Sex-related differences in pulmonary physiologic outcome measures in a high-risk birth cohort

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Background: Sex influences the risk of wheezing illnesses and the prevalence of asthma throughout childhood.
Objective: To better understand the mechanisms of these effects, we analyzed longitudinal relationships between sex, lung physiology, and asthma in the Childhood Origins of ASThma birth cohort study.
Methods: Childhood Origins of ASThma birth cohort study children were followed prospectively from birth and assessed annually. Results of spirometry, fractional exhaled nitric oxide (FENO), mannitol provocation testing, and ^{3}He gas magnetic resonance imaging were assessed by sex using multivariate models including age, asthma diagnosis, and wheezing histories.
Results: Girls had higher prebronchodilator forced expiratory volume in 0.5 seconds/forced vital capacity values than did boys (mean difference, 0.017; 95% CI, 0.000-0.034; $P = .05$) of equivalent age. Postbronchodilator findings were more pronounced, with boys demonstrating reduced forced expiratory volume in 0.5 seconds/forced vital capacity values than did girls of equivalent age (mean difference, 0.032; 95% CI, 0.014-0.049; $P = .0005$). Conversely, girls were noted to have higher ventilation defects on ^{3}He magnetic resonance imaging than did boys ($P = .01$). No differences were noted in the rate of positive responses to mannitol provocation or FENO measurements.
Conclusions: Lower airflow values are present by spirometry for prepubertal boys than for age-matched girls; however, greater ^{3}He ventilation defects were noted in girls. This could represent a greater degree of subclinical air trapping in prepubertal girls because residual volumes are not detected on standard spirometric readings. No differences were noted between the 2 sexes with airway hyperresponsiveness (mannitol provocation testing) or inflammation (FENO). Prospective peripubertal follow-up will determine whether these differences persist or change with the *de novo* expression and remission of asthma based on sex and age. (J Allergy Clin Immunol 2015;136:282-7.)

Key words: *Sex, gender, asthma, pulmonary physiology, spirometry, helium MRI, ventilation defects, mannitol, airway hyperreactivity, fractional exhaled nitric oxide*

Previous studies have demonstrated sex differences in asthma over time, with males exhibiting a higher prevalence early in life.[1] This finding reverses sometime during the time of puberty, and then females develop higher incidence and prevalence rates that persist into the adult years. Mechanisms responsible for these findings remain elusive, but could be related to changes in lung physiology related to sex and age.

Childhood Origins of ASThma (COAST) is a prospective high-risk birth cohort study designed to evaluate the interactions among age, patterns of immune dysfunction, and viral infections with respect to the subsequent development of asthma and allergic diseases.[2] Previously, we identified sex differences in patterns of immune development (as measured by cytokine responses of PBMCs) that were associated with wheezing illnesses. In children with recurrent wheezing during the first 3 years of life, boys were found to have increased levels of IFN-γ, IL-5, and IL-13 responses at age 3 years compared with age-matched girls.[3] Boys also had increased rates of allergic sensitization, total IgE levels, and peripheral eosinophil counts at age 3 years.[3] Genotyping of the IFN-γ single nucleotide polymorphisms by Loisel et al[4] identified an interaction by sex in which male heterozygotes for specific single nucleotide polymorphisms (rs2069727 and rs2430561) had the highest risk for asthma development, with heterozygote girls conversely showing the lowest risk. These findings suggest specific genotype-by-sex effects on asthma risk that are associated with distinct cytokine response profiles.[4]

Other research groups have further evaluated prepubertal physiologic sex differences in pulmonary function,[5] fractional

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Abbreviations used
COAST: Childhood Origins of ASThma
FENO: Fractional exhaled nitric oxide
$FEV_{0.5}$: Forced expiratory volume in 0.5 seconds
FVC: Forced vital capacity
MRI: Magnetic resonance imaging

exhaled nitric oxide (FENO),[6] and methacholine airway responsiveness.[7] These findings indicate that sex affects several aspects of lung physiology. To prospectively identify relationships among sex, lung physiology, and asthma, we longitudinally evaluated spirometry and FENO within the COAST study, and, in addition, conducted cross-sectional analysis of mannitol airway responsiveness and structure function relationships using hyperpolarized ^{3}He gas magnetic resonance imaging (MRI).

METHODS

After obtaining informed consent, 289 participants were enrolled at birth in the COAST study beginning in November 1998 and ending in May 2000. At least 1 parent was required to have respiratory allergies and/or physician-diagnosed asthma. A total of 285 participants remained enrolled at 1 year, with 259 enrolled at year 6 and 217 enrolled at year 11 (Table I). Details of the study design have been described previously.[2,8] The University of Wisconsin Human Subjects Committee approved the study.

Children were diagnosed with asthma if they fulfilled at least 1 of the following criteria[9]: (1) physician-diagnosed asthma; (2) frequent albuterol use for coughing or wheezing episodes as prescribed by a physician; (3) use of a prescribed daily controller medication; (4) an implemented step-up plan, including the use of albuterol or inhaled corticosteroids during illness as prescribed by a physician; and (5) use of prednisone for an asthma exacerbation.

Spirometry testing procedures

Spirometry was performed using the Jaeger Masterscope System on study participants aged 4 to 11 years (n = 240). Reproducibility and acceptability standards developed by the American Thoracic Society and modified through the Childhood Asthma Research and Education Network were followed.[10,11]

Fractional exhaled nitric oxide

FENO testing was performed on study participants aged 5 to 11 years (n = 226). Measurements (in parts per billion) were performed via the online NIOX system according to American Thoracic Society standards adapted for children.[12] The expiratory flow rate was 0.05 L/s, with exhalation times of at least 6 seconds. *Acceptability* was defined as 3 measurements within 10% or 2 measurements within 5%. Measurements were taken before the performance of spirometry.

Mannitol bronchoprovocation

Mannitol bronchoprovocation was performed at age 11 years (171 participants attempted, 144 completed). Dry powder mannitol (Aridol; Pharmaxis, Frenchs Forest, Australia) was administered in cumulative doses (0, 5, 10, 20, 40, 80, 160, 160, 160 mg). A *positive challenge* was defined as the dose causing a 15% fall in FEV_1.[13]

Magnetic resonance imaging

Pulmonary MRI with inhaled ^{3}He gas was performed on children aged 9 to 10 years (n = 44) as previously described.[14] Briefly, 19 female and 25 male participants were recruited from the highest and lowest quartiles of FEV_1 measured at age 8 years ($FEV_1 \geq 109.9\%$ of predicted value or $FEV_1 \leq 91.4\%$ of predicted value) and from those with or without a moderate-to-severe rhinovirus illness before age 1 year in approximately equal numbers. Quartile cutoffs were selected to increase the chances of observing differences in imaging among the mild asthma phenotypes of the cohort. ^{3}He was polarized using spin-exchange optical pumping in a commercial polarizer (HeliSpin; GE Healthcare, Durham, NC). The polarized ^{3}He gas (~30% polarization) was diluted with nitrogen to produce a volume adjusted for each subject (14% of the subjects' total lung capacity). Imaging studies were performed on a 1.5-T clinical magnetic resonance scanner (SignaHDx; GE Healthcare, Waukesha, Wis) with broadband imaging capability. Fast MRI acquisitions were used to limit breath-hold time to 15 seconds or less. The anoxic dose of ^{3}He and N_2 did not cause any adverse effects. A ventilation defect was assigned a score on the basis of the extent of lobar involvement (1, <25%; 2, 26% to 50%; 3, 51% to 75%; 4, at least 76% of a lobe). The total ventilation defect score used for analysis was the summation of scores across the whole lung.

Statistical analysis

Spirometry measurements were compared by sex using longitudinal mixed effect models, with fixed effects for year, sex, "asthma ever" (ages 6, 8, or 11 years) diagnosis, and their interactions and a random effect for participant to account for repeated measures over the years. FENO measurements were compared by sex using a longitudinal mixed effect model, with fixed effects for sex, year, asthma ever, and their interactions and a random effect for participant. Mannitol bronchoprovocation data were compared by sex and current asthma using logistic regression. Ventilation defect scores were square-root transformed for analysis and compared by sex using linear models, with sex, current asthma, and total lung capacity as covariates. In the absence of significant sex-by-asthma and sex-by-year interactions, the *P* value for the main effect for sex is reported. A 2-sided *P* value of less than .05 was regarded as statistically significant.

RESULTS

Spirometry

For spirometric values obtained between the ages 5 and 11 years, girls had higher prebronchodilator forced expiratory volume in 0.5 seconds ($FEV_{0.5}$)/forced vital capacity (FVC) values than did boys (mean difference, 0.017; 95% CI, 0.000-0.034; $P = .05$) Postbronchodilator findings were more pronounced, with boys demonstrating reduced $FEV_{0.5}$/FVC values than did girls of equivalent age (mean difference, 0.032; 95% CI, 0.014-0.049; $P = .0005$). Similar sex findings were noted for FEV_1/FVC (postbronchodilator mean difference, 0.015; 95% CI, 0.002-0.028; $P = .02$). There was no evidence that the sex difference varied over time (sex-by-year interaction *P* values >.2). There was no evidence that the effect of asthma differed between girls and boys (asthma-by-sex interaction *P* values >.2) (Fig 1).

Fractional exhaled nitric oxide

FENO measurements increased significantly from ages 6 to 11 years for both sexes ($P < .0001$); however, there were no significant differences noted between boys and girls ($P = .49$) (Fig 2). The effect of asthma on FENO did not differ significantly between boys and girls (sex-by-asthma interaction $P = .12$), and there was no evidence of a sex-by-year interaction ($P = .68$). Before puberty, FENO measurements were higher in both sexes for children with asthma who also had concurrent allergic sensitization ($P = .0009$).

Mannitol bronchoprovocation

Of 171 children who attempted the mannitol challenge, 144 children completed the procedure, resulting in a procedure

TABLE I. Demographic characteristics of COAST subjects

Characteristic	Girls		Boys	
Asthma ever (6, 8, or 11 y)	No	Yes	No	Yes
N	74	38	84	63
Maternal age at birth (y)	31.7 ± 5.3	31.5 ± 5.2	31.5 ± 4.4	30.7 ± 5.3
White	89	74	88	90
Black	4	18	4	8
Hispanic or Latino	3	8	2	2
Other	4	0	6	0
Breast-fed at birth	91	82	92	95
Breast-fed at age 3 mo	73	63	75	76
Passive smoke exposure year 1	24	39	20	22
Positive mannitol challenge year 11*	22	38	20	35
Ventilation defect score year 9/10†	11.5 ± 8.6	17.7 ± 10.3	6.4 ± 6.5	10.8 ± 6.8
FENO year 6	9.2 ± 8.8	12.7 ± 9.7	10.9 ± 8.5	10.4 ± 7.9
FENO year 11	14.1 ± 8.9	25.6 ± 24.5	16.9 ± 15.0	21.4 ± 23.9
FEV_1/FVC pre-BD year 5	0.90 ± 0.06	0.90 ± 0.07	0.92 ± 0.04	0.89 ± 0.07
FEV_1/FVC pre-BD year 11	0.83 ± 0.07	0.81 ± 0.08	0.82 ± 0.06	0.79 ± 0.06
$FEV_{0.5}$/FVC pre-BD year 5	0.70 ± 0.08	0.69 ± 0.06	0.70 ± 0.07	0.67 ± 0.07
$FEV_{0.5}$/FVC pre-BD year 11	0.64 ± 0.08	0.62 ± 0.08	0.62 ± 0.06	0.58 ± 0.08
FEV_1/FVC post-BD year 6	0.90 ± 0.06	0.94 ± 0.04	0.93 ± 0.05	0.97 ± 0.01
FEV_1/FVC post-BD year 11	0.86 ± 0.06	0.87 ± 0.04	0.85 ± 0.04	0.81 ± 0.07
$FEV_{0.5}$/FVC post-BD year 6	0.73 ± 0.11	0.73 ± 0.06	0.72 ± 0.09	0.75 ± 0.05
$FEV_{0.5}$/FVC post-BD year 11	0.66 ± 0.06	0.68 ± 0.05	0.64 ± 0.05	0.61 ± 0.10

Values are % or mean ± SD.
BD, Bronchodilation.
*Grouped by asthma diagnosis at the time of mannitol challenge visit.
†Grouped by asthma diagnosis at the time of imaging visit.

completion success rate of 84%. Out of all participants with asthma (n = 57), 23 had a positive mannitol challenge, corresponding to a sensitivity of 40%. Out of all subjects without asthma (n = 87), 69 had a negative challenge, corresponding to a specificity of 79%. In terms of airway hyperresponsiveness, there were no sex differences observed in positive mannitol responses performed at age 11 years ($P = .75$) (sex-by-asthma interaction $P = .97$) (Fig 3).

^{3}He MRI and ventilation defect scores

Participants diagnosed with asthma had a higher ventilation defect score ($P = .03$) than did participants without asthma. Girls were noted to have a higher ventilation defect scores than did boys ($P = .01$) regardless of asthma diagnosis status (sex-by-asthma interaction $P = .68$) (Fig 4).

DISCUSSION

Sex strongly influences the risk of wheezing illnesses and the incidence and prevalence of asthma throughout childhood. Although some of these effects can be attributed to sex-related effects on immune development,[3] the goal of the present study was to define longitudinal associations between sex and pulmonary physiologic outcome measures. It has been previously established that during fetal development, girls demonstrate earlier lung development and maturation, with surfactant production and mature lung phospholipid profiles occurring ahead of their male counterparts.[1] In addition, compared with boys, large airway growth in girls outpaces surrounding parenchymal development.[1] The end result is that young males have narrower airways than do young females shortly after birth. The impact of these early life airway alterations has not clearly been linked to phenotypic manifestations (such as asthma) in the developing child and adolescent, though they have spurred ongoing interest in determining their influence on disease outcomes.

Our data showing higher postbronchodilation $FEV_{0.5}$/FVC and FEV_1/FVC in girls than in boys are consistent with the notion that boys may have narrower airways relative to lung volume, and are consistent with studies from multiple cohorts reporting higher FEV_1/FVC in girls.[15] Our data also show that a diagnosis of asthma at any age is associated with an additional reduction in postbronchodilation $FEV_{0.5}$/FVC and FEV_1/FVC that is similar in boys and girls. Rasmussen et al[16] reported reduced postbronchodilation FEV_1/FVC ratios in 18- and 26-year-olds with a history of asthma in a cohort that had been followed since age 9 years. The lowest ratios were associated with male sex, airway hyperresponsiveness, and lower prebronchodilation FEV_1/FVC at younger ages. Belgrave et al[17] reported similar patterns of higher specific airway resistance in males and in children with a diagnosis of asthma; however, they did not report findings of postbronchodilator assessment. In a 4- to 6-year follow-up of asthmatic children in the Childhood Asthma Management Program, deterioration of postbronchodilation FEV_1/FVC was greatest in those children with the greatest airflow limitation at the time of enrollment and in those with eosinophilic inflammation.[18] Thus, our data show that this indicator of progressive airflow limitation is already present at age 5 years. Continued monitoring of the COAST cohort through adolescence will provide a more complete picture of how the asthmatic airways evolve from childhood to adulthood.

FENO has been used as a surrogate marker to assess airway inflammation in asthma.[19] Buchvald et al[6] looked to establish normative values for FENO through a multicenter study measuring FENO in 405 healthy subjects (191 boys and 214 girls) between the

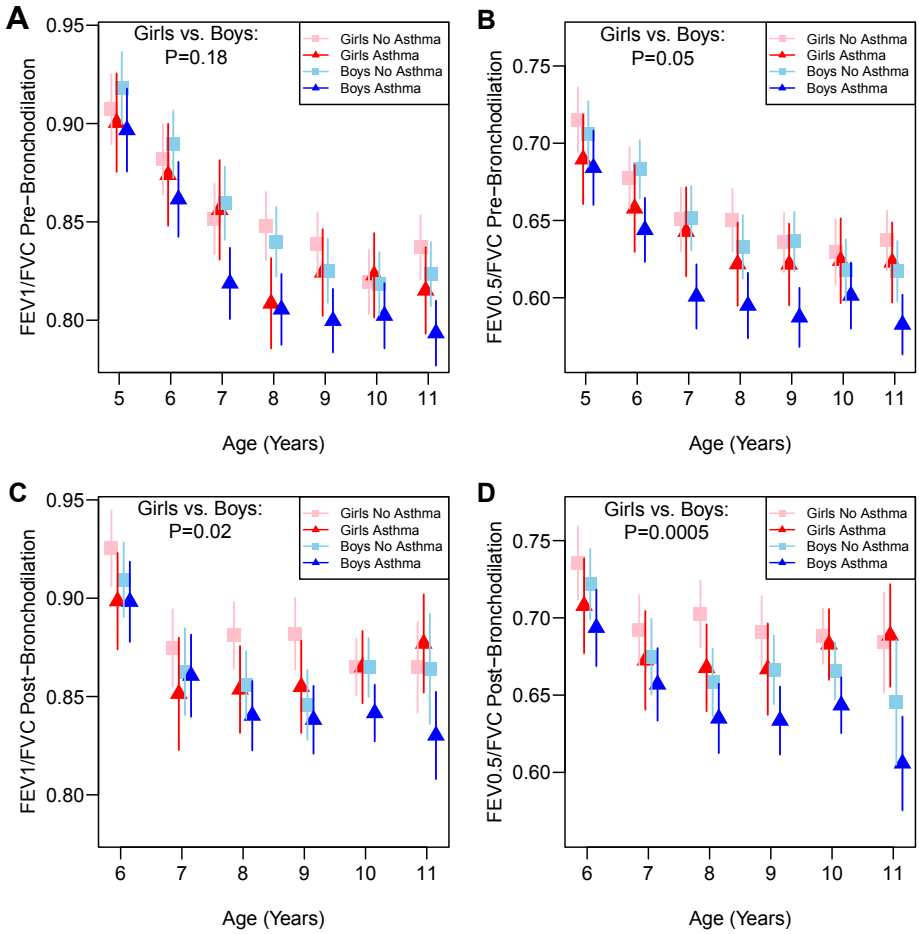

FIG 1. A-D, Spirometry. Effects of sex and asthma diagnosis on pre- and postbronchodilation $FEV_{0.5}$/FVC and FEV_1/FVC in children aged 5 to 11 years.

ages of 4 and 17 years. Although they found that FENO increased significantly with age for both sexes, they found no difference in measured FENO values between sexes. These data were echoed in our high-risk cohort; FENO values increased with age but did not vary by sex.

Airway hyperresponsiveness can be evaluated using a number of different bronchoprovocation agents, and increased responsiveness is a characteristic physiologic feature of asthma. Using methacholine as the challenging agent, Tantisira et al[7] in the Childhood Asthma Management Program reported similar results for methacholine PC_{20} values in boys and girls before puberty, but a significant divergence in reactivity (boys became less reactive) postpuberty. In contrast to methacholine, which acts directly on bronchial smooth muscle, mannitol is an indirect challenge agent that appears to affect airway diameter through the creation of a hyperosmolar environment,[20] and was chosen for use in COAST because of the thought that it is a more specific test of airway responsiveness than methacholine. In COAST, mannitol had low sensitivity (40%) and modest specificity (79%) for asthma diagnosis. Similar to results reported with methacholine, we found no sex differences in mannitol airway responsiveness before puberty. It will be of interest to determine whether sex differences also emerge postpuberty using indirect airway challenge in the COAST cohort.

^{3}He MRI of ventilation is a useful tool for viewing subclinical degrees of airway narrowing and gas trapping without the need for exposure to harmful radiation. The size and extent of *local ventilation defects*, defined as lung regions with limited or no hyperpolarized gas signal intensity, has been shown to correlate with asthma severity and decreased lung function.[21] Previously within the COAST population, Cadman et al[14] used both gas spin density and diffusion-weighted helium MRI techniques to show that children with a history of asthma had lower mean gas diffusion lengths (a measure proportional to acinar size) and increased ventilation defect scores than did those without asthma. These associations were also true for children with early life wheezing due to rhinovirus infections.[14] In the present study, we extended these findings and demonstrated the presence of distinct sex differences, noting that girls demonstrated higher ventilation defect scores than did boys ($P = .01$) regardless of asthma diagnosis status (Fig 4). These findings suggest a greater degree of subclinical airway narrowing and air trapping in prepubertal girls than that found in age-matched boys. The higher defect scores in girls may foreshadow more pronounced decreases in lung function and worsening asthma severity in girls than in boys postadolescence. Thus, this innovative imaging modality may offer early detection of regional airway narrowing that cannot be detected by standard spirometry. Prospective

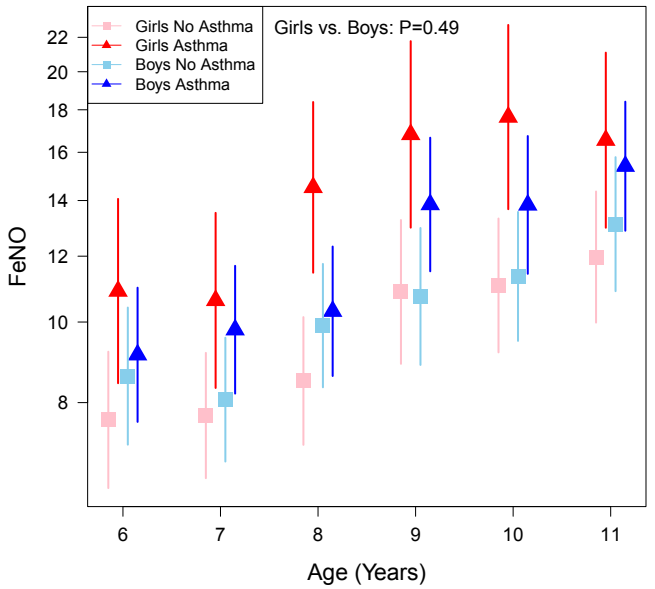

FIG 2. FENO. No significant differences in FENO were noted between sexes for ages 6 to 11 years.

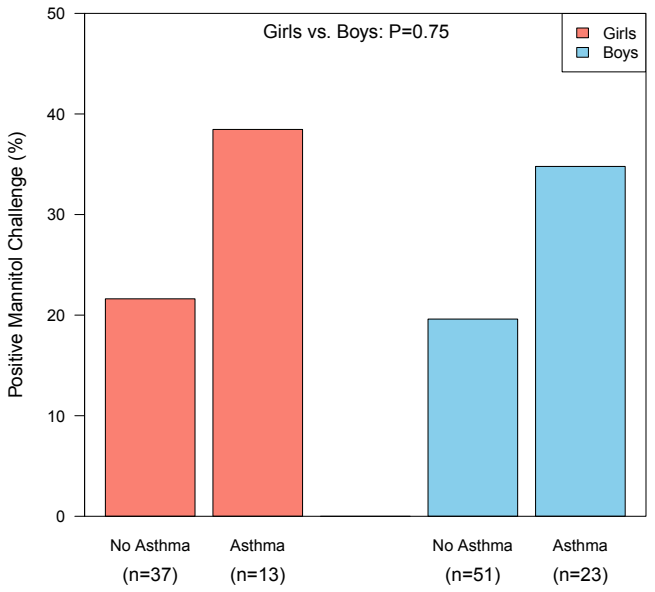

FIG 3. Mannitol challenge. No significant differences were noted between sexes for positive mannitol challenges regardless of asthma status.

follow-up extending through puberty is needed to determine whether these differences persist or change on the basis of sex and age.

In conclusion, the findings of our study confirm previous work in healthy and asthmatic populations noting prepubertal sex differences in selected pulmonary physiologic outcome measures. In addition, our findings expand on these observations by using different methods of bronchoprovocation (ie, mannitol) and novel imaging techniques (eg, ventilation defect assessment). As the children participating in this study enter adolescence, pre- and postpubertal airway physiologic evaluations will provide additional insights as to how the sex-specific changes in lung

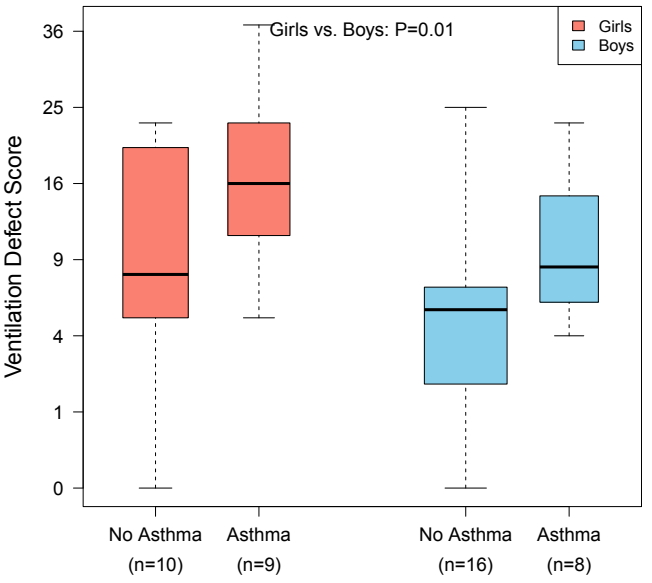

FIG 4. ^{3}He MRI ventilation defect scores. Girls had higher ventilation defect scores than did boys, regardless of asthma diagnosis.

physiology identified in this study track with the expression, remission, and progression of asthma.

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Clinical implications: The sex of an individual influences the inception, remission, and severity of asthma throughout life. The results of this study provide insight into sex-related physiologic differences that accompany the clinical developmental expression of asthma during childhood and adolescence.

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