Outcome of Asthma and Wheezing in the First Six Years of Life: Follow-up through Adolescence

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Online Data Supplement

Online Methods

Subject Characteristics and Assessment of Wheeze

Healthy infants were enrolled at birth in the Tucson Children's Respiratory Study in Tucson, Arizona (n=1246) between 1980 and 1984 [E1E,2]. Parents of participating subjects were patients of Group Health Medical Associates, a large health maintenance organization in Tucson, Arizona, and were contacted shortly after their children were born. At the time of enrollment, parents completed a questionnaire describing their ethnicity, history of physician-diagnosed asthma, years of education, current age and smoking history. They were instructed to take their child to the pediatrician whenever the child had any of a defined set of signs and symptoms of lower respiratory tract illness (LRI; deep or "wet" chest cough, wheezing, hoarseness, stridor, or shortness of breath). The pediatricians obtained a detailed history at the time of such illnesses and recorded all relevant signs and symptoms (including wheezing on auscultation). The occurrences of lower respiratory tract illnesses were followed prospectively for the first three years of life. If the subject had at least one physician confirmed wheezing lower respiratory illness in the first three years of life they were considered to have wheezed before age three.

When the children reached a mean age of 6.3 years (SD=0.9), parents again answered a questionnaire about the child's respiratory illnesses. In that questionnaire, current wheezing was defined as at least one episode of wheezing during the previous year.

Four phenotypes characterizing preschool age wheeze were then defined according to the presence or absence of 'at least one physician-diagnosed wheezing lower respiratory illness in the first three years of life' and 'parent-reported wheeze during the past year for the child at age

six': no wheeze from birth to age 6 (never wheezers), wheezing LRI before age 3 only (transient early wheezers), wheeze at age 6 only (late onset wheezers) and wheezing LRI before age 3 and wheeze at age 6 (persistent wheezers) [E3]. The current study was limited to subjects with complete wheeze information through age 6 (n=826).

In the current study, the prevalence of wheeze at ages 8 (mean 8.6 years (SD=0.7), n=672, 11 (10.9 years (SD=0.6), n=767), 13 (13.5years (SD=0.6), n=582) and 16 (16.6years (SD=0.6), n=617) was obtained from a parent-completed questionnaire that asked if the child had 'ever wheezed' and 'how often during the past year?' had the child wheezed, similar to the previous assessment at age 6. Wheeze was categorized into three groups at each age: no wheeze, infrequent wheeze (1-3 episodes) or frequent wheeze (≥4 episodes) during the previous year.

Pulmonary Function Tests

As reported previously, during the first year of life, 176 infants underwent pulmonary function testing (PFT)[E3]. A detailed description of the selection criteria and the medical and social characteristics of these infants, as compared with those who were not tested, was reported earlier[E4]; the frequency of a family history of asthma or allergies did not differ significantly between the infants who underwent pulmonary-function testing and those who were not tested. Of the 176 infants initially tested, 125 were tested before any lower respiratory tract illness occurred; complete follow-up data to the age of six years were available for these infants. Their mean age at the time of testing was 2.4 months (SD=2.0).

Partial expiratory flow-volume curves were obtained by the chest-compression technique [E5]. Briefly, informed consent was obtained from the parents, and the children were usually sedated with chloral hydrate (50 to 60 mg per kilogram of body weight). A plastic bag connected to a pressure reservoir was tightly wrapped around the child's chest and abdomen. A mask connected to a pneumotachygraph was sealed around the child's mouth and nose, and tidal flow-volume loops were displayed on a monitor. At end-tidal inspiration, the bag was rapidly inflated to a known pressure, compressing the child's chest and forcing air out of the lungs. The flow at the end-tidal expiration point was recorded from the forced flow-volume loops. This maneuver was repeated with increments in pressure of 5 to 10 cm of water. The maximal pressure applied to the thorax was the pressure at which no further increase in flow was obtained; this value -- the maximal expiratory flow at functional residual capacity ($V'_{max}FRC$, expressed in milliliters per second) -- was recorded and used in the analysis. $V'_{max}FRC$ is believed to reflect the size of the intrapulmonary airways. At the time of the six-year survey, partial expiratory flow-volume curves were obtained with maneuvers to measure voluntary maximal expiratory flow[E6]. Tidal flow-volume loops were recorded on a computer screen as described above. As the child approached end-tidal inspiration, he or she was encouraged to expel air forcefully, and a partial flow-volume curve was obtained. $V'_{max}FRC$ was calculated from at least three acceptable expirations; the highest value obtained was used in our analyses.

V′maxFRC

Pulmonary function tests at age 11 were performed with a custom-built pneumotach-based system running software on a portable computer and PFTs at age 16 were performed with a portable Schiller Spirovit SP-1 (Schiller AG, Baar, Switzerland). Both systems were calibrated

with a Jones syringe (Jones Medical Instruments Co, Oak Brook, IL). Forced vital capacity (FVC, milliliters), forced expiratory volume in one second (FEV_1, milliliters) and the forced expiratory flow between 25-75% of the FVC (FEF_{25-75}, milliliters/second) were measured[E7].

Atopy

Children were skin prick tested at ages 6, 11 and 16. Extracts of six common aeroallergens in the Tucson area and diluent were obtained from Hollister-Stier Laboratories (Everett, WA, USA) including: *Alternaria alternata*, Bermuda grass (*Cynodon dactylon*), careless weed (*Amaranthus palmeri*), and olive (*Olea europaea*), mesquite (*Prosopis glandulosa*) and mulberry trees (*Morus alba*) (Hollister-Stier Laboratories, Everett, Washington). All tests were read at 20 min and the sizes of the wheals elicited by each allergen were recorded as the sum of the 2 diameters at right angles to each other (mm). Wheal sizes of 3 mm or more after subtraction of diluent control size were considered positive. A child was considered atopic if he or she had at least one positive skin-test reaction to an aeroallergen. Parents were skin prick tested to the same allergens when the child was six years old. Total serum IgE (IU/ml) was measured by Phadebas Radioimmunosorbent Test (Pharmacia Diagnostics, Piscataway, NJ) at age 11 and by Autocap (Pharmacia) at age 16. Complete peripheral blood counts were performed at ages 11 and 16 and the percent of circulating eosinophils was recorded.

Statistics

PFT measures included $V'_{max}FRC$ (ml/s) at ages 2.4 months and 6 years, and forced vital capacity (FVC, ml), forced expiratory volume in one second (FEV_1, ml) and the forced

expiratory flow between 25-75% of the FVC (FEF_{25-75},ml/s) at ages 11 and 16. All PFT measures were approximately normally distributed.

Multiple regression was used to test for differences in lung function between the preschool wheeze phenotypes at ages 11 and 16 adjusting for current wheeze, height, weight, age and gender. In addition, to take into account the serial correlation between the paired lung function values at ages 11 and 16 and the data from subjects with lung function measured at only one time (11 or 16) we used a random effects model (general linear mixed model)[E8]. Time dependent covariates included in the model were height, weight, age and current wheeze. The preschool wheeze phenotypes and gender were the fixed covariates.

Z-scores of forced expiratory flows were calculated for the cross sectional analyses of flow at ages 2.4 months, 6, 11 and 16 years for the preschool wheeze phenotypes in order to make the two measures of flow comparable. $V'_{max}FRC$ at age 2.4 months and 6 years were adjusted for height as previously described [E3] and these values were z-scored for this analysis. FEF_{25-75} at ages 11 and 16 years were adjusted for height in a multiple regression and the standardized residuals (z-scored residuals) used as the outcome measure. Oneway analysis of variance with Bonferroni's multiple range test was used to assess differences between groups.

To test whether there was a significant change in flow (slope) between ages 6 and 16 another random effects model was used with z-scored flow as the outcome. Time dependent covariates included in the model were height, weight and age. Fixed covariates were preschool wheeze phenotypes, gender and frequent wheeze. Frequent wheeze at ages 11 and 16 was defined as: no

frequent wheeze at either age (11−16−), frequent wheeze at age 11 only (11+16−), frequent wheeze at age 16 only (11−16+), and frequent wheeze at both ages (11+16+); children with infrequent wheeze were combined with the no wheeze group and children with missing wheeze information were included in a separate category. An age by preschool wheeze phenotype interaction term was included in the random effects model to assess changes in flow between ages 6 and 16.

Proportions were compared using contingency tables and chi-square statistics. Generalized estimating equations (GEE) were used to assess the relation between the preschool wheeze phenotypes and longitudinal wheeze at ages 8, 11, 13 and 16[E9].

References

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Table E1. Background characteristics of study subjects with and without pulmonary function testing (PFT) at ages 11 and/or 16 and preschool wheeze information.

Characteristics of the Study Subjects	Preschool Wheeze information and PFT % (total n)	Preschool Wheeze information and no PFT % (total n)	Incomplete Preschool Wheeze information with or without PFT % (total n)	Chi-square p value
Enrolled Child Characteristics:				
Male	50.9 (595)	45.5 (231)	48.8 (420)	2df, p=0.4
Preschool Wheeze:	(595)	(231)		
Never	49.2	57.1	-	
Transient early	20.5	18.2	-	
Late onset	16.0	12.6	-	
Persistent	14.3	12.1	-	3df, p=0.2
Wheeze at age 11:	(586)	(176)	(187)	
No	71.7	75.0	76.5	
Infrequent	18.1	13.6	17.6	
Frequent	10.2	11.4	5.9	4df, p=0.2
Wheeze at age 16:	(495)	(111)	(129)	
No	72.3	82.0	74.4	
Infrequent	20.2	12.6	18.6	
Frequent	7.5	5.4	7.0	4df, p=0.3
Skin Test Positive Age 6	40.0 (543)	34.4 (108)	32.7 (110)	2df, p=0.2
Parental Characteristics Assessed at Enrollment:				
Anglo/White Parents	60.5 (595)	66.7 (231)	52.4 (420)*	2df, p=0.001
MD Diagnosed Asthma:				
Maternal	10.6 (587)	12.3 (227)	10.9 (341)	2df, p=0.8
Paternal	13.6 (560)	12.3 (219)	9.2 (315)	2df, p=0.2
Smoking:				
Maternal	15.5 (595)	17.8 (230)	20.8 (418)*	2df, p=0.09
Paternal	28.1 (588)	31.9 (226)	35.9 (412)*	2df, p=0.03
Age ≤28years:				
Maternal	59.7 (595)	53.7 (231)	68.5 (419)*	2df, p<0.001
Paternal	40.0 (587)	42.5 (228)	57.2 (414)*	2df, p<0.001
Education >12 years:				
Maternal	72.3 (595)	72.6 (230)	60.1 (416)*	2df, p<0.001
Paternal	73.2 (583)	75.8 (227)	63.1 (407)*	2df, p<0.001

*p<0.05 compared to children with complete preschool wheeze information through age 6 as previously published[3]

Table E2. Multvariate results for FEF_{25-75} (ml/s), FEV_1 (ml) and FEV_1/FVC ratio (%) at ages 11 and 16 for the preschool wheeze phenotypes, current wheeze and gender; adjusted for height, weight, and age. Model-based predicted coefficients represent deviations from the reference group, for example, persistent wheezers had a predicted FEF_{25-75} that was 200ml/s lower than never wheezers at age 11 and if they were also frequent wheezers at age 11, predicted FEF_{25-75} was 353 ml/s lower than children with no wheeze during the previous year, for an estimated total deficit of 553ml/s. Lung function reference values for male never wheezers with no current wheeze at the mean weight (40.6kg), height (145cm) and age (10.9years) for the 537 subjects included in the model at age 11 were: 2434ml/sec for FEF_{25-75}, 2305ml for FEV_1 and 83.1% for the FEV_1/FVC ratio. Similarly, the age 16 the lung function reference values, calculated at the mean weight (67.3kg), height (171cm) and age (16.7years) for the 418 subjects included in the model at age 16 were: 3763ml/sec for FEF_{25-75}, 3751ml for FEV_1 and 82.5% for the FEV_1/FVC ratio. The preschool wheeze phenotypes were defined as follows: no wheeze from birth to age 6 (never wheeze), wheezing LRI before age 3 only (transient early wheeze), wheeze at age 6 only (late onset wheeze) and wheezing LRI before age 3 and wheeze at age 6 (persistent wheeze). Infrequent wheeze was defined as 1-3 episodes of wheeze during the previous year and frequent wheeze was defined as 4 or more episodes of wheeze during the previous year.

	Age 11 (n=537)					
	FEF_{25-75} (ml/s) Coefficient (95%CI)	p	FEV_1 (ml) Coefficient (95%CI)	p	FEV_1/FVC (%) Coefficient (95%CI)	p
Preschool Wheeze Phenotypes:						
Never Wheeze	Ref		Ref		Ref	
Transient Early	-191 (-321 to -60)	0.004	-52 (-110 to 5.9)	0.08	-1.2 (-2.6 to 0.07)	0.07
Late Onset	-59 (-208 to 91)	0.4	-21 (-88 to 45)	0.5	-0.1 (-1.6 to 1.4)	0.9
Persistent	-200 (-363 to -36)	0.02	-89 (-162 to -16)	0.02	-1.5 (-3.1 to 0.2)	0.08
Current Wheeze:						
No Wheeze	Ref		Ref		Ref	
Infrequent	-164 (-302 to -25)	0.02	-57 (-119 to 4.2)	0.07	-1.3 (-2.7 to 0.1)	0.06
Frequent	-353 (-538 to -170)	<0.001	-99 (-181 to -17)	0.02	-3.8 (-5.7 to -2.0)	<0.001
Gender:						
Male	Ref		Ref		Ref	
Female	86 (-16 to 188)	0.1	-57 (-102 to -12)	0.01	2.0(0.9 to 3.0)	<0.001

	Age 16 (n=418*)					
	FEF_{25-75} (ml/s) Coefficient (95%CI)	p	FEV_1 (ml) Coefficient (95%CI)	p	FEV_1/FVC (%) Coefficient (95%CI)	p
Preschool Wheeze Phenotypes:						
Never Wheeze	Ref		Ref		Ref	
Transient Early	-348 (-542 to -153)	0.001	-96 (-197 to 4.9)	0.06	-2.9 (-4.4 to -1.3)	<0.001
Late Onset	-115 (-338 to 107)	0.3	-19 (-132 to 95)	0.7	-1.0 (-2.9 to 0.6)	0.2
Persistent	-272 (-512 to -31)	0.03	-67 (-191 to 58)	0.3	-2.9 (-4.9 to -1.0)	0.003
Current Wheeze:						
No Wheeze	Ref		Ref		Ref	
Infrequent	-56 (-252 to 140)	0.6	-109 (-210 to -7.9)	0.04	0.6 (-1.0 to 2.2)	0.4
Frequent	-564 (-876 to -252)	<0.001	-214 (-376 to -53)	0.01	-4.5 (-7.0 to -2.0)	0.001
Gender:						
Male	Ref		Ref		Ref	
Female	-58 (-255 to 140)	0.6	-297 (-399 to -195)	<0.001	2.4 (0.8 to 4.0)	0.003

* N=415 for FEF_{25-75} at age 16.

Table E3. Inhaled steroid use by children with wheeze during the previous year at ages 11 and 16.

		Inhaled Steroid Use % (n+)		
Wheeze during the previous year:	n	Occasionally	Frequently	Daily
Age 11				
Infrequent	130	7.7 (10)	1.5 (2)	1.5 (2)
Frequent	80	6.3 (5)	5.0 (4)	7.5 (6)
Age 16				
Infrequent	111	3.6 (4)	0.9 (1)	0.9 (1)
Frequent	41	9.8 (4)	7.3 (3)	0.0 (0)