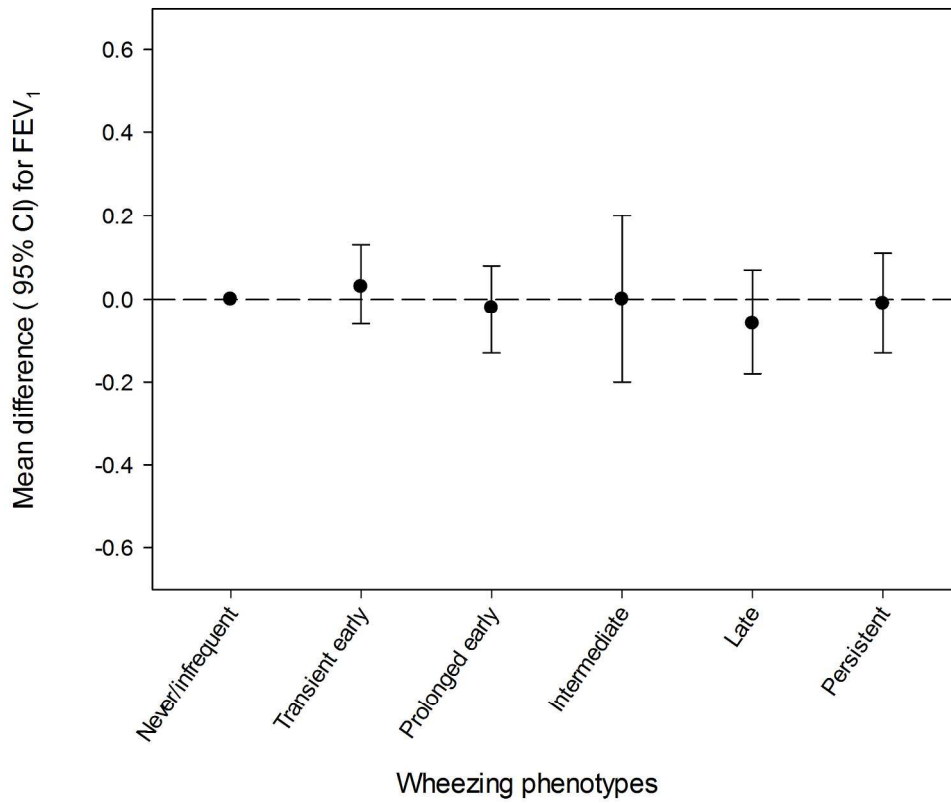206x181mm (300 x 300 DPI)
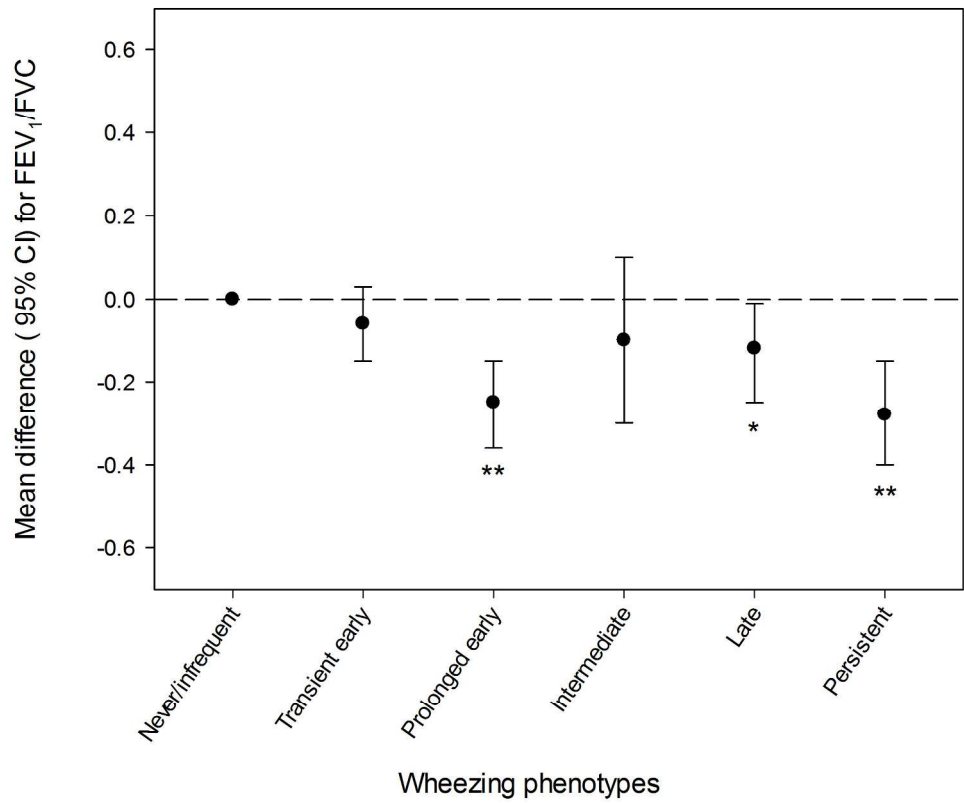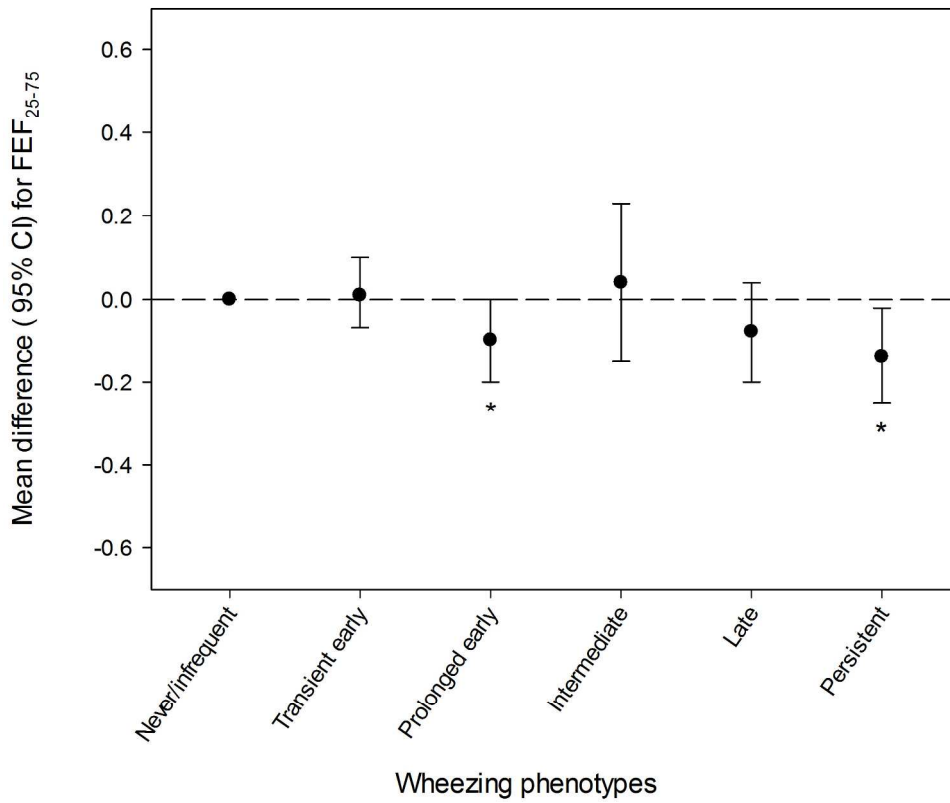
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Online supplementary file

Wheezing phenotypes in the first 7 years of life, fractional exhaled nitric oxide

and lung function in adolescence. The ALSPAC Study.

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Table S1. Association of wheezing phenotypes with physician-diagnosed asthma in 14-15
year old adolescents.

Values are odds ratios (OR) with their 95% confidence Interval (95% CI), compared with
never/infrequent wheeze. Models are adjusted for maternal age at delivery, parity, anxiety,
smoking during pregnancy, history of asthma and allergy, children's sex, low birth weight,
preterm birth, breastfeeding and childhood tobacco smoke exposure.

	Ever physician-diagnosed asthma at 14-15 years	
	n = 5,742	
	n/total (%)	OR (95% CI)
Wheezing phenotype		
Never/infrequent	458/3,589 (12.8)	(1.00 (reference)
Transient early	151/701 (21.6)	1.88 (1.55, 2.30)
Prolonged early	191/527 (36.3)	3.89 (3.17, 4.76)
Intermediate-onset	131/148 (88.5)	52.65 (31.44, 88.18)
Late-onset	232/365 (63.6)	11.91 (9.41, 15.06)
Persistent	350/412 (85.0)	38.88 (29.11, 51.91)

Table S2 (Figure 1). Association of wheezing phenotypes with lung function in 14-15 year old adolescents.

Values are mean differences of sex-, age- and height-adjusted standard deviation units of primary (A) and bronchodilator reversibility (B) lung function measurements with their 95% confidence intervals (95% CI), compared with never/infrequent wheeze. FEV_1, forced expiratory volume in 1 second; FEV_1/FVC, forced expiratory volume in 1 second / forced vital capacity; FEF_{25-75}, mid forced expiratory flow. Models are adjusted for maternal age at delivery, parity, anxiety, smoking during pregnancy, history of asthma and allergy, children's sex, low birth weight, preterm birth, breastfeeding and childhood tobacco smoke exposure.

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	Lung function (SDU) at 14-15 years								
	FEV$_1$ $n = 4,072$			**FEV$_1$/FVC** $n = 4,072$			**FEF$_{25-75}$** $n= 4,145$		
	Total	Mean (SD)	Mean difference (95%CI)	Total	Mean (SD)	Mean difference (95%CI)	Total	Mean (SD)	Mean difference (95%CI)
Phenotype									
Never/infrequent	2,567	0.02 (1.00)	0 (reference)	2,567	0.10 (0.93)	0 (reference)	2,617	0.08 (0.99)	0 (reference)
Transient early	500	0.02 (1.01)	-0.01 (-0.10, 0.09)	500	-0.01 (0.99)	-0.11 (-0.20, -0.01)	509	0.02 (0.99)	-0.07 (-0.17, 0.02)
Prolonged early	373	-0.09 (0.99)	-0.11 (-0.22, 0.01)	373	-0.24 (1.09)	-0.34 (-0.45, -0.23)	377	-0.19 (0.99)	-0.28 (-0.38, -0.17)
Intermediate-onset	96	-0.11 (0.95)	-0.14 (-0.34, 0.07)	96	-0.25 (1.22)	-0.36 (-0.56, -0.16)	97	-0.22 (1.09)	-0.30 (-0.51, -0.10)
Late-onset	261	-0.10 (1.03)	-0.12 (-0.25, 0.01)	261	-0.12 (1.00)	-0.22 (-0.34, -0.09)	264	-0.15 (0.97)	-0.24 (-0.36, -0.11)
Persistent	275	-0.12 (1.05)	-0.15 (-0.27, -0.02)	275	-0.40 (1.24)	-0.5 (-0.62, -0.38)	281	-0.33 (1.04)	-0.42 (-0.54, -0.29)
	Bronchodilator reversibility (SDU) at 14-15 years								
	FEV$_1$ $n = 3,540$			**FEV$_1$/FVC** $n = 3,540$			**FEF$_{25-75}$** $n= 3,701$		
	Total	Mean (SD)	Mean difference (95%CI)	Total	Mean (SD)	Mean difference (95%CI)	Total	Mean (SD)	Mean difference (95%CI)
Never/infrequent	2,225	-0.04 (0.46)	0 (reference)	2,225	-0.02 (0.71)	0 (reference)	2,345	-0.04 (0.57)	0 (reference)
Transient early	443	-0.03 (0.52)	0.02 (-0.03, 0.07)	443	-0.01 (0.88)	0.01 (-0.06, 0.09)	456	-0.05 (0.54)	-0.01 (-0.07, 0.04)
Prolonged early	322	0.02 (0.45)	0.06 (0.01, 0.12)	322	0.07 (0.76)	0.09 (0.01, 0.18)	331	0.03 (0.52)	0.06 (0, 0.13)
Intermediate-onset	83	0.07 (0.57)	0.11 (0.01, 0.22)	83	0.10 (0.59)	0.13 (-0.04, 0.29)	86	0.14 (0.53)	0.18 (0.06, 0.3)
Late-onset	227	0.06 (0.48)	0.10 (0.04, 0.17)	227	0.06 (0.70)	0.08 (-0.02, 0.18)	235	0.10 (0.56)	0.14 (0.06, 0.21)
Persistent	240	0.08 (0.47)	0.13 (0.06, 0.19)	240	0.15 (0.66)	0.18 (0.08, 0.28)	248	0.10 (0.51)	0.14 (0.06, 0.20)

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Table S3 (Figure 2). Association of wheezing phenotypes with lung function change between mid-childhood (8-9 years) and adolescence (14-15 years).

Values are mean differences of sex-, age- and height-adjusted adjusted standard deviation units with their 95% confidence intervals (95% CI), compared with never/infrequent wheeze. FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FEF_{25-75}, mid forced expiratory flow; SD, standard deviation; SDU, standard deviation unit. Models are adjusted for maternal age at delivery, parity, anxiety, smoking during pregnancy, history of asthma and allergy, children's sex, low birth weight, preterm birth, breastfeeding and childhood tobacco smoke exposure, and additionally for lung function standard deviations scores in mid-childhood.

	Change in lung function (SDU) from 8 - 15 years								
	FEV_1 n = 3,471			FEV_1/FVC n = 3,392			FEF_{25-75} n = 3,573		
	Total	Mean (SD)	Mean difference (95%CI)	Total	Mean (SD)	Mean difference (95%CI)	Total	Mean (SD)	Mean difference (95%CI)
Phenotype									
Never/infrequent	2,189	-0.02 (1.07)	0 (reference)	2,138	-0.02 (1.00)	0 (reference)	2,257	-0.04 (0.99)	0 (reference)
Transient early	428	0.09 (1.07)	0.03 (-0.06, 0.13)	418	0.01 (1.01)	-0.06 (-0.15, 0.03)	441	0.06 (0.93)	0.01 (-0.07, 0.10)
Prolonged early	321	0.10 (1.05)	-0.02 (-0.13, 0.08)	314	-0.07 (1.14)	-0.25 (-0.36, -0.15)	326	0.05 (0.92)	-0.10 (-0.20, -0.00)
Intermediate-onset	82	0.16 (1.25)	0.00 (-0.20, 0.20)	79	0.26 (1.43)	-0.10 (-0.20, 0.10)	84	0.28 (1.13)	0.04 (-0.15, 0.23)
Late-onset	218	0.01 (1.13)	-0.06 (-0.18, 0.07)	214	0.03 (1.12)	-0.12 (-0.25, -0.01)	223	0.03 (0.99)	-0.08 (-0.20, 0.04)
Persistent	233	0.12 (1.11)	-0.01 (-0.13, 0.11)	229	0.02 (1.21)	-0.28 (-0.40, -0.15)	242	0.07 (0.92)	-0.14 (-0.25, -0.02)

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Table S4 (Figure 3) Association of wheezing phenotypes with Fractional exhaled Nitric

Oxide (FeNO) in 14-15 year old adolescents.

Values are ratios with their 95% confidence interval (95% CI), compared with

never/infrequent wheeze, and geometric means. Models are adjusted for maternal age at

delivery, parity, anxiety, smoking during pregnancy, history of asthma and allergy, children's

sex, low birth weight, preterm birth, breastfeeding and childhood tobacco smoke exposure.

| | FeNO at 14-15 years | | |
| | *n = 2,767* | | |
	Total	Geometric mean (SD)	Ratio of geometric mean (95%CI)
Phenotype			
Never/infrequent	1.781	26.1 (1.97)	1.00 (reference)
Transient early	342	26.3 (1.99)	0.96 (0.88, 1.05)
Prolonged early	248	26.8 (2.16)	0.94 (0.85, 1.04)
Intermediate-onset	62	54.6 (2.61)	1.93 (1.59, 2.34)
Late-onset	156	43.4 (2.53)	1.60 (1.42, 1.81)
Persistent	178	37.0 (2.34)	1.37 (1.22, 1.54)

Table S5. Association of wheezing phenotypes with clinical cut-off (≥ 20 ppb) of Fractional

exhaled Nitric Oxide in 14-15 year old adolescents.

Values are odds ratios with their 95% confidence intervals (95% CI), compared with

never/infrequent wheeze. Models are adjusted for maternal age at delivery, parity, anxiety,

smoking during pregnancy, history of asthma and allergy, children's sex, low birth weight,

preterm birth, breastfeeding and childhood tobacco smoke exposure.

	FeNO ≥ 20 ppb at 14-15 years	
	n = 2,767	
	n/total (%)	OR (95%)
Phenotype		
Never/infrequent	1,053/1,781 (59.1)	1.00 (reference)
Transient early	204/342 (59.6)	0.97 (0.75, 0.25)
Prolonged early	143/248 (57.7)	0.86 (0.64, 1.16)
Intermediate-onset	47/62 (75.8)	2.31 (1.18, 4.51)
Late-onset	116/156 (74.3)	2.03 (1.36, 3.05)
Persistent	119/178 (66.9)	1.45 (1.01, 2.08)

Table S6. Association of wheezing phenotypes with Fractional exhaled Nitric Oxide and the

use of inhaled corticosteroids in 14-15 year old adolescents.

Values are ratios of geometric means with their 95% confidence intervals (95% CI),

compared with never/infrequent wheeze. Models are adjusted for maternal age at delivery,

parity, anxiety, smoking during pregnancy, history of asthma and allergy, children's sex, low

birth weight, preterm birth, breastfeeding and childhood tobacco smoke exposure. *Missing

data on use of inhaled corticosteroid (ICS) (n = 820).

		FeNO at 14-15 years Ratio of geometric means (95% CI)		
		Total group	**Non - ICS use***	**ICS use***
		n = 2,767	*n = 1,810*	*n = 137*
Phenotype				
Never/infrequent	1,782	1.00 (reference)	1.00 (reference)	1.00 (reference)
Transient early	342	0.96 (0.88, 1.05)	0.98 (0.88, 1.08)	0.73 (0.35, 1.53)
Prolonged early	247	0.94 (0.85, 1.04)	0.97 (0.86, 1.09)	0.47 (0.23, 0.93)
Intermediate-onset	61	1.93 (1.59, 2.34)	1.63 (1.24, 2.15)	1.38 (0.74, 2.57)
Late-onset	156	1.60 (1.42, 1.81)	1.52 (1.31, 1.78)	1.10 (0.60, 2.00)
Persistent	177	1.37 (1.22, 1.54)	1.19 (1.02, 1.39)	0.91 (0.53, 1.55)