

Association Between Menarche and Increased Bronchial Hyper-Responsiveness During Puberty in Female Children and Adolescents

Young-Ho Kim, MD,¹ Eun Lee, MD, PhD,² Hyun-Ju Cho, MD,³ Song-I Yang, MD, PhD,⁴
 Young-Ho Jung, MD, PhD,⁵ Hyung Young Kim, MD,⁶ Ju-Hee Seo, MD,⁷ Hyo-Bin Kim, MD, PhD,⁸
 So-Yeon Lee, MD, PhD,⁴ Dae-Jin Song, MD, PhD,⁹ Woo-Kyung Kim, MD, PhD,¹⁰
 Gwang-Cheon Jang, MD, PhD,¹¹ Jung-Yeon Shim, MD, PhD,¹² Eun-Jin Kim, PhD,¹³
 Joo-Shil Lee, PhD,¹⁴ Ji-Won Kwon, MD, PhD,^{15*} and Soo-Jong Hong, MD, PhD^{3**}

Summary. Purpose: Bronchial hyper-responsiveness (BHR) is a key feature of asthma. The degree of BHR in children may be altered by several factors. We evaluated the prevalence of BHR according to age and gender in pediatric and adolescent population and analyzed the associated factors for gender differences. Methods: Among the 2,067 subjects, methacholine challenge tests were performed in 1,820 children from one elementary and one middle school in Seoul, Korea. A total of 1,725 subjects between 6 and 14 years old were included in the analysis. The prevalence of BHR, defined as a provocative concentration that induced a 20% reduction of FEV₁ (PC₂₀) that was less than 8 mg/ml, was evaluated according to age and gender. Gender differences associated with BHR prevalence at each age were calculated and multiple logistic regression analyses were performed to identify factors associated with BHR by gender. Results: The prevalence of BHR (PC₂₀ ≤ 8 mg/ml) for each gender decreased with age ($P < 0.001$). Although the prevalence of BHR linearly decreased in males with age, females showed an increase after 11 years of age. BHR in males was associated with a younger age (aOR, 0.797; 95%CI, 0.678–0.925), a higher blood eosinophil counts (%) (aOR, 1.160; 95%CI, 1.047–1.284), atopy (aOR, 2.091; 95%CI, 1.003–4.359), and a lower FEV₁/FVC ratio (aOR, 0.947; 95%CI,

¹Department of Pediatrics, Gyeongsang National University Changwon Hospital, Changwon, Korea.

²Department of Pediatrics, Inje University Haeundae Paik Hospital, Busan, Korea.

³Department of Pediatrics, Childhood Asthma Atopy Center, Environmental Health Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

⁴Department of Pediatrics, Hallym University Sacred Heart Hospital, Anyang, Korea.

⁵Department of Pediatrics, CHA University School of Medicine, Seongnam, Korea.

⁶Department of Pediatrics, Pusan National University Yangsan Hospital, Yangsan, Korea.

⁷Department of Pediatrics, Dankuk University Hospital, Cheonan, Korea.

⁸Department of Pediatrics, Sanggye Paik Hospital, Inje University College of Medicine, Seoul, Korea.

⁹Department of Pediatrics, Korea University Guro Hospital, Seoul, Korea.

¹⁰Department of Pediatrics, Seoul Paik Hospital, Inje University College of Medicine, Seoul, Korea.

¹¹Department of Pediatrics, National Health Insurance Corporation Ilsan Hospital, Goyang, Korea.

¹²Department of Pediatrics, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea.

¹³Division of Allergy and Respiratory Diseases, National Institute of Health, Korea Center for Disease Control and Prevention, Cheongju, Korea.

¹⁴National Institute of Health, Korea Center for Disease Control and Prevention, Cheongju, Korea.

¹⁵Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam, Korea.

*Correspondence to: Ji-Won Kwon, MD, Department of Pediatrics, Seoul National University Bundang Hospital, 82, Gumi-ro, 173 beon-gil, Bundang-gu, Songnam-si, Gyeonggi-do 463-707, Korea.
 E-mail: pedas@snuh.org

**Correspondence to: Soo-Jong Hong, MD, PhD, Childhood Asthma Atopy Center, Research Center for Standardization of Allergic Diseases, Department of Pediatrics, Asan Medical Center, University of Ulsan, College of Medicine, Pungnap 2-dong, Songpa-gu, Seoul 138-736, Korea.
 E-mail: sjhong@amc.seoul.kr

Received 24 September 2015; Revised 18 January 2016; Accepted 11 February 2016.

DOI 10.1002/ppul.23433
 Published online in Wiley Online Library
 (wileyonlinelibrary.com).

0.901–0.995), and $FEF_{25-75\%}$ (aOR, 0.980; 95%CI, 0.961–0.999). In females, BHR was significantly associated with a younger age (aOR, 0.845; 95%CI, 0.747–0.957), lower FEV_1 (%) (aOR, 0.961; 95%CI, 0.938–0.984), and menarche (aOR, 3.674; 95%CI, 1.226–11.012). Conclusion: BHR declined with age in the Korean pediatric population. A younger age and reduced lung function were common factors related to BHR in both genders. Additionally, atopy was related to BHR in males, whereas sexual maturation was related to BHR in females. These findings have important clinical implications for evaluating of childhood BHR and asthma related to gender.

Pediatr Pulmonol. © 2016 Wiley Periodicals, Inc.

Key words: bronchial hyper-responsiveness; menarche; gender; atopy; child.

Funding source: Research of Korea Centers for Disease Control and Prevention, Number: 014-ER5602-00.

INTRODUCTION

Bronchial hyper-responsiveness (BHR) is a characteristic feature of asthma, and varying degrees of airflow limitation have been associated with BHR. Known early-life risk factors for BHR in school-aged children include a history of allergic disease in infancy, low birth weight in infancy and a higher socioeconomic status of the parents at the time of the child's birth.¹ The persistence of BHR has been associated with female adult, asthma severity at diagnosis, a familial history of allergic disease, and allergic sensitization. Allergic sensitization has also been linked to persistent asthma symptoms.^{2–4} Some demographic factors such as age, gender, and body mass index (BMI) may influence BHR and the subsequent development of asthma symptoms.^{1,5–7} Therefore, examining the prevalence and associated risk factors of BHR in children, including asthmatics, is important.

Several studies have reported that the prevalence of childhood asthma decreases during puberty.^{8–10} Although asthma is more prevalent in males during childhood than in females, this association is reversed in adults, suggesting that a gender-dependent mechanism may affect the persistence of asthma during the transition from childhood to adulthood.^{11–15} The relationship between BHR and signs of puberty, such as changes in the concentration of sex hormones, has been reported, although the exact underlying mechanism is not yet known.¹⁶ Recently, we reported that the prevalence of BHR in elementary school children declined with age. A younger age, preterm birth, eosinophilia, sensitization to mites, lower lung function, and high FeNO levels were independently associated with BHR.¹⁷ In this present study, we extended our study population from elementary school children to middle school adolescents during and after puberty. We analyzed data to evaluate the prevalence and associated risk factors of BHR according to age and gender.

MATERIALS AND METHODS

Study Population

A total of 2,067 children from an elementary school ($n = 1300$) and a middle school located in Seoul ($n = 767$)

were enrolled in 2011 and 2012. A survey questionnaire, blood tests to measure serum total IgE levels, eosinophil counts (%), skin prick tests for common allergens, pulmonary function tests, and methacholine challenge test (MCT) were performed after informed consent was provided by each of the study participants. The methacholine challenge test was carried out successfully in 1,820 children. Because few subjects were 15 or 16 years of age, data for these ages were excluded in the analysis. Finally, 1,725 subjects between 6 and 14 years of age were included in the analysis. This present study was approved by the institutional review boards of the Korea Centers for Disease Control and Prevention and Asan Medical Center, University of Ulsan College of Medicine (2010-0064).

Methacholine Bronchial Provocation Test

We used a modified five-breath dosimeter method according to the American Thoracic Society (ATS) guidelines for the MCT. The functions of the dosimeter and nebulizer were checked annually by engineers before surveys and technical support was provided at each survey spot. A Rosenthal-French dosimeter (Laboratory for Applied Immunology, Baltimore, MD) and a DeVilbiss 646 nebulizer (DeVilbiss Healthcare, Somerset, PA) were used to generate aerosol. A technician checked the pressure of dosimeter daily, and the nebulizer output was checked by inspection. Methacholine was supplied from Sigma-Aldrich (St Louis, MO). Normal saline was used for a baseline and stepwise concentrations (0.62, 1.25, 2.5, 5, 12.5, and 25 mg/ml) of methacholine solutions were followed with challenges.¹⁸ Subjects who took medication for the treatment of acute respiratory infection were excluded from the analysis. Ultimately, 1,820 subjects were eligible to participate in the MCT. We defined the presence of BHR as when the provocative concentration of 20% reduction in FEV_1 (PC_{20}) was less than 8 mg/ml.

Survey Questionnaire

The Korean version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was

used to evaluate the prevalence of wheezing or asthma symptoms.¹⁸ The questionnaire was delivered to parents via individual envelopes and were collected by the health teacher at each subject's school. Other detailed questions were included related to parental allergies, socioeconomic status, exposure to passive smoking, and the onset of puberty. Menarche was defined when menstrual bleeding first developed in females.

Skin Prick and Pulmonary Function Tests

Histamine and normal saline were used as positive and negative controls, respectively, in skin prick tests. We defined atopic sensitization when the wheal size induced by a specific allergen was larger than 3 mm or at least as large as the size of the positive control. A total of 13 inhaled allergens, including house dust mites (*Dermatophagoides pteronyssinus* and *farinae*), dog dander, cat epithelium, cockroaches, molds (*Alternaria alternata* and *Aspergillus fumigatus*), pollens (grass pollen mixture, ragweed, mugwort, alder, oak, and birch), and food allergens (peanut, egg white, cow milk, and soybean) were used for skin prick tests (Allergopharma GmbH & Co., Reinbek, Germany). Pulmonary function tests were performed according to the ATS (American Thoracic Society)/ERS (European Respiratory Society) guidelines.¹⁹ A portable micro-spirometer (Microspiro HI-298; Chest Corporation, Tokyo, Japan) was used to measure the forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV₁), and the FEV₁/FVC ratio. This portable device has good accuracy for measuring FEV₁ and FVC in general practice and epidemiologic survey.²⁰

Statistical Analysis

SPSS 17.0 (IBM SPSS, Chicago, IL) was used for statistical analyses. Descriptive statistics were used to express continuous variables as the means and standard deviation. To compare characteristics between the BHR (+) and BHR (−) groups, the Student's *t*-test was used for continuous variables, and the χ^2 test was used for categorical variables. Multiple logistic regression analyses were conducted to investigate the factors associated with BHR in females after adjusting for age, BMI, educational degree of the mother, asthma history of the parents, atopic sensitization, eosinophil counts (%), total serum IgE level, FEV₁ (%), and environmental tobacco smoke (ETS). A *P*-value <0.05 was considered to denote a statistically significant difference.

RESULTS

Characteristics of the Study Subjects

A total 1,725 children (811 males and 914 females) were enrolled. The mean age of the study participants was 10.20 ± 2.59 years. There were no gender differences in

the parental history of asthma, maternal education level, exposure to passive smoking, FEV₁ (%), FEF_{25–75%} (%), BHR prevalence, wheezing or asthma prevalence, and current atopic dermatitis (AD) prevalence. The BMI, blood eosinophil counts (%), atopic sensitization, and current AR prevalence were greater in males compared with females (*P*<0.05). Age and the FEV₁/FVC ratio (%) were lower in males (*P*<0.05) (Table 1).

Assessments of the Subject's Characteristics According to the Presence of BHR

The prevalence of BHR (PC₂₀ ≤ 8 mg/ml) was 9.3% (males, 8.1%; females, 10.3%). We compared differences in asthma prevalence with the occurrence of other allergic diseases, blood eosinophil counts (%), total serum IgE levels, atopic sensitization, and lung function according to the presence or absence of BHR in each gender. The prevalence of wheezing (ever), blood eosinophil counts (%), total serum IgE levels, and atopic sensitization were greater in the male BHR (+) group compared with the male BHR (−) group (*P*<0.05). The FEV₁ (%) and FEF_{25–75%} (%) were lower in the male BHR (+) group compared with the male BHR (−) group (*P*<0.05). Eosinophil counts (%), total serum IgE levels, and atopic sensitization were greater in the female BHR (+) group compared with the female BHR (−) group (*P*<0.05). Finally, FEV₁ (%), the FEV₁/FVC ratio (%), and FEF_{25–75%} (%) were lower in the female BHR (+) group compared with the female BHR (−) group (*P*<0.05) (Table 2).

Prevalence of BHR According to Age and Gender

We investigated the prevalence of BHR according to gender and age. The prevalence of BHR in both genders declined with age (trend *P*<0.001). However, in females, the prevalence of BHR then began to increase tendency after 11 years of age. There was an overall reduction in BHR for boys, except for small increase between 8 and 9 years of age and then again at 14 years. However, this increase was not as great as it was for girls at 11 years of age (Fig. 1).

Numbers of Subjects With Menarche According to Age

Because the pubertal period of females generally began in the final years of elementary school, we also evaluated the degree of sexual maturation, such as menarche, in females. Females from first- to third- grade were not asked about their sexual maturity, including menstruation. We observed that 98.2% of females experienced an onset of menarche between 11 and 14 years of age. This age period of increased prevalence of BHR was similar to that of menarche in females (Table 3).

TABLE 1—Characteristics of the Study Subjects

	Males (n = 811)	Females (n = 914)	P value
Demographic factors			
Age (years), mean $\pm$ SD	10.06 $\pm$ 2.55	10.32 $\pm$ 2.62	0.041
BMI (kg/m ²), mean $\pm$ SD	19.32 $\pm$ 3.85	18.61 $\pm$ 3.37	<0.001
Parental history of asthma	4.3% (31/724)	4.2% (33/782)	0.953
Maternal educational degree			0.388
Below high school	43.6% (339/778)	44.3% (389/878)	
Below university	51.5% (401/778)	52.2% (458/878)	
Below graduate school	4.9% (38/778)	3.5% (31/878)	
Exposure to ETS, n (%)	44.5% (357/802)	46.0% (414/901)	0.553
Atopic factors			
Blood eosinophil (%)	3.35 $\pm$ 2.36	2.56 $\pm$ 1.97	<0.001
Total serum IgE (IU/ml)	204.1 $\pm$ 303.1	188.6 $\pm$ 340.6	0.329
Atopic sensitization	48.9% (387/902)	37.5% (338/902)	<0.001
Lung function			
FEV ₁ (%)	96.50 $\pm$ 11.17	96.35 $\pm$ 10.97	0.773
FEV ₁ /FVC ratio (%)	92.25 $\pm$ 5.55	95.06 $\pm$ 4.70	<0.001
FEF _{25–75%} (%)	95.71 $\pm$ 20.85	97.64 $\pm$ 20.19	0.067
Airway responsiveness			
PC ₂₀ $\leq$ 8 mg/ml	8.1% (66/811)	10.2% (94/914)	0.125
Allergic disease prevalence			
Wheezing ever	13.6% (109/804)	11.6% (105/907)	0.216
Wheezing within 12 months	4.4% (35/800)	4.3% (39/905)	0.947
Asthma diagnosis, ever	6.5% (52/801)	5.1% (46/907)	0.208
Current asthma	1.4% (11/797)	1.0% (9/901)	0.467
Current AR	24.6% (189/767)	17.0% (148/872)	<0.001
Current AD	9.8% (78/795)	11.5% (102/897)	0.299

AR, allergic rhinitis; AD, atopic dermatitis; BMI, body mass index; ETS, environmental tobacco smoke; FEV₁, forced expiratory volume in 1 sec; FVC, forced vital capacity.

Gender-Specific and Other Risk Factors Associated With BHR

We investigated the factors associated with BHR in each gender to test for a possible gender influence. In multiple logistic regression analysis after adjusting for

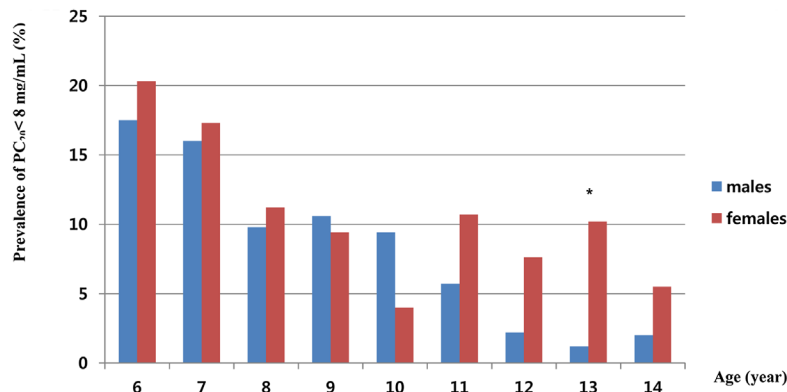
age, BMI, the maternal education level, a parental history of asthma, blood eosinophil counts (%), total serum IgE levels, atopic sensitization, FEV₁ (%), and exposure to environmental tobacco smoke, boys were more likely to have BHR at a younger age (aOR, 0.797; 95%CI, 0.687–0.925), higher blood eosinophil counts (aOR, 1.160;

TABLE 2—Comparison of the Prevalence of Asthma/Wheezing, Atopic Tendency, and Lung Functions According to the Presence of BHR in the Study Subjects of Each Sex

	Males			Females		
	BHR (–) (PC ₂₀ > 8, n = 745)	BHR (+) (PC ₂₀ $\leq$ 8, n = 66)	P value*	BHR (–) (PC ₂₀ > 8, n = 820)	BHR (+) (PC ₂₀ $\leq$ 8, n = 94)	P value*
Wheezing ever	12.4% (96/774)	25.8% (17/66)	0.002	11.9% (97/813)	8.5% (8/94)	0.326
Wheezing within 12 months	4.0% (31/773)	7.7% (5/65)	0.160	4.4% (36/811)	3.2% (3/94)	0.789
Asthma diagnosis, ever	5.8% (45/773)	10.6% (7/66)	0.122	4.8% (39/813)	7.5% (7/94)	0.268
Current asthma	1.2% (9/770)	3.1% (2/65)	0.209	1.0% (8/807)	1.1% (1/94)	1.000
Current AR	24.1% (177/736)	33.3% (21/63)	0.101	16.3% (127/781)	23.1% (21/91)	0.101
Current AD	9.8% (75/767)	10.6% (7/66)	0.829	11.3% (91/804)	11.8% (11/93)	0.884
Blood eosinophil (%)	3.19 $\pm$ 2.26	4.97 $\pm$ 3.53	<0.001	2.45 $\pm$ 1.85	3.57 $\pm$ 2.63	<0.001
serum total IgE (IU/ml)	193.6 $\pm$ 284.4	324.2 $\pm$ 453.6	0.029	179.1 $\pm$ 334.5	270.6 $\pm$ 382.0	0.016
Atopic sensitization	48.0% (367/764)	66.7% (44/66)	0.004	35.4% (287/810)	55.4% (51/92)	<0.001
FEV ₁ (%)	96.78 $\pm$ 11.14	95.48 $\pm$ 11.26	0.364	96.81 $\pm$ 11.04	92.31 $\pm$ 9.48	<0.001
FEV ₁ /FVC ratio (%)	92.43 $\pm$ 5.44	90.37 $\pm$ 6.19	0.004	95.19 $\pm$ 4.74	93.92 $\pm$ 4.69	0.026
FEF _{25–75%} (%)	96.78 $\pm$ 21.02	86.43 $\pm$ 17.15	<0.001	98.78 $\pm$ 19.88	87.55 $\pm$ 20.29	<0.001

BHR, bronchial hyper-responsiveness; FEV₁, forced expiratory volume in 1 sec; AR, allergic rhinitis; AD, atopic dermatitis.

*The Student's *t*-test was used for continuous variables and χ^2 test for categorical variables.



	6 years	7 years	8 years	9 years	10 years	11 years	12 years	13* years	14 years
M (no.)	11/63	17/106	10/102	10/94	8/85	5/88	2/90	1/82	2/101
F (no.)	16/79	18/104	10/89	9/96	4/100	9/84	8/106	13/128	7/128

Fig. 1. Prevalence of BHR according to age and gender ($PC_{20} \leq 8$ mg/ml). The P value for the trend was <0.001 in both genders. The prevalence of BHR in males continuously decreased with age, in contrast to females for which the value partially rebounded after 11 years of age. Differences in BHR prevalence between the two genders that were found to be statistically significant ($P < 0.05$) were indicated by an asterisk.

95%CI, 1.047–1.284), atopic sensitization (aOR, 2.091; 95%CI, 1.003–4.359), a lower FEV_1/FVC ratio (%) (aOR, 0.947; 95%CI, 0.901–0.995), and a lower $FEF_{25-75\%}$ (%) (aOR, 0.980; 95%CI, 0.961–0.999). In girls, a younger age (aOR, 0.845; 95%CI, 0.747–0.957) and lower FEV_1 (%) (aOR, 0.961; 95%CI, 0.938–0.984) were associated with increased BHR. Additionally, we found that menarche was significantly associated with BHR and showed the highest odds ratio in girls (aOR, 3.647; 95% CI, 1.226–11.012) (Table 4).

DISCUSSION

The prevalence of BHR appears to decline in both genders with age; however, in girls, the pattern of BHR prevalence showed a tendency to rebound from 11 to

14 years of age. In multivariate analyses, a younger age and lower lung function were risk factors for BHR in both sexes. After adjustments, atopy was a significant factor associated with BHR in boys, but not girls. Menarche was a female-specific risk factor for BHR that had the highest odds ratio. There might be a critical relationship between the stabilization of the BHR after puberty in females and menarche. Thus, our present findings may have important clinical implications and provide valuable basic knowledge for childhood BHR and asthma related to sex.

The ATS guidelines recommend a cut-off point that ranges from 4 to 16 mg/ml in adults and older children for a PC_{20} in the MCT to determine a positive BHR (borderline) result.²¹ Because growing children and adolescents have variable physical parameters, lung functions, atopic status and other factors that influence BHR, a cautious approach to better understand age-dependent factors is needed to define BHR in this age group. When determining the BHR cut-off, the number of positive BHR results and the false-positive or false-negative rates are issues in epidemiologic studies. Among these issues, we carefully considered the BHR cut-off and ultimately elected to use 8 mg/ml.

In our present study, the prevalence of BHR declines with age even after adjusting for confounding factors that include BMI, socioeconomic status, a family history of asthma, atopy, and lung functions. Understanding that there are normal developmental changes in the prevalence of BHR in growing children will help to define and understand associations of asthma and other respiratory conditions with BHR. Previous studies have shown that BHR changes according to age, sex, and other factors.²²

TABLE 3—Numbers of Subjects Who Have Reached Menarche According to Age

Age	Menarche		Total
	No	Yes	
6	0	0	0
7	0	0	0
8	0.4% (1/261)	0	1
9	23.0% (60/261)	0.3% (1/344)	61
10	36.4% (95/261)	1.5% (5/344)	100
11	21.1% (55/261)	7.6% (26/344)	81
12	10.7% (28/261)	22.7% (78/344)	106
13	5.8% (15/261)	32.9% (113/344)	128
14	2.7% (7/261)	35.2% (121/344)	128
Total	100.0% (261/261)	100.0% (344/344)	605

TABLE 4—Adjusted Odds Ratios (aOR) of Associated Factors for BHR ($PC_{20} \leq 8$ mg/ml) in Each Gender

Variables	Total subjects with BHR (n = 160/1,725) aOR ¹	Males with BHR (n = 66/811) aOR ¹	Females with BHR (n = 94/914) aOR ¹
Age	0.851 (0.774–0.937)	0.797 (0.687–0.925)	0.845 (0.747–0.957)
Sex, female	1.736 (1.163–2.591)	NA	NA
BMI	0.981 (0.917–1.048)	0.911 (0.823–1.008)	1.073 (0.979–1.177)
Asthma history of parents	1.235 (0.532–2.865)	1.072 (0.286–4.025)	1.199 (0.394–3.643)
Maternal educational degree	0.846 (0.601–1.192)	0.924 (0.552–1.549)	0.785 (0.496–1.244)
Wheezing ever	0.940 (0.539–1.640)	1.267 (0.594–2.702)	0.675 (0.288–1.580)
Wheezing within 12 months	0.956 (0.389–2.349)	1.183 (0.322–4.346)	0.846 (0.241–2.977)
Asthma diagnosis ever	1.322 (0.642–2.719)	1.459 (0.527–4.037)	1.227 (0.427–3.529)
Current asthma	1.959 (0.490–7.829)	2.757 (0.447–7.015)	1.217 (0.121–2.205)
Current AR	1.148 (0.725–1.817)	0.998 (0.511–1.948)	1.273 (0.666–2.432)
Current AD	0.568 (0.295–1.095)	0.553 (0.187–1.634)	0.556 (0.241–1.281)
Breast milk feeding	1.007 (0.672–1.508)	0.872 (0.464–1.640)	1.262 (0.732–2.175)
Older sibling	0.955 (0.651–1.403)	1.228 (0.679–2.218)	0.794 (0.475–1.329)
Antibiotics use within 1 yr	0.750 (0.502–1.122)	1.126 (0.623–2.036)	0.536 (0.301–0.954)
Keeping pet	1.370 (0.689–2.724)	1.082 (0.354–3.308)	1.467 (0.605–3.560)
Blood eosinophil (%)	1.140 (1.055–1.232)	1.160 (1.047–1.284)	1.121 (0.998–1.260)
Serum total IgE (IU/ml)	1.364 (0.902–2.063)	1.033 (0.519–2.054)	1.623 (0.965–2.728)
Atopic sensitization	1.719 (1.099–2.689)	2.091 (1.003–4.359)	1.643 (0.927–2.912)
FEV ₁ (%)	0.972 (0.954–0.989)	0.976 (0.949–1.004)	0.961 (0.938–0.984)
FEV ₁ /FVC ratio (%)	0.969 (0.935–1.005)	0.947 (0.901–0.995)	0.988 (0.937–1.042)
FEF _{25–75%} (%)	0.984 (0.971–0.997)	0.980 (0.961–0.999)	0.987 (0.970–1.004)
Exposure to ETS	0.995 (0.668–1.481)	1.113 (0.601–2.060)	0.946 (0.559–1.600)
Pubic hair	NA	NA	2.551 (0.891–7.306)
Ejaculation	NA	NA	NA
Menarche	NA	NA	3.674 (1.226–11.012)

aOR, adjusted odds ratio; AR, allergic rhinitis; AD, atopic dermatitis; BMI, body mass index; ETS, environmental tobacco smoke; FEV₁, forced expiratory volume in 1 sec.

¹Logistic regression analysis was performed after adjusting for age, sex, BMI, educational degree of the mother, asthma history of parents, atopic sensitization, eosinophil (%), total serum IgE, FEV₁ (%), and ETS.

Others have observed that BHR declines with age and the prevalence of BHR differs in children who live in different regions within western countries.^{23,24} Our current study establishes that these previous results are applicable to Asian populations and may also be used to determine the BHR prevalence among Korean children.

A relationship between asthma susceptibility and severity and both sex hormones and gender differences has been previously proposed.²⁵ Another group showed that female gender, atopy, and airway hyper-responsiveness are associated with the persistence of asthma from childhood to adulthood.²⁶ The association between the prevalence of BHR and menarche remains controversial. Some studies have indicated that the BHR of females after puberty is more severe than for males in the asthma group,^{22,24,27} while other studies have reported no significant association between puberty and BHR.^{16,28,29} In this present study, we also found a gender difference in the prevalence of BHR that varied according to age in a general pediatric and adolescent population that included Korean/Asian population.

The female sex hormone estrogen may be an important factor that regulates airway function and inflammation. BHR in female mice increased after ovariectomy or the administration of an estrogen antagonist; moreover,

estrogen treatment reduces BHR.³⁰ Estrogen and progesterone have been reported to positively affect lung function through life in women.³¹ A relationship between hormonal cycles and asthma exacerbation may be explained by changes in the levels of these hormones or a change in their ratio. A few studies that investigated the contribution of sex hormones to airway inflammation arrived at less than robust conclusion, as estrogens and progesterone were described to have both pro- and anti-inflammatory effects.³² Sex hormones contribute to the release of histamine from mast cells and enhance eosinophil adhesion and degranulation.³³ Some reports proposed a stimulatory effect on BHR, although other studies have reported anti-inflammatory effects of estrogen.^{34,35} The mechanism whereby progesterone affects asthma and airway inflammation is not well understood and the positive and negative effects of this hormone on airway inflammation are currently the subject of debate. Additionally, progesterone has been shown to enhance BHR and eosinophilic airway inflammation in a mouse model of asthma.^{32,36}

Several previous studies have investigated factors associated with BHR and reduced lung function, such as eosinophilia, high total IgE levels, and atopy.^{4,17,23} Our present findings replicate associations of those factors

with BHR in Korean male children. Interestingly, although lower lung function was commonly associated with BHR in both genders, atopy was not found to be significant in females. This finding differs compared from those of previous studies. Indeed, most previous studies were carried out in an asthma patients cohort,^{16,27} whereas our present study analyzed a general population. Lung function testing, blood samples, and specific tests for detecting specific IgE may help to assess BHR in the general pediatric population. Additionally, sexual maturation should be considered in evaluation of BHR in females.

There were some limitations to this present study of note. First, the study subjects were only enrolled from Seoul, which is the most populated area in Korea. A nation-wide study with more subjects will be needed to determine whether there is gender difference associated with the prevalence of BHR at puberty throughout the country or whether this varies in different regions or environments. Second, we did not measure the levels of sex hormones or evaluate pubertal development in our present survey using Tanner stages; therefore, a follow-up questionnaire about sexual changes, including the presence of menarche or pubarche, might address this limitation. Third, BHR testing was performed at different years in each school; however, all surveys were conducted in the same season each year by the same investigators and examiners to minimize potential bias. Taken together, our findings have established the relationship between menarche and BHR, but could not establish the cause and effect of asthma incidence.

In summary, our present study has estimated the prevalence of BHR according to age and gender in a Korean pediatric population. Menarche was found to be an independent risk factor associated with a rebound of the BHR prevalence in pubertal females. We predicted that there may be a crucial relationship between them, although the exact relationship between menarche and BHR to methacholine remains to be fully elucidated. Future longitudinal studies designed to show the effect of menarche on BHR are needed. Sex hormones should also be assessed to investigate their influence on changes in BHR in these studies.

ACKNOWLEDGMENTS

This research was supported by a grant (2014-ER5602-00) from the Research of Korea Centers for Disease Control and Prevention.

REFERENCES

1. Arshad SH, Kurukulaaratchy RJ, Fenn M, Matthews S. Early life risk factors for current wheeze, asthma, and bronchial hyper-responsiveness at 10 years of age. *Chest* 2005;127:502–508.

2. Homer RJ, Elias JA. Airway remodeling in asthma: therapeutic implications of mechanisms. *Physiology* 2005;20:28–35.
3. Subbarao P, Mandhane PJ, Sears MR. Asthma: epidemiology, etiology and risk factors. *CMAJ* 2009;181:E181–E190.
4. Kim BS, Jin HS, Kim HB, Lee SY, Kim JH, Kwon JW, Kim BJ, Yu J, Yoo S, Hong SJ. Airway hyperresponsiveness is associated with total serum immunoglobulin E and sensitization to aeroallergens in Korean adolescents. *Pediatr Pulmonol* 2010;45:1220–1227.
5. Yoo S, Kim HB, Lee SY, Kim BS, Kim JH, Yu J, Kim BJ, Hong SJ. Association between obesity and the prevalence of allergic diseases, atopy, and bronchial hyperresponsiveness in Korean adolescents. *Int Arch Allergy Immunol* 2011;154:42–48.
6. Riiser A, Hovland V, Carlsen KH, Mowinkel P, Lodrup Carlsen KC. Does bronchial hyperresponsiveness in childhood predict active asthma in adolescence? *Am J Respir Crit Care Med* 2012;186:493–500.
7. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, Cowan JO, Herbison GP, Silva PA, Poulton R. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. *N Engl J Med* 2003;349:1414–1422.
8. Sears MR. Growing up with asthma. *BMJ* 1994;309:72–73.
9. Weiss ST, Sparrow D, O'Connor GT. The interrelationship among allergy, airways responsiveness, and asthma. *J Asthma* 1993;30:329–349.
10. Bronnimann S, Burrows B. A prospective study of the natural history of asthma. Remission and relapse rates. *Chest* 1986;90:480–484.
11. Skobeloff EM, Spivey WH, St Clair SS, Schoffstall JM. The influence of age and sex on asthma admissions. *JAMA* 1992;268:3437–3440.
12. Anderson HR, Pottier AC, Strachan DP. Asthma from birth to age 23: incidence and relation to prior and concurrent atopic disease. *Thorax* 1992;47:537–542.
13. Martin AJ, McLennan LA, Landau LI, Phelan PD. The natural history of childhood asthma to adult life. *Br Med J* 1980;280:1397–1400.
14. Dodge RR, Burrows B. The prevalence and incidence of asthma and asthma-like symptoms in a general population sample. *Am Rev Respir Dis* 1980;122:567–575.
15. Crawford WA, Beedham CG. The changing demographic pattern in asthma related to sex and age. A study of 13,651 patients on sodium cromoglycate (Intal). *Med J Aust* 1976;1:430–434.
16. Nicolai T, Illi S, Tenborg J, Kiess W, v Mutius E. Puberty and prognosis of asthma and bronchial hyper-reactivity. *Pediatr Allergy Immunol* 2001;12:142–148.
17. Kim MS, Kim YH, Suh DI, Koh YY, Kim BJ, Kim HB, Lee SY, Song DJ, Kim WK, Jang GC, et al. The prevalence of bronchial hyperresponsiveness in elementary school children and its associated factors. *Allergy Asthma Respir Dis* 2014;2:171.
18. Kim BS, Jin HS, Kim HB, Lee SY, Kim JH, Kwon JW, Kim BJ, Yu J, Yoo S, Hong SJ. Airway hyperresponsiveness is associated with total serum immunoglobulin E and sensitization to aeroallergens in Korean adolescents. *Pediatr Pulmonol* 2010;45:1220–1227.
19. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000;161:309–329.
20. Dompeling E, van Schayck CP, Folgering H, van den Hoogen HJ, van Weel C. Accuracy, precision and linearity of the portable flow-volume meter Microspiro HI-298. *Eur Respir J* 1991;4:612–615.
21. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, Van der Grinten CP, Gustafsson P,

- et al. Standardisation of spirometry. *Eur Respir J* 2005;26:319–338.
22. Paoletti P, Carrozzi L, Viegi G, Modena P, Ballerin L, Di Pede F, Grado L, Baldacci S, Pedreschi M, Vellutini M, et al. Distribution of bronchial responsiveness in a general population: effect of sex, age, smoking, and level of pulmonary function. *Am J Respir Crit Care Med* 1995;151:1770–1777.
 23. Forastiere F, Corbo GM, Dell’Orco V, Pistelli R, Agabiti N, Kriebel D. A longitudinal evaluation of bronchial responsiveness to methacholine in children: role of baseline lung function, gender, and change in atopic status. *Am J Respir Crit Care Med* 1996;153:1098–1104.
 24. Manfreda J, Sears MR, Becklake MR, Chan-Yeung M, Dimich-Ward H, Siersted HC, Ernst P, Sweet L, Van Til L, Bowie DM, et al. Geographic and gender variability in the prevalence of bronchial responsiveness in Canada. *Chest* 2004;125:1657–1664.
 25. Melgert BN, Ray A, Hylkema MN, Timens W, Postma DS. Are there reasons why adult asthma is more common in females? *Curr Allergy Asthma Rep* 2007;7:143–150.
 26. Becklake MR, Kauffmann F. Gender differences in airway behaviour over the human life span. *Thorax* 1999;54:1119–1138.
 27. Tantisira KG, Colvin R, Tonascia J, Strunk RC, Weiss ST, Fuhlbrigge AL. Airway responsiveness in mild to moderate childhood asthma: sex influences on the natural history. *Am J Respir Crit Care Med* 2008;178:325–331.
 28. Wassmer G, Jorres RA, Heinrich J, Wjst M, Reitmair P, Wichmann HE. The association between baseline lung function and bronchial responsiveness to methacholine. *Eur J Med Res* 1997;2:47–54.
 29. Ulrik CS, Backer V. Longitudinal determinants of bronchial responsiveness to inhaled histamine. *Chest* 1998;113:973–979.
 30. Matsubara S, Swasey CH, Loader JE, Dakhama A, Joetham A, Ohnishi H, Balhorn A, Miyahara N, Takeda K, Gelfand EW, et al. Estrogen determines sex differences in airway responsiveness after allergen exposure. *Am J Respir Cell Mol Biol* 2008;38:501–508.
 31. Haggerty CL, Ness RB, Kelsey S, Waterer GW. The impact of estrogen and progesterone on asthma. *Ann Allergy Asthma Immunol* 2003;90:284–291.
 32. van den Berge M, Heijink HI, van Oosterhout AJ, Postma DS. The role of female sex hormones in the development and severity of allergic and non-allergic asthma. *Clin Exp Allergy* 2009;39:1477–1481.
 33. Hamano N, Terada N, Maesako K, Numata T, Konno A. Effect of sex hormones on eosinophilic inflammation in nasal mucosa. *Allergy Asthma Proc* 1998;19:263–269.
 34. Carey MA, Card JW, Bradbury JA, Moorman MP, Haykal-Coates N, Gavett SH, Graves JP, Walker VR, Flake GP, Voltz JW, et al. Spontaneous airway hyperresponsiveness in estrogen receptor-alpha-deficient mice. *Am J Respir Crit Care Med* 2007;175:126–135.
 35. Tai P, Wang J, Jin H, Song X, Yan J, Kang Y, Zhao L, An X, Du X, Chen X, et al. Induction of regulatory T cells by physiological level estrogen. *J Cell Physiol* 2008;214:456–464.
 36. Mitchell VL, Gershwin LJ. Progesterone and environmental tobacco smoke act synergistically to exacerbate the development of allergic asthma in a mouse model. *Clin Exp Allergy* 2007;37:276–286.