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Genetic variation of IL13 as a risk factor of reduced lung function in children and adolescents: A cross-sectional population-based study in Korea☆

Heung-Woo Park [a,b], Jong-Eun Lee [c], Se-Hoon Kim [a,b], Yoon-Keun Kim [a,b,d], Kyung-Up Min [a,b], You-Young Kim [a,b], Sang-Heon Cho [a,b,*]

[a] *Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea*
[b] *Institute of Allergy and Clinical Immunology, Seoul National University College of Medicine, Seoul, Republic of Korea*
[c] *DNA Link Inc., Seoul, Republic of Korea*

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Summary
Background: Previous investigations have suggested that genetic variations are associated with reduced lung function in early childhood. This study was conducted to evaluate the association between IL13 + 2044G → A, the functionally relevant single nucleotide polymorphism (SNP) in the gene coding IL13, and lung function in early childhood.
Patients and methods: A total of 1900 subjects aged 10–18 years living in Korea, were randomly recruited. Lung function test and methacholine bronchial provocation test were performed. Multiple regression analysis adjusting for sex, age, height, atopy, and history of passive smoking was done to evaluate effect of IL13 + 2044G → A on lung function.
Results: Mean (±SD) forced expiratory volume in 1 s (FEV_1) was 2.66 L (±0.60) in subjects with the AA or AG genotype ($n = 982$) and 2.75 L (±0.57) in subjects with the GG genotype ($n = 918$). IL13 + 2044G → A showed a significant association with FEV_1 [in the minor allele dominant model (GG *vs.* AG + AA), $P < 0.001$]. Interestingly, the association between FEV_1 and IL13 + 2044G → A remained still significant in subgroup analysis according to the presence of AHR ($P < 0.001$ in subjects without AHR and $P = 0.002$ in subjects with AHR). Moreover, FEV_1/FVC (forced vital capacity) ratio also showed a significant association with IL13 + 2044G → A in both groups ($P < 0.001$ in subjects without AHR and $P < 0.001$ in subjects

Abbreviations: AHR, airway hyperresponsiveness; BMI, body mass index; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; SNP, single nucleotide polymorphism.

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* Corresponding author: Department of Internal Medicine, Seoul National University, 28 Yongondong, Chongno-gu, Seoul 110-744, Republic of Korea. Tel.: +82 2 2072 2971; fax: +82 2 762 9662.
E-mail address: shcho@plaza.snu.ac.kr (S.-H. Cho).
[d] Present address: Department of Life Science, Pohang University of Science and Technology, Pohang, Republic of Korea.

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with AHR). This cross-sectional study demonstrates that IL13 + 2044G → A is significantly associated with a reduced lung function in Korean children and adolescents.

Introduction

Longitudinal studies of children and adolescent have demonstrated that increased airway responsiveness to stimuli like cold air[1] and methacholine,[2,3] atopy,[2,3] and the presence of asthma[4,5] are associated with airway limitations and impaired age-related growth of lung function. Recently, in addition to those factors, genetic variations in IL12B[6] and ADAM33[7] have been shown to be associated with reduced lung function in early childhood.

Interleukin 13 (IL13) is a pleomorphic cytokine and causes mucous metaplasia, enhanced mucin gene expression, increased tissue hyaluronic acid accumulation, and subepithelial fibrosis.[8] In particular, it is well known that genetic polymorphisms and expression levels of IL13 are closely related to pulmonary fibrosis,[9,10] chronic obstructive pulmonary disease,[11,12] and airway remodeling in asthma.[13,14] However, relatively little is known about the effects of genetic variations in IL13 on the lung functions of children and adolescents.

In the present cross-sectional study, we selected a nonsynonymous coding single nucleotide polymorphism (SNP) in IL13 [+2044G → A (R130Q); rs20541], which has been widely studied[15] and shown to be functionally relevant in the previous study.[16] Moreover, this SNP was reported to be associated with allergic diseases in Korean population.[17,18] We sought to determine whether IL13 + 2044G → A is associated with airway limitation manifested by a low forced expiratory volume in 1 s (FEV_1) adjusting for covariates, such as age, gender, height, atopy and history of passive smoking in randomly recruited Korean children and adolescents.

Materials and methods

Study subjects

All subjects enrolled in this study provided written informed consent, and the study protocol was approved by the institutional review board at Seoul National University Hospital. A total of 2864 ethnically identical participants aged 10–18 years were randomly recruited through schools located on the southern part of Jeju Island in Korea, of whom, 1900 (66.3%) were enrolled in this study. All of them resided in rural areas, and most of their parents lived by fishing or cultivating fruit trees. Skin prick test using 11 common aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, dog fur, cat fur, *Aspergillus*, *Alternaria*, tree pollen mixture, grass pollen mixture, mugwort, ragweed, and cockroach; Allergopharma, Germany) was also performed as previously described.[19] Subjects who had received oral antihistamine during the 5 days prior to skin prick test or had dermographism were excluded. Atopy was defined as a positive skin prick test response (allergen/histamine ratio >1.0 plus a mean wheal size >4 mm) to one or more allergens. All 1900 subjects responded to a questionnaire concerning risk factors, e.g., passive smoking, and a family history of allergic diseases. None of the study subjects had been treated or had used oral or inhaled bronchodilators. Subjects who had contracted an upper respiratory tract infection during the 2 week period prior to study commencement were also excluded.

Pulmonary function test and methacholine bronchial provocation test

FEV_1 and forced vital capacity (FVC) values were determined using a microspirometer (Micro Medical Limited, UK) in a standing position without a nose-clamp, but otherwise according to the guidelines issued by the European Respiratory Society.[20] Each measurement consisted of at least three maximal expiratory maneuvers from total lung capacity to residual volume with variations of less than 5% and the highest FEV_1 and FVC values were used in the analyses. Methacholine bronchial provocation test was performed as previously described,[21] and positive airway hyperresponsiveness (AHR) was defined as a methacholine PC_{20} of <16 mg/mL. The slope of the dose-response curve (DRS), defined as the percentage fall in FEV_1 at the last dose divided by the log of the last concentration of methacholine (mg/mL), was calculated for a continuous index of airway responsiveness to methacholine.[22]

Genotyping

Genotyping for IL13 + 2044G → A was done using the high throughput single base-pair extension method (SNP-IT™ assay) using an SNPstream25K system, which was customized to automatically genotype DNA samples in 384 well plates and provide a colorimetric readout (Orchid Biosciences, NJ) as previously described.[23]

Statistical analysis

Association analysis was performed for IL13 + 2044G → A using a genetic model approach; a minor allele dominant model (AG + AA *vs.* GG). Multiple regression analysis adjusting for sex, age, height, atopy, and history of passive smoking was done to evaluate the effect of IL13 + 2044G → A on FEV_1 and FEV_1/FVC ratio. Compliance with the Hardy–Weinberg equilibrium was tested using 2×2 χ^2 tests. Statistical analyses were performed using SPSS version 12.0 (SPSS, Inc., Chicago, IL). *P* values of less than 0.05 were regarded significant.

Results

The characteristics of study population are presented in Table 1. IL13 + 2044G → A did not deviate from Hardy–Weinberg equilibrium in our study subjects ($P > 0.05$).

Table 1 Characteristics of subjects enrolled in the study.

Characteristics	Number of positivity
Age, mean (range)	14.8 (10–18)
Male (%)	954 (50.2)
BMI (Kg/m^2)[a]	19.5 (±3.1)
Atopy (%)	703 (37.0)
AHR (%)[b]	475 (25.0)
History of passive smoking (%)	1313 (69.1)
Family history of allergic diseases (%)	376 (19.8)

[a] BMI = body mass index, mean ± SD.
[b] AHR = airway hyperresponsiveness.

A total of 982 subjects were genotyped as AA or AG (51.7%) and 918 subjects as GG. Methacholine AHR showed a significant association with IL13 + 2044G → A. Two hundred and seventy two subjects with the AG or AA genotype (27.8%) and 203 subjects with the GG genotype (22.1%) showed positive results ($P = 0.014$). In terms of DRS, IL13 + 2044G → A also showed a significant association adjusting for sex, age, height, atopy, and history of passive smoking [in the minor allele dominant model (AG + AA *vs.* GG), $P = 0.022$]. Meanwhile, no association between IL13 + 2044G → A and atopy was found [375 subjects with the AG or AA genotypes (37.7%) *vs.* 328 subjects with the GG genotype (36.2%); $P = 0.515$]. So far, there has been no report concerning the equation predicting FEV_1 in normal Korean children and adolescents. In addition, a quarter of subject enrolled in the present study showed a positive methacholine AHR, which might be a barrier for deriving a prediction equation directly from them. Considering this, we decided to evaluate the association between IL13 + 2044G → A and raw values of FEV_1, itself based on multiple regression analysis adjusting for covariates, such as sex, age, height, atopy, history of passive smoking. Mean FEV_1 (±SD) was 2.66 L (±0.60) in subjects with the AA or AG genotype and 2.75 L (±0.57) in subjects with the GG genotype. Table 2 shows the results of regression analysis. Sex, age, height, and IL13 + 2044G → A (in the minor allele dominant model) were significantly associated with FEV_1 in simple regression analysis. All the variables except age remained significant after multiple regression analysis (Table 2). Because AHR was one of the factors related with airway limitation,[2,3] and the prevalence of AHR in the present study was significantly associated with IL13 + 2044G → A, subgroup analysis was performed according to the presence of AHR. IL13 + 2044G → A including sex and height showed a significant association with FEV_1 in both groups by multiple regression analysis ($P < 0.001$ in subjects without AHR and $P = 0.002$ in subjects with AHR, Table 3). Mean FEV_1/FVC ratio (±SD) was 0.92 (±0.07) in subjects with the AA or AG genotype and 0.97 (±0.04) in subjects with the GG genotype. Interestingly, FEV_1/FVC ratio was also significantly associated with IL13 + 2044G → A adjusting for sex, age, height, atopy, and history of passive smoking (Table 4) and subgroup analysis according to the presence of AHR showed a significant association in both groups (Table 5).

Table 2 Results of regression analysis for FEV_1.

Explanatory variable	Simple regression analysis		Multiple regression analysis[a]	
	Coefficient	*P* value	Coefficient	*P* value
Sex	−0.517	<0.001	−0.295	<0.001
Age	0.283	<0.001	0.038	0.023
Height	0.722	<0.001	0.596	<0.001
Atopy	0.040	0.080	0.011	0.439
Passive smoking	0.030	0.487	0.010	0.439
IL13 + 2044G → A[b]	0.073	0.001	0.092	<0.001

[a] *R* square = 0.606.
[b] Minor allele dominant model [AG + AA (dummy variable = 1) *vs.* GG (dummy variable = 2)].

Discussion

In this cross-sectional study, we found that IL13 + 2044G → A is significantly associated with a reduced lung function, as manifested by a low $FEV_{1\%}$ and FEV_1/FVC ratio adjusting for covariates, such as sex, age, height, atopy, and history of passive smoking, in Korean children and adolescents. These significant associations were found not only in subjects with AHR but also in subjects without AHR. Collectively, these findings suggest that reduced early-lung function is in part a genetically determined trait.

As mentioned earlier, the presences of asthma and AHR are markers of impaired growth of lung function in children and adolescents.[2–5] A previous study on Korean children showed that IL13 + 2044G → A was significantly associated with methacholine AHR and asthma development[18] like results of the present study. Therefore, it is likely that methacholine AHR rather than IL13 + 2044G → A is associated with a reduced lung function. To test this, we performed subgroup analysis and found that IL13 + 2044G → A showed a significant association with reduced lung functions in subjects with AHR or without AHR. Interestingly, Leung et al. showed that IL13 + 2044G → A, combined with ADRB2 R16G and STAT6 + 1570C → T significantly influenced lung function growth in Chinese asthmatic children

Table 3 Results of multiple regression analysis for FEV_1 according to the presence of airway hyperresponsiveness (AHR).

Explanatory variable	AHR[a] (+, *n* = 475)		AHR[b] (−, *n* = 1425)	
	Coefficient	*P* value	Coefficient	*P* value
Sex	−0.305	<0.001	−0.289	<0.001
Age	0.030	0.424	0.039	0.038
Height	0.542	<0.001	0.617	<0.001
Atopy	−0.003	0.921	0.012	0.477
Passive smoking	0.053	0.097	−0.004	0.823
IL13 + 2044G → A[c]	0.102	0.002	0.089	<0.001

[a] R square = 0.524.
[b] *R* square = 0.626.
[c] Minor allele dominant model [AG + AA (dummy variable = 1) *vs.* GG (dummy variable = 2)].

Table 4 Results of regression analysis for FEV_1/FVC ratio.

Explanatory variable	Simple regression analysis		Multiple regression analysis[a]	
	Coefficient	*P* value	Coefficient	*P* value
Sex	0.001	0.999	0.017	0.484
Age	0.079	0.001	0.047	0.055
Height	0.077	0.001	0.065	0.013
Atopy	−0.048	0.036	−0.036	0.092
Passive smoking	0.011	0.646	0.147	0.883
IL13 + 2044G → A[b]	0.409	<0.001	0.411	<0.001

[a] *R* square = 0.181.
[b] Minor allele dominant model [AG + AA (dummy variable = 1) *vs*. GG (dummy variable = 2)].

after 4.5-year observation.[24] A recent report revealed that a combined effect of *in utero* exposure to smoking and IL13 + 2044G → A on persistent wheezing in British childfren.[25] In addition, it was reported that IL13 + 2044G → A, combined with IL4RA Q551R was significantly associated with rapid decline in lung functions in adult smokers.[26] Moreover, FEV_1/FVC ratio was significantly associated with IL13 + 2044G → A in the present study. As indicated before, decreased FEV_1/FVC ratio in childhood was a risk factor for airway obstruction in young adults.[27] These findings suggest that IL13 may be a ''pulmonary function gene'' as well as an ''AHR gene'' and IL13 + 2044G → A may play an important role.

A previous study found that constitutive over-expression of IL13 induced prominent epithelial hypertrophy in small and conducting airways, mucus production with enhanced airway mucin gene expression, and collagen deposition in the subepithelial region and in the adventitia of the small and large airways which are hall markers of airway remodeling in asthma.[8] In addition, a longitudinal population study conducted by Rasmussen et al.[5] suggested that airway remodeling as determined by ratio of FEV_1 to vital capacity begins in childhood and continues into adult life. Although bronchial biopsies were not performed to search for clues of airway remodeling because they are impossible in epidemiologic studies, it is plausible that genetic variation in IL13 results in reduced lung function found in the present study, by promoting airway remodeling.

Table 5 Results of multiple regression analysis for FEV_1/FVC according to the presence of airway hyperresponsiveness (AHR).

Explanatory variable	AHR[a] (+, *n* = 475)		AHR[b] (−, *n* = 1425)	
	Coefficient	*P* value	Coefficient	*P* value
Sex	0.049	0.312	0.005	0.865
Age	0.054	0.271	0.042	0.131
Height	−0.016	0.766	0.096	0.002
Atopy	−0.069	0.106	−0.024	0.323
Passive smoking	0.018	0.676	−0.004	0.857
IL13 + 2044G → A[c]	0.411	<0.001	0.416	<0.001

[a] *R* square = 0.169.
[b] *R* square = 0.183.
[c] Minor allele dominant model [AG + AA (dummy variable = 1) *vs*. GG (dummy variable = 2)].

The results of genetic association studies only become solid when the functional relevancy of the genetic polymorphisms of interest are proven and when independent replication confirms initial results. Previous studies have consistently witnessed functional consequences of IL13 + 2044G → A, i.e., enhancements of STAT6 phosphorylation and CD23 expression in monocytes and hydrocortisone-dependent IgE switching in B cells,[16] and AHR induction in a murine model of asthma.[28] Moreover, an important role of IL13 + 2044G → A in determining lung function was proven in Asian population[24] as well as different ethnic populations.[25,26] These findings plus a sufficiently large sample size of the present study make it possible to generalize our results that lung function during early life is in part associated with genetic components. Although differences according to genetic variations are minimal in early life, these differences may become greater when individuals grow up or confer particular susceptibilities to respiratory diseases. If patterns of lung function during early childhood and subsequent impairments of lung function in adult life can be predicted by analyzing genetic variations, physicians would be able to select high-risk individuals and better plan primary prevention.

Conflict of interest disclosure statement

This statement accompanies the article ''Genetic variation of IL13 as a risk factor of reduced lung function in children and adolescents: A cross-sectional population-based study in Korea,'' authored (co-authored) by Sang-Heon Cho (Heung-Woo Park, Jong-Eun Lee, Se-Hoon Kim, Yoon-Keun Kim, Kyung-Up Min, and You-Young Kim) and submitted to *Respiratory Medicine* as an original article. Below all authors have disclosed relevant commercial associations that might pose a conflict of interest:

Consultant arrangements: None.
Stock/other equity ownership: None.
Patent licensing arrangements: None.
Grants/research support: None.
Employment: None.
Speakers' bureau: None.
Expert witness: None.
Other: None.

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