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Characterization of Longitudinal Wheeze Phenotypes from Infancy to Adolescence in Project Viva, a Pre-Birth Cohort Study

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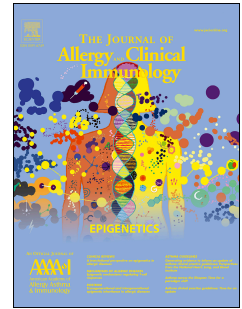
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1 **Characterization of Longitudinal Wheeze Phenotypes from Infancy to Adolescence in** 2 **Project Viva, a Pre-Birth Cohort Study**

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29 **Key words:** Wheeze, wheeze trajectory, longitudinal wheeze phenotype

30 **Capsule summary:** Using data from infancy to adolescence, our analysis of longitudinal
 31 wheeze phenotypes allowed us to visualize four longitudinal wheeze trajectories
 32 (never/infrequent wheeze, mid-childhood onset wheeze, early transient wheeze, and persistent
 33 wheeze).

34

35 *To the Editor:*

36 Longitudinal trajectories of symptom data, particularly for episodic outcomes, are especially
37 useful for capturing dynamic phenotypes that vary by stage of life. Wheeze is a prevalent airway
38 symptom in infancy and early childhood that may be transitory in nature and is known to be an
39 important predictor of later wheeze and development of asthma.(1) While longitudinal wheeze
40 trajectories overlap with asthma case status, patterns of symptom heterogeneity over time may
41 further delineate the dynamics of disease progression and/or remittance of asthma, and may also
42 reveal phenotypes of airways disease that are distinct from clinically diagnosed asthma.

43 Previous studies have characterized wheeze trajectories from infancy to mid-childhood or
44 adolescence.(2–4) The total number of trajectories identified and their specific patterns vary
45 somewhat by population and whether or not children were followed into the teen years.
46 However, most of these longitudinal analyses identified transient, persistent and mid-childhood
47 onset wheeze categories.(2–4) Studies of prenatal and early life exposures that predict wheeze
48 trajectories will ultimately have clinical import, in that they will help identify potential
49 interventions for prevention of adverse wheeze phenotypes (i.e. persistent wheeze). Additional
50 susceptibility factors (including genetic susceptibility) may also contribute to wheeze trajectory
51 outcomes.

52 Our aim was to characterize wheeze trajectories from infancy to adolescence in Project Viva,
53 a Massachusetts based pre-birth cohort study,(5) and to determine associations between early life
54 factors and select genetic variants with these longitudinal phenotypes. This work is an extension
55 of our previous wheeze trajectory analysis in Project Viva that assessed wheeze annually on nine

occasions up to a mean age of 9.3 years (range 9 to 11 years for final time point).(6) In the current study, we included wheeze assessments at three additional adolescent time points (mean ages of 13.2, 14.4 and 15.4 years) which may be critical transition periods in expression of asthma symptoms. Caregivers responded yes or no to the question “Since your child was [age at last annual questionnaire], had he/she ever had wheezing (or whistling in the chest)?”. We analyzed data from 1,033 participants (9,704 observations) who had data on at least one wheeze outcome from three different time windows (less than 5 years, 5-10 years, and over 10 years of age) resulting in a median number of 10 wheeze assessments (IQR = 8 to 11). Characteristics of all study subjects are shown in Supplemental Table 1. (Characteristics for full study cohort vs. those with longitudinal wheeze trajectories is shown in Supplemental Table 2). We imputed missing independent predictor variables to optimize power (see supplemental methods), and used the imputed data in our trajectory analysis. We characterized wheeze trajectories using a semi-parametric latent class growth model (LCGM), as implemented in SAS (v 9.4) PROC TRAJ. Intercept and slope parameters were estimated as fixed effects for each individual trajectory, and polynomial terms were tested to allow for flexibility in trajectory modeling (i.e. changes in the direction and/or magnitude of the trajectory over time). We selected the optimum number of trajectories for our cohort by considering factors such as model fit (AIC/BIC), sufficient participant numbers within each trajectory group, posterior inclusion probabilities, and biological plausibility of the trajectories (see supplemental table 3). We sequentially tested polynomial terms (quadratic, then cubic), but kept only those polynomial terms that reached statistical significance at $p < 0.05$. We examined prenatal and early life factors and single nucleotide polymorphisms (SNPs) as predictors of being in a particular wheeze trajectory group using multinomial logistic regression. Relationships between SNPs and wheeze trajectories were

assessed in participants of non-Hispanic white race/ethnicity who had genotype data (N=459); sample sizes were insufficient to perform genetic association analyses in the other racial/ethnic groups.

Our final LCGM trajectory model showed four independent latent trajectories of wheeze: never/infrequent wheeze (62%), mid-childhood onset wheeze (10%), early transient wheeze (22%), and persistent wheeze (6%) (Figure 1). The mid-childhood onset wheeze category that emerged in this analysis of infancy through adolescence had not been previously noted in our analysis through age 9.(2) Mean posterior probabilities for each class were high (0.91 for never/infrequent wheeze, 0.85 for mid-childhood onset wheeze, 0.82 for early transient wheeze and 0.90 for persistent wheeze). Stratification by sex produced very similar wheeze trajectories for males vs. females (data not shown).

In addition to characterizing the wheeze trajectories themselves, we also examined how complementary phenotypes assessed at the “early teen” study visit (mean age 12.9 years) were distributed across the wheeze trajectory groups. Using ANOVA we observed significant differences in IgE and FeNO levels across the wheeze trajectory groups (p values <0.0001 for both biomarkers). Compared to participants in the never/infrequent wheeze trajectory, those grouped within the mid-childhood onset and persistent wheeze trajectories had elevated levels of the airway inflammatory biomarker FeNO (increases of 81% (95% CI 54% to 112%) and 60% (95% CI 30% to 95%), respectively, as compared to the never/infrequent wheeze category). Those in the mid-childhood onset wheeze category also had elevated total serum IgE (increases of 206% (95% CI 99% to 369%) as compared to never/infrequent wheeze). Supplementary Figures 1 and 2 show plots of these biomarkers by group on the natural log scale. Levels of FeNO were similar for the persistent wheeze and mid-childhood onset wheeze groups (p=0.5 for

comparison), however there was suggestive association for higher IgE levels in the mid-childhood onset vs. persistent wheeze groups ($p=0.07$ for comparison). Median levels and interquartile ranges for these biomarkers by trajectory group are shown in supplemental table 4. Prevalence of current asthma assessed at the early teen visit (defined as mother's report of a doctor's diagnosis of asthma plus report of current wheeze or asthma medication use) was high amongst individuals classified into the mid-childhood onset and persistent wheeze trajectories (81% and 87% respectively), but low for those in the transient wheeze (6%) and never/infrequent wheeze (5%) trajectories.

We identified a number of early life factors associated with the wheeze trajectories (Table 1). As expected, maternal history of asthma was associated with the persistent and mid childhood onset wheeze trajectories and also showed an association with transient wheeze. Participants with an early atopic phenotype (diagnosis of eczema in infancy) were also more likely to fall into the trajectory for mid-childhood onset wheeze. Bronchiolitis in infancy showed the strongest associations with the two wheeze trajectories with the highest rates of wheeze in early life (early transient wheeze and persistent wheeze), but was also associated with mid-childhood onset wheeze. Acetaminophen intake in pregnancy (categorized as no intake, 1-9 times in pregnancy and 10 or more times in pregnancy), which has previously been associated with early and mid-childhood asthma in our cohort, showed odds ratios above one for all wheeze trajectories compared to the never/infrequent trajectory, with statistically significant odds ratios for both mid-childhood onset and persistent wheeze. Participants of black or "other" race/ethnicity were at increased risk for persistent wheeze. We did not observe clear associations between maternal smoking patterns and wheeze trajectory group. Since bronchiolitis may be an intermediary on the causal pathway between breastfeeding and wheeze trajectory outcome (see directed acyclic

graph (DAG) in Supplemental Figure 3), we did not include the bronchiolitis variable in our model that assessed breastfeeding as an independent predictor of wheeze trajectory (Model 2 in Table 1). Breastfeeding duration was not associated with wheeze trajectory group.

In our prior analysis of wheeze trajectories (up to age 9), we observed interactions of sex with race or with parental history of asthma when predicting odds of trajectory phenotypes. While we did not detect any interactions for race and sex in the current analysis, we did detect a potential interaction of sex by paternal history of asthma ($p=0.05$). Male participants with a paternal history of asthma were more likely to fall into the “mid childhood onset” wheeze category (OR =2.40, 95% CI 1.07 to 5.37) as compared to female participants (OR=1.07, 95% CI 0.34 to 3.35). We did not see a later onset trajectory with an increase of incident asthma in females, which in some other studies has been detected in follow-up of participants beyond the age of our cohort.

With respect to genetic predictors of our wheeze trajectories, we chose to interrogate the top two genetic loci (*GSDMB/ORMDL3* and *CDHR3*) from a compendium of twenty-five asthma risk alleles.(7) This analysis focused only on SNPs selected *a priori* rather than testing SNPs from across the genome. From the chromosome 17q21 *GSDMB/ORMDL3* locus, we tested two polymorphisms (rs7216389 in *GSDMB* and a proxy SNP (rs12603332) for rs4065275 in *ORMDL3* ($R^2=0.98$), which has been shown to regulate IL-2 production in T cells).(8) For *CDHR3* we tested a proxy SNP (rs10258293) for rs6967330 ($R^2=0.85$). In addition to its association with asthma risk, this variant in the *CDHR3* locus also confers increased risk of susceptibility to viral respiratory infections, a leading cause of wheeze in early life.(9) We hypothesized that the *GSDMB/ORMDL3* risk variants would be associated with the two wheeze trajectories with the largest percentages of individuals with current asthma in early adolescence (persistent wheeze and mid-childhood onset wheeze) and that the *CDHR3* risk variant would

confer increased risk of the trajectories with higher rates of wheeze in early life (transient and persistent wheeze). Using an additive genetic model, we observed an increased odds (OR=2.22 per risk allele, 95%CI= 1.04 to 4.77) of persistent wheeze for the proxy *ORMDL3* variant rs12603332 (C allele), but no clear association for transient or mid-childhood onset wheeze phenotypes. The *GSDMB* and *CDHR3* polymorphisms were not associated with any wheeze patterns. Of note, the *CDHR3* polymorphism is related to increased susceptibility to human rhinovirus C (RV-C) specifically, but not to other common respiratory viral infections (RV-A, RV-B and RSV),(11) which are also triggers for wheeze in early life. Since we do not have data on specific types of respiratory infection in early life, we were unable to account for this risk factor in our analysis.

In summary, the persistent wheeze trajectory observed in our cohort was strongly associated with parental history of asthma, non-white race/ethnicity, bronchiolitis in early life, and a genetic polymorphism in a known asthma susceptibility loci (*ORMDL3*). The mid-childhood wheeze trajectory showed associations with atopic phenotypes (eczema in infancy and total serum IgE in adolescence). For the transient wheeze phenotype, the strongest measure of association was for bronchiolitis in infancy; however it is important to note that bronchiolitis was associated with all of the more adverse wheeze trajectories (mid-childhood, persistent and transient) as compared to never/infrequent wheeze

In conclusion, using data from infancy to adolescence, our analysis of longitudinal wheeze phenotypes allowed us to visualize four longitudinal wheeze trajectories, including one not previously observed for Project Viva in a past trajectory analysis up to age 9 years. The trajectories observed had some common early life predictors (maternal asthma, bronchiolitis), as

well as unique predictors, such as an *ORMDL3* genetic variant (for persistent wheeze in white children) and atopic phenotypes (for mid-childhood wheeze onset).

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Figure 1 Legend:

Figure 1. Longitudinal wheeze trajectories from infancy to adolescence (N=1033 participants, 9704 observations)

Table 1. Multinomial Multiple Logistic Regression Results for Prenatal/Early Life Factors, Genetic Variants and Wheeze Phenotypes (vs. Never/Infrequent Wheeze)

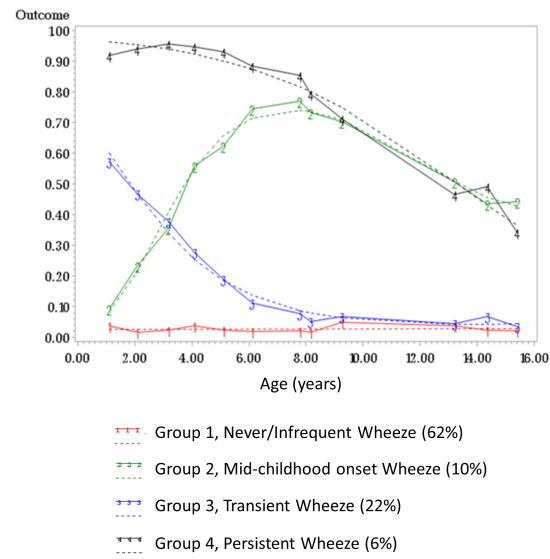
	Never/infrequent N=637	Wheeze trajectory group (Total N=1033)		
		Mid-childhood onset N=100	Transient N=232	Persistent N=64
	Adjusted OR (95% CI)			
Model 1 ^a				
Mother/father				
Pregnancy smoking status (v. never)				
. Former	1.0 (ref)	1.10 (0.64, 1.89)	1.06 (0.71, 1.58)	0.86 (0.39, 1.87)
. Smoked during pregnancy	1.0 (ref)	1.04 (0.48, 2.25)	0.62 (0.33, 1.19)	0.54 (0.18, 1.64)
Prenatal acetaminophen intake (per unit increase in score)**	1.0 (ref)	1.42 (1.04, 1.94)	1.27 (1.00, 1.60)	1.60 (1.01, 2.53)
Maternal asthma (yes v. no)	1.0 (ref)	1.92 (1.03, 3.61)	2.15 (1.32, 3.49)	5.87 (2.85,12.12)
Paternal asthma (yes v. no)	1.0 (ref)	1.81 (0.96, 3.42)	1.28 (0.75, 2.18)	4.10 (1.90, 8.85)
Child				
Female (v. male)	1.0 (ref)	0.60 (0.38, 0.92)	0.50 (0.36, 0.69)	0.63 (0.34, 1.16)
Child race/ethnicity (v. white)				
. Black	1.0 (ref)	1.54 (0.83, 2.87)	0.88 (0.51, 1.50)	4.08 (1.78, 9.38)
. Hispanic	1.0 (ref)	3.87 (1.53, 9.78)	2.48 (1.12, 5.51)	2.33 (0.53,10.30)
. Other	1.0 (ref)	1.19 (0.61, 2.30)	1.12 (0.69, 1.81)	3.63 (1.68, 7.83)
Bronchiolitis in infancy (yes v. no)	1.0 (ref)	2.67 (1.12, 6.33)	9.26 (5.28,16.25)	33.22 (14.83,74.42)
Eczema in infancy (yes v. no)	1.0 (ref)	2.11 (1.30, 3.43)	1.19 (0.78, 1.79)	1.85 (0.93, 3.68)
Model 2 ^b				
Breastfeeding duration (per month)	1.0 (ref)	1.00 (0.95, 1.05)	0.97 (0.93, 1.00)	0.95 (0.90, 1.02)
Model 3 ^c				
Genetic variants (among white children only)	N=297	N=32	N=113	N=17
GSDMB rs7216389, (T allele)	1.0 (ref)	1.36 (0.80, 2.29)	1.19 (0.87, 1.62)	1.86 (0.90, 3.87)
ORMDL3 rs12603332 (C allele), (proxy for rs4065275)	1.0 (ref)	1.37 (0.80, 2.32)	1.12 (0.82, 1.53)	2.22 (1.04, 4.77)
CDHR3 rs10258293 (C allele), (proxy for rs6967330)	1.0 (ref)	1.07 (0.54, 2.14)	1.06 (0.71, 1.59)	0.90 (0.38, 2.12)

^aAll characteristics included in the multiple regression model with adjustment for breastfeeding; reference category for wheeze trajectories was never/infrequent wheeze

^bAdjusted for Model 1 characteristics (with the exception of bronchiolitis); reference category for wheeze trajectories was never/infrequent wheeze *Prenatal

Acetaminophen Intake Score (0=Never; 1=1-9 times; 2 = greater than or equal to 10 times) ^cModel adjusted for genotype principal components; Genetic Variant analysis conducted only in subset of participants of white race/ethnicity with genotype data (N=459); ORs for additive genetic effect

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SUPPLEMENT

Supplemental Methods*Project Viva Cohort Description.*

Project Viva is a prospective pre-birth cohort study. Mothers were recruited 1999-2002 at their first prenatal appointment at Atrius Harvard Vanguard Medical Associates, a multispecialty group practice in Massachusetts. This cohort consisted of 2128 singleton live born infants. At enrollment mothers completed questionnaires reporting history of maternal and paternal asthma, and maternal smoking habits. On postpartum questionnaires, mothers reported child race/ethnicity (“other” race ethnicity included participants whose mothers reported “other” for race/ethnicity, those with reported Asian or Amerindian race/ethnicity, and those with a report of “more than one race”). We obtained child sex and date of birth from hospital birth records. At in-person and mailed visits, mothers were invited to respond to a questionnaire about the child (including diagnosis of bronchiolitis, eczema and breastfeeding duration) at birth, six months, one year and at approximately one-year intervals thereafter. Breastfeeding total duration was quantified as number of months the infant was breastfed. For eczema, we asked mothers at the 1-year visit whether they have ever been told by a health care professional that their infant had eczema (atopic dermatitis).

Of the 2128 participants at enrollment, 1033 had sufficient data for wheeze trajectory analysis through adolescence (a comparison of characteristics of full cohort vs those included in the wheeze trajectory analysis is shown in Supplemental Table 4; participants with wheeze trajectory analysis were comprised of a higher percentage of whites, and fewer were exposed to maternal smoking in pregnancy).

Imputation of Independent Variables and Covariates for Statistical Analysis

We imputed missing independent predictor variables in the final model to optimize power. We used Proc MI in SAS version 9.4 (Cary, NC) and imputed 50 values for each missing observation to create 50 “completed” datasets. We combined multivariable modeling estimates from multinomial logistic regression using Proc MIANALYZE.

Fractional Exhaled Nitric Oxide (FeNO) Measurement at Early Teen Visit

Nitric oxide (NO) is a mediator involved in chronic inflammatory diseases and Th2-mediated immune responses. Measurement of NO in exhaled air, as fraction exhaled nitric oxide (FeNO), is a non-invasive biomarker of airway inflammation that correlates with airway eosinophilia. In 827 participants, we measured FeNO levels with a portable electrochemical device (NIOX MINO; Aerocrine AB, Stockholm, Sweden), which is in agreement with published procedures for FeNO measurement. Before FeNO measurement, participants breathed in through a nitric oxide scrubbing filter and exhaled out into the room air twice before inhaling a third time through the filter and exhaling into the FeNO analyzer. Exhaled FeNO measurements were conducted without a nose clip at a flow rate of 50 mL/s. (The last 3 seconds of the exhalation was used for FeNO measurement to ensure quantification of lower rather than upper airway FeNO). In accordance with American Thoracic Society guidelines, we did not use nose clips because blocking the nasal passages can cause exhaled nitric oxide to accumulate in the nose and potentially leak into the exhaled air stream through the posterior nasopharynx. This procedure was performed twice, so that each subject had two FeNO measurements. The mean of these two measurements was used in data analysis. FeNO was log-transformed prior to data analysis.

IgE Measurement at Early Teen Visit

Of the 1033 participants with sufficient wheeze data for trajectory analysis, there were 577 who also had assessment for total serum IgE. Total serum IgE was log-transformed prior to data analysis. Total serum IgE was measured ImmunoCAP (Phadia, Uppsala, Sweden) and levels were compared across wheeze trajectory groupings. Specific IgEs were also assessed, however the study of specific sensitization patterns by wheeze trajectory is beyond the scope of this analysis. Sensitization rates for specific allergens (specific IgE levels ≥ 0.35 IU/ml) at the early teen visit were as follows: 58% were sensitized to dust mite allergen (*D. Farinae*), 42% to cat, 38% to dog, 7% to *Aspergillus*, 11% to *Alternaria*, 43% to oak pollen, 42% to silver birch, 32% to rye grass, and 26% to common ragweed.

Genotyping.

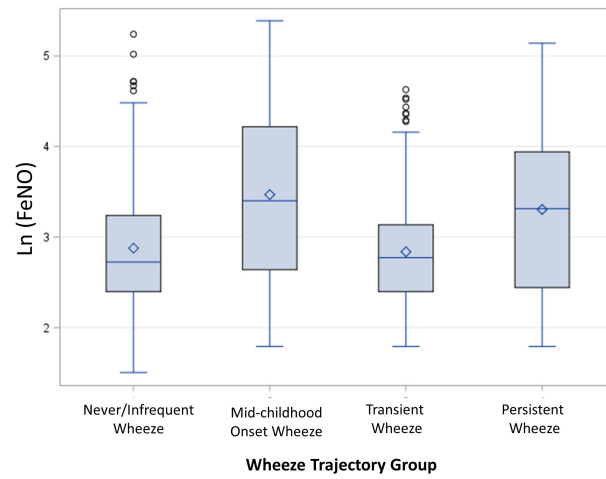
Genotyping was performed at the Channing Division of Network Medicine using Illumina Illumina MEGAex microarray chips (Illumina, Inc., San Diego, CA). All of the SNPs considered met basic genotype quality control standards (minor allele frequency $\geq 1.0\%$, Hardy Weinberg equilibrium of at least 0.0001, and a SNP call rate of $\geq 98\%$).

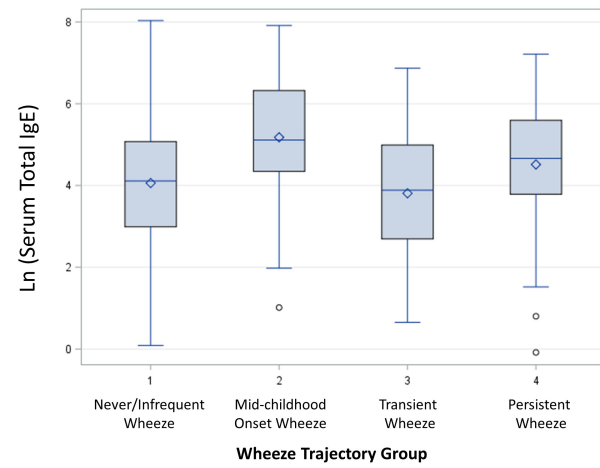
Supplemental Figure Legends:

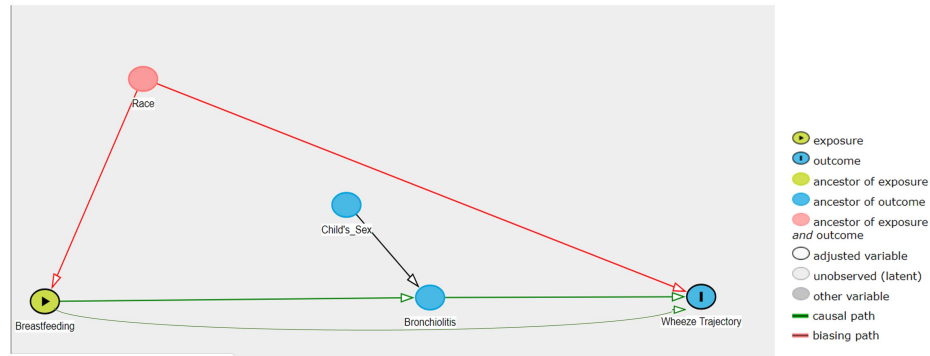
Supplemental Figure 1. Distributions of log-transformed FeNO at Early Teen Visit (average age 12.9 years) by wheeze trajectory group (N=827 participants)

Supplemental Figure 2. Distributions of log-transformed total serum IgE at the Early Teen Visit (average age 12.9 years) by wheeze trajectory group (N=577 participants)

Supplemental Figure 3. Directed Acyclic Graph (DAG) showing the causal pathway from breastfeeding to wheeze trajectory







Supplemental Table 1. Characteristics of participants overall (based on multiple imputation values) and according to wheeze trajectory grouping (N=1033)*

Characteristic	Overall	Never/ infrequent	Wheeze trajectory group		
			Mid-childhood onset	Transient	Persistent
	N=1033	637 (61.7%)	100 (9.7%)	232 (22.5%)	64 (6.2%)
%					
Mother/father					
Pregnancy smoking status					
. Never	71	72	68	69	72
. Former	21	20	22	23	19
. Smoked during pregnancy	8	8	10	8	9
Maternal Asthma					
. No	88	91	84	85	69
. Yes	12	9	16	15	31
Maternal Acetaminophen Intake in Pregnancy					
. Never	302	32	23	26	24
. 0-9 times	500	47	49	50	53
. >10 times	231	21	29	24	24
Paternal Asthma					
. No	89	91	85	89	74
. Yes	11	9	15	11	26
Child					
Female					
. No	51	45	58	63	56
. Yes	49	55	42	37	44
Child race/ethnicity					
. White	68	72	61	69	44
. Black	13	13	17	10	23
. Hispanic	4	3	8	6	5
. Other	14	13	13	14	28
Bronchiolitis in infancy					
. No	87	96	90	71	48
. Yes	13	4	10	29	52
Eczema in infancy					
. No	77	80	65	78	62
. Yes	23	20	35	22	38
Breastfeeding duration, months: Mean (SD)	6.6 (4.5)	6.8 (4.5)	6.7 (4.7)	6.2 (4.5)	5.9 (4.5)

*Variables include multiple imputed data

Supplemental Table 2. Characteristics of full cohort vs. participants with longitudinal wheeze trajectory categorization.

Characteristic	All Participants* N=2128	Participants with Wheeze Trajectory N=1033
		%
<i>Mother/father</i>		
Pregnancy smoking status**		
. Never	68	71
. Former	19	21
. Smoked during pregnancy	12	8
Maternal Asthma		
. No	86	88
. Yes	13	12
Maternal Acetaminophen Intake in Pregnancy		
. Never	28	28
. 0-9 times	43	46
. >10 times	22	22
Paternal Asthma		
. No	85	89
. Yes	11	11
<i>Child</i>		
Female		
. No	52	51
. Yes	48	49
Child race/ethnicity**		
. White	63	68
. Black	17	13
. Hispanic	6	4
. Other	13	14

*Data reflects missing values **p<0.05 for comparison

Supplemental Table 3. Model Criteria and Groupings for Trajectory Models

Model	AIC	BIC	Participant N per group	Group Mean Posterior Probability
3 Trajectory Model	-3338.92	-3366.09	Group 1=649 Group 2=228 Group 3=156	Group 1=0.92 Group 2=0.80 Group 3=0.93
4 Trajectory Model	-3285.40	-3319.98	Group 1=637 Group 2=100 Group 3=232 Group 4=64	Group 1=0.91 Group 2=0.85 Group 3=0.82 Group 4=0.90
5 Trajectory Model	-3294.44	-3341.37	Group 1=183 Group 2=509 Group 3=120 Group 4=25 Group 5=196	Group 1=0.84 Group 2=0.81 Group 3=0.93 Group 4=0.79 Group 5=0.78

Supplemental Table 4. Distributions of IgE and FeNO for each of the wheeze trajectory groups

	Wheeze trajectory group			
	Never/infrequent	Mid-childhood onset	Transient	Persistent
	637 (61.7%)	100 (9.7%)	232 (22.5%)	64 (6.2%)
<i>Log-transformed IgE Levels (median (IQR))</i>	61.0 (19.9 to 160.0)	166.0 (77.0 to 556.0)	48.7 (14.8 to 147.0)	106.0 (44.1 to 269.0)
<i>Log-transformed FeNO Levels (median (IQR))</i>	15.5 (11.0-25.5)	30.0 (15.0 to 68.0)	14.5 (11.0 to 22.5)	29.5 (12.0 to 59.5)

Journal Pre-proof