ORIGINAL ARTICLE

Association of a 4-Locus Gene Model Including *IL13*, *IL4*, *FCER1B*, and *ADRB2* With the Asthma Predictive Index and Atopy in Chinese Han Children

Bai S[1,*], Hua L[1,*], Wang X[2], Liu Q[1], Bao Y[1]

[1]Department of Pediatric Pulmonology, Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
[2]Department of Pediatrics, Shanghai EverBetter Pubin Children's Hospital, Shanghai, China
*Shasha Bai and Li Hua contributed equally to this work.

J Investig Allergol Clin Immunol 2018; Vol. 28(3): 407-413
doi: 10.18176/jiaci.0272

Abstract

Background: Asthma is a complex and heterogeneous disease. We found gene-gene interactions between *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108 in asthmatic Chinese Han children. This 4-locus set constituted an optimal statistical interaction model.
Objective: We examined associations between the 4-gene model (*IL13*, *IL4*, *FCER1B*, and *ADRB2*) and the Asthma Predictive Index (API) and atopy in Chinese Han children.
Methods: Four single-nucleotide polymorphisms in the 4 genes were genotyped in 385 preschool children with wheezing symptoms using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. The *t* test and χ^2 tests were used for the analysis.
Results: Significant correlations were found between the 4-locus gene model and a stringent and loose API (both $P<.0001$). Additionally, a high-risk asthma genotype was a risk factor for a positive API (stringent API, OR=4.08; loose API, OR=2.36). We also found a statistically significant association between the 4-locus gene model and atopy ($P<.01$, OR=2.09).
Conclusions: Our results indicated that the 4-locus gene model consisting of *L13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108 was associated with the API and atopy. These findings provide evidence that this gene model can be used to determine a high risk of developing asthma and atopy in Chinese Han children.

Key words: Asthma Predictive Index. Atopy. Gene model. Single-nucleotide polymorphism.

Resumen

Antecedentes: El asma es una enfermedad compleja y heterogénea. En este estudio, encontramos que las interacciones gen-gen entre IL13 rs20541, IL4 rs2243250, ADRB2 rs1042713 y FCER1B rs569108, en niños asmáticos de nacionalidad china Han, constituyen un modelo estadístico óptimo de interacción.
Objetivo: Este estudio examinó un modelo de las asociaciones de cuatro genes (IL13, IL4, FCER1B y ADRB2) con el Índice Predictivo de Asma (IPA) y la atopia en niños Han chinos.
Métodos: Se genotiparon cuatro polimorfismos de un solo nucleótido (SNP) en los cuatro genes, en 385 niños en edad preescolar con síntomas de sibilancias, utilizando espectrometría de masas con desorción/ionización mediante láser asistida por Matriz (MALDI). Para el análisis estadístico de utilizaron el test t de Student y el c2.
Resultados: Se encontraron correlaciones significativas entre el modelo génico de los cuatro locus y el valor de IPA estricto y laxo (ambos P <0,0001). Además, el genotipo de riesgo alto de asma fue un factor de riesgo para IPA positivo (IPA estricto: OR = 4,08, IPA laxo: OR = 2,36). También, encontramos una asociación estadísticamente significativa entre el modelo génico de los cuatro locus, con atopia (P <0,01, OR = 2,09).
Conclusiones: Nuestros resultados indicaron que el modelo génico de cuatro locus compuesto por L13 rs20541, IL4 rs2243250, ADRB2 rs1042713 y FCER1B rs569108 estaba asociado con IPA y atopia. Estos hallazgos proporcionan la evidencia de la utilidad de este modelo génico para determinar el riesgo alto de desarrollar asma y atopia en niños chinos Han.

Palabras clave: Índice Predictivo de Asma. Atopia. Modelo génico. Polimorfismo de nucleótido simple.

Introduction

Asthma is a common respiratory disease in childhood and is characterized by chronic airway inflammation, airway hyperresponsiveness, and reversible airflow obstruction [1,2]. The pathogenesis of asthma is complex and involves multiple inflammatory cells and cytokines. Genes that encode these cytokines are key factors for the development of asthma. Our previous case-control study reported that 4 single-nucleotide polymorphisms (SNPs)—*IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108—not only had a significant individual effect on asthma (G allele of rs20541, T allele of rs2243250, A allele of rs1042713, and G allele of rs569108), but also interact to confer a higher risk of asthma in Chinese Han children. These SNPs were chosen to build a 4-way gene-gene interaction model to determine asthma susceptibility using the multifactor dimensionality reduction method [3]. Furthermore, a number of SNPs in *ADRB2*, IL-13, IL-4, and *FCER1B* are associated with elevated IgE levels and the development of both atopy and asthma [4-7]. However, it is unknown whether this 4-locus gene model is correlated with atopy.

Most wheezing symptoms occur in children of preschool age, and approximately 50% of children experience a wheezing-related disorder during the first 6 years of life. However, only a fraction of children experience symptoms of asthma later in life [8]. Therefore, this period is important in the development of asthma, and certain gene variants might be associated with asthma or a wheezing phenotype. Although transient wheezing is often nonatopic, recurrent wheezing in young children who have signs of atopy is a precursor of asthma [9,10]. Currently, there is no specific diagnostic standard for asthma in children younger than 6 years in China. The 2016 Chinese guideline for childhood asthma [11] recommends that the diagnosis of asthma in children <6 years of age is based on the following criteria: (*1*) symptom patterns (recurrent wheezing, exercise-induced cough or wheezing, intermittent nocturnal cough that is not caused by viral infection, and continued wheezing after the age of 3 years) and (*2*) therapeutic response to an asthma controller and/or reliever treatment [12]. Therefore, identifying children who have the potential to develop persistent asthma and initiate treatment early is critical. The Asthma Predictive Index (API) serves as a clinically applicable tool for identifying preschool children with recurrent wheezing at high risk for subsequent asthma [13,14]. Researchers have found that combining data on the expression of the inflammation-related genes TLR4 and TNF-α with application of API correctly predicts asthma in preschool children with wheezing [15].

In this study, we genotyped the following 4 SNPs in candidate genes: *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108. We attempted to determine whether potential correlations existed between the gene model and API and atopy. Additionally, we aimed to provide a scientific basis for early screening of children at high risk for asthma and to improve primary disease prevention.

Methods

Study Population

A total of 385 preschool children with wheezing symptoms were recruited. We obtained written informed consent from their guardians. All patients were from the asthma outpatient clinic and ward of the Children's Respiratory Department at Xinhua Hospital, Shanghai, China and had had at least 1 episode of wheezing symptoms (ie, a continuous high-pitched sound with a musical quality emitting from the chest during expiration). The interval between 2 wheezing episodes was at least 7 days without respiratory symptoms [16]. The study exclusion criteria consisted of the following: congenital pulmonary disease, congenital heart disease, congenital vascular malformation, congenital immune defects, foreign body aspiration, and tuberculosis of the bronchial lymph nodes. Complete allergen testing results were available for 250 of the 385 patients. The study was approved by the Ethics Committee of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Ethics Approval Number: XHEC-D-2016-393) and was conducted according to the principles of the Declaration of Helsinki.

Evaluation of API and Atopy

We developed 2 indices to classify children as potentially at risk for asthma at school age, namely, the stringent API and the loose API. The stringent API included frequent wheezing during the first 3 years of life and either 1 major risk factor (parental history of asthma or eczema) or 2 of 3 minor risk factors (eosinophilia, wheezing without colds, and allergic rhinitis). The loose API included any wheezing during the first 3 years of life and the same combination of risk factors described previously (Table 1) [14].

The serum specific IgE levels for a panel of locally relevant aeroallergens (including *Dermatophagoides farinae, Dermatophagoides pteronyssinus*, cockroach, cat or dog hair, and ragweed) and food allergens (including milk, egg, peanut, and seafood) were measured using fluorescent enzyme immunoassay. A specific IgE concentration ≥0.35 kIU/L was considered positive, and children with at least 1 positive

Table 1. Asthma Predictive Index[a]

Major Criteria	Minor Criteria
1. Parental asthma[b]	1. Allergic rhinitis[c]
2. Eczema[d]	2. Wheezing without colds
	3. Eosinophilia (>4%)

[a]Loose index for the prediction of asthma: Early wheezing plus at least 1 of 2 major criteria or 2 of 3 minor criteria. Stringent index for the predication of asthma: early frequent wheezing plus at least 1 of 2 major criteria or 2 of 3 minor criteria.
[b]History of physician-diagnosed asthma.
[c]Physician-diagnosed allergic rhinitis, as reported in questionnaires at ages 2 or 3.
[d]Physician-diagnosed atopic dermatitis, as reported in questionnaires at ages 2 or 3.

allergen-specific IgE test response were classified as atopic [17,18].

Genotyping and Grouping

Genomic DNA was isolated from an oral mucosal swab using a magnetic genomic DNA extraction kit (EmerTher). Multiplex polymerase chain reaction (PCR) was used to detect the genotypes of the 4 loci (*IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108). The PCR cycling conditions were as follows: 95°C for 2 minutes; 45 cycles at 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 60 seconds; and, finally, 72°C for 5 minutes. The conditions used for the SAP enzyme digestion reaction were 37°C for 40 minutes and then termination at 85°C for 5 minutes. The conditions used for iPLEX were 95°C for 30 seconds; 5 inner cycles at 52°C for 5 seconds and at 85°C for 5 seconds; and 40 outer cycles at 94°C for 5 seconds, 52°C for 5 seconds, and 85°C for 5 seconds. After completing the multiplex PCR, we used matrix-assisted laser desorption ionization time-of-flight mass spectrometry to discriminate between the 4 SNPs. The genotyping results were validated by blind retesting of 10% of the total number of samples, and the concordance rate reached 99.5%. The call rate for each SNP was greater than 99%.

In the present analysis, the different risk genotypes for asthma were defined according to the number of risk allele homozygotes. The control group comprised patients with no risk homozygotes (ie, *IL13* rs20541 AA or GA, *IL4* rs2243250 CC or TC, *ADRB2* rs1042713 GG or AG, and *FCER1B* rs569108 AA or AG). Those with different genotype combinations who had a significantly higher risk of asthma (if $P<.05$ and OR>1) than the reference group were defined as being at higher genetic risk for asthma. The others were defined as being at lower genetic risk for asthma. The grouping results of the different genotype combinations are shown in Table 2.

Statistical Analysis

A total of 385 preschool children with wheezing symptoms were divided into 2 groups according to the classification method. The results were expressed as proportions or mean (SD). Patient characteristics were compared using the *t* test or ANOVA for numerical data. The χ^2 or Fisher exact test was used to compare categorical variables. All the SNPs were examined for Hardy-Weinberg equilibrium using the χ^2 test. The allele frequencies were estimated using the gene-counting method. Associations between API and atopy and the different groups were assessed using the Pearson χ^2 test or the Fisher exact test, as appropriate. All comparisons were 2-tailed, and *P* values <.05 were considered statistically significant. The statistical analyses were conducted using the SPSS package version 20.0 (IBM Corp).

Results

Study Population

The study population comprised 385 preschool children with wheezing symptoms. According to the classification method 174 patients were at high genetic risk of asthma and the others were at low genetic risk of asthma. No significant differences were observed between the 2 groups in terms of age. However, more male children exhibited wheezing in these 2 groups than female children. The percentage of males in the high-risk genotype group was lower than that in the low-risk genotype group, although this difference was not statistically significant. Moreover, significant differences were found with respect to age at onset of wheezing ($P<.01$). More children in the high-risk genotype group began to wheeze in infancy than those in the low-risk group. There were no statistically significant differences between the 2 groups in terms of a confirmed personal history of allergy and family atopy (Table 3).

Table 2. High- and Low-Risk Genotype of Asthma According to the 4 Genotypes Studied

	Genotype			
Groups	*IL13* rs20541	*IL4* rs2243250	*ADRB2* rs1042713	*FCER1B* rs569108
Low-risk genotype	AA or GA	CC or TC	GG or AG	AA or AG
	AA or GA	CC or TC	GG or AG	GG
	AA or GA	CC or TC	AA	AA or AG
	AA or GA	TT	GG or AG	AA or AG
	GG	CC or TC	GG or AG	AA or AG
	AA or GA	CC or TC	AA	GG
	GG	CC or TC	GG or AG	GG
High-risk genotype	AA or GA	TT	GG or AG	GG
	AA or GA	TT	AA	AA or AG
	GG	CC or TC	AA	AA or AG
	GG	TT	GG or AG	AA or AG
	AA or GA	TT	AA	GG
	GG	CC or TC	AA	GG
	GG	TT	GG or AG	GG
	GG	TT	AA	AA or AG
	GG	TT	AA	GG

Table 3. Baseline Demographic Characteristics in Preschoolers With High-Risk and Low-Risk Genotypes

Variable	High-Risk Group	Low-Risk Group	*P* Value
Number	174	211	NA
Mean (SD) age, mo	42.3 (21.37)	44.2 (21.40)	.39
Males, No. (%)	113 (64.9)	148 (70.1)	.28
Age at onset of wheezing, No. (%)	58 (58)	42 (42)	
<1 y			
1-3 y	70 (46.4)	81 (53.6)	.001
> 3 y	46 (34.3)	88 (65.7)	
Personal history of allergy, No. (%)[a]	159 (91.4)	184 (87.2)	.191
Family history of atopy, No. (%)[b]	110 (63.2)	125 (59.2)	.426

Abbreviation: NA, not available.
[a]Personal history of allergy was defined as confirmed rhinitis, eczema, and food allergy.
[b]Family atopy was defined as confirmed rhinitis, asthma, and eczema in at least 1 family member [34].

Associations Between the Loose and Stringent API and Different Risk Genotype Groups

All 4 SNPs examined were in Hardy-Weinberg equilibrium (*P*>.05). As shown in Table 4, the percentage of patients with the positive API was significantly higher in the high-risk genotype group than that in the low-risk genotype group regardless of whether the comparison was with the loose API or with the stringent API (both *P*<.0001). Furthermore, a high genetic risk of asthma was a risk factor for a positive loose and stringent API (loose API, OR=2.36; stringent API, OR=4.08) (Table 4).

Association Between Different Groups and Atopy

More atopic patients were found in the high-risk genotype group than in the low-risk genotype group; this difference was statistically significant (*P*<.01, OR=2.09) (Table 5).

Discussion

This is the first study to report the association between a 4-locus gene model consisting of *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108 and API and atopy.

Table 4. Association Between Loose and Stringent API and Genotype Risk Group

	Loose API		Stringent API	
Group	Positive, No. (%)	Negative, No. (%)	Positive, No. (%)	Negative, No. (%)
High-risk group	119 (68.4%)	55 (31.6%)	95 (54.6%)	79 (45.4%)
Low-risk group	101 (47.9%)	110 (52.1%)	48 (22.7%)	16 (77.3%)
P Value	5.12E-5		1.22E-10	
OR (95%CI)	2.36 (1.55-3.58)		4.08 (2.63-6.33)	

Abbreviations: API, Asthma Predictive Index; CI, confidence interval; OR, odds ratio.

Table 5. Association Between Genotype Risk Group and Atopy

Group	Positive, No. (%)	Negative, No. (%)	*P* Value	OR (95%CI)
High-risk group	79 (71.8%)	31 (28.2%)	.006	2.09 (1.22-3.55)
Low-risk group	77 (55%)	63 (45%)		

Abbreviations: CI, confidence interval; OR, odds ratio.

It is also the first study to use a gene model to examine atopic wheezing in Chinese Han preschool children.

Epidemiologic studies indicate that the development of asthma and allergic sensitization is determined early in life [19]. During this period, wheezing is heterogeneous, and only 30% of preschool children with recurrent wheezing still have asthma at the age of 6 years [20]. However, it is difficult to identify young children with wheezing symptoms who will have asthma at school age. The API was first proposed by the Tucson Children's Respiratory Study team in the year 2000 [14]. The index is based on factors that were found during the first 3 years of life and was able to predict continued wheezing at school age. The API is attractive for use in clinical practice because it is simple to assess and requires no additional equipment or expertise [21]. One study that validated the API reported that a patient with a positive loose API indicated a 4-fold higher probability of developing asthma than a negative patient. Additionally, a positive stringent API indicated an 8-fold higher probability of developing asthma [22]. In our study, we found that more children had a positive API in the high-risk genotype group than in the low-risk genotype group, independently of whether the comparison was with a loose API or with a stringent API. Furthermore, the correlation between a stringent API and the 4-locus gene model was stronger (stringent API, $P<.0001$, OR=4.08; loose API, $P<.0001$, OR=2.36). These findings suggested that children with high-risk genotypes for asthma are susceptible to developing asthma. In addition, more male children exhibited wheezing than female children in both groups, consistent with the phenomenon that boys are more susceptible to wheezing than girls before the age of 6 years [23]. Interestingly, a significantly higher proportion of children in the high-risk genotype group began to wheeze at a younger age, especially during their first year of life, thus indicating an association between the gene model and age at onset of wheezing.

Asthma is generally considered a multifactorial disease involving immunologic, genetic, environmental, and other factors. Its chronic airway inflammation mechanism and type have been widely demonstrated. Atopy is present in most children with asthma and is more common in those with severe asthma [24]. Central to the process of allergic asthma is an enhanced T_H2 response, which increases secretion of interleukin (IL) 5, IL-4, and IL-13. IL-4 drives B-cell isotype class switching and IgE synthesis, and IgE binds to high-affinity IgE receptors on mast cells and leads to mast cell activation following allergen-mediated IgE cross-linking [25]. Our previous study reported that *IL13* R130Q was associated with childhood asthma and could promote the production of IgE [26]. The results of interactions between *IL13* and *IL4*RA demonstrated a significant association with plasma total IgE [27]. Moreover, β_2 adrenoceptor agonists are used to relieve bronchoconstriction by modulating airway smooth muscle. A study from Korea reported that 4-locus gene-gene interactions between *B2ADR*-46A>G, *CCR3*-520T>G, *CysLTR1*-634C>T, and *FCER1B*-109T>C had substantial effects on aspirin-intolerant asthma [28]. Our results revealed that the 4-locus gene model was significantly associated with atopy and that the high-risk genotype was a risk factor for atopy ($P<.01$, OR=2.09).

We demonstrated the predictive role of the 4-locus gene model for asthma in young children and, for the first time, showed that it was associated with atopy in Chinese Han patients. We hypothesized that the 4-locus gene model consisting of *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108 could be used to differentiate between atopic asthma and nonatopic asthma. Our results are consistent with the data from a 5-locus gene model in Filipino cases [29].

Our study was subject to a series of limitations. First, all patients with wheezing symptoms were from the asthma outpatient clinic and ward of the Children's Respiratory Department, and this may have resulted in data bias. Second, we did not have a healthy control group. In our future studies, healthy children will be recruited, and genetic differences will be compared between wheezing children and healthy controls. Third, the method used to clarify different risk genotype groups did not fully consider the role of single risk alleles, and the use of only homozygous risk alleles may weaken the role of individual risk alleles for the disease. Therefore, any future studies should include other approaches, such as the genetic risk score (GRS). The GRS is an emerging method for exploring correlations between SNPs and clinical phenotypes of complex diseases. It integrates weak effects of multiple SNPs and dramatically enhances the predictability of complex diseases using gene polymorphisms [30]. The GRS has been widely applied in genetic studies of common complex diseases, such as diabetes, coronary heart disease, asthma, and others [31-33]. The results from the Wheezing Illnesses Study Leidsche Rijn (WHISTLER) cohort illustrated that an adult-derived GRS for allergy predicted the risk of developing allergies during childhood [33].

We intend to conduct a series of validation tests for the gene model in different ethnic groups, birth cohorts, lung function groups, and asthma phenotypes. We believe that this 4-locus model is extensively validated, and we consider it apt for use in clinical practice.

In conclusion, the results of this study suggest that the 4-locus gene model comprising *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108 was significantly associated with API and atopy. Our findings provide evidence of the usefulness of the model for determining a high-risk genotype for developing asthma and atopy. The gene model may become a useful tool for objectively predicting the risk of asthma in Chinese Han preschool children after comprehensive validation studies.

Acknowledgments

We are grateful to Jiakui Pan and his team for their contributions to the study. We thank Jiayan Luan for her assistance with sample collection. We also heartily thank all the study participants and the parents of the minor participants.

Funding

Scientific Research Fund of Shanghai Municipal Commission of Health and Family Planning, number: 20164Y0136.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Habernau MA, Del PAV, Rodríguez Vidigal FF, Bobadilla GP. Role of periostin in uncontrolled asthma in children. (DADO study). J Investig Allergol Clin Immunol. 2017;27:291-8.
2. Troy NM, Hollams EM, Holt PG, Bosco A. Differential gene network analysis for the identification of asthma-associated therapeutic targets in allergen-specific T-helper memory responses. BMC Medical Genomics. 2016;9:9.
3. Hua L, Zuo XB, Bao YX, Liu QH, Li JY, Lv J, et al. Four-locus gene interaction between IL13, IL4, FCER1B, and ADRB2 for asthma in Chinese Han children. Pediatr Pulmonol. 2016;51:364-71.
4. Scichilone N, Caponetto C, Fagone E, Benfante A, Paternò A, Heffler E, et al. The Arg/Arg polymorphism of the ADRB2 is associated with the severity of allergic asthma. J Allergy Clin Immunol Pract. 2016;4:1251-2.
5. Miyake Y, Tanaka K, Arakawa M. Relationship between polymorphisms in IL4 and asthma in Japanese women: the Kyushu Okinawa Maternal and Child Health Study. J Investig Allergol Clin Immunol. 2013;23:242-7.
6. Liu Q, Hua L, Fang D, Lin Q, Zhu Y, Gan X, et al. Interleukin-13 and RANTES polymorphisms in relation to asthma in children of Chinese Han nationality. Asian Pac J Allergy Immunol. 2013;31:247-52.
7. Oh KY, Kang MJ, Choi WA, Kwon JW, Kim BJ, Yu J, et al. Association between serum IgE levels and the CTLA4 +49A/G and FCER1B -654C/T polymorphisms in Korean children with asthma. Allergy Asthma Immunol Res. 2010;2:127-33.
8. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med. 1995;332:133-8.
9. Dijk FN, de Jongste JC, Postma DS, Koppelman GH. Genetics of onset of asthma. Curr Opin Allergy Clin Immunol. 2013;13:193-202.
10. Lynch SV, Wood RA, Boushey H, Bacharier LB, Bloomberg GR, Kattan M, et al. Effects of early-life exposure to allergens and bacteria on recurrent wheeze and atopy in urban children. J Allergy Clin Immunol. 2014;134:593-601.
11. Hong J, Bao Y, Chen A, Li C, Xiang L, Liu C, et al. Chinese guidelines for childhood asthma 2016: Major updates, recommendations and key regional data. J Asthma. 2017:1-9.
12. Hong J, Bao Y. Emphasis on standardized diagnosis and treatment of bronchial asthma in Children. Zhonghua Er Ke Za Zhi. 2016;54:161-2.
13. Wi CI, Park MA, Juhn YJ. Development and initial testing of Asthma Predictive Index for a retrospective study: an exploratory study. J Asthma. 2015;52:183-90.
14. Castro-Rodríguez JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. Am J Respir Crit Care Med. 2000;162:1403-6.
15. Klaassen EMM, van de Kant KDG, Jöbsis Q, van Schayck OCP, Smolinska A, Dallinga JW, et al. Exhaled biomarkers and gene expression at preschool age improve asthma prediction at 6 years of age. Am J Respir Crit Care Med. 2015;191:201-7.
16. Blanken MO, Rovers MM, Molenaar JM, Winkler-Seinstra PL, Meijer A, Kimpen JLL, et al. Respiratory syncytial virus and recurrent wheeze in healthy preterm infants. N Engl J Med. 2013;368:1791-9.
17. Leung TF, Sy HY, Ng MC, Chan IH, Wong GW, Tang NL, et al. Asthma and atopy are associated with chromosome 17q21 markers in Chinese children. Allergy. 2009;64:621-8.
18. Savenije OE, Mahachie John JM, Granell R, Kerkhof M, Dijk FN, de Jongste JC, et al. Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with wheezing phenotypes and asthma in childhood. J Allergy Clin Immunol. 2014;134:170-7.
19. de Vos G, Milush JM, Aaron J, Pichardo Y, York VA, Nazari R, et al. Peripheral CD8+ T-cell levels are decreased in atopic wheezing children aged less than 4 years. J Investig Allergol Clin Immunol. 2012;22:442-4.
20. Burbank AJ, Szefler SJ. Current and future management of the young child with early onset wheezing. Curr Opin Allergy Clin Immunol. 2017;17:146-52.
21. Castro-Rodriguez JA. The Asthma Predictive Index: a very useful tool for predicting asthma in young children. J Allergy Clin Immunol. 2010;126:212-6.
22. Leonardi NA, Spycher BD, Strippoli MP, Frey U, Silverman M, Kuehni CE. Validation of the Asthma Predictive Index and comparison with simpler clinical prediction rules. J Allergy Clin Immunol. 2011;127:1466-72.
23. Leynaert B, Sunyer J, Garcia-Esteban R, Svanes C, Jarvis D, Cerveri I, et al. Gender differences in prevalence, diagnosis and incidence of allergic and non-allergic asthma: a population-based cohort. Thorax. 2012;67:625-31.
24. Del Giacco SR, Bakirtas A, Bel E, Custovic A, Diamant Z, Hamelmann E, et al. Allergy in severe asthma. Allergy. 2017;72:207-20.
25. Russell RJ, Brightling C. Pathogenesis of asthma: implications for precision medicine. Clin Sci (Lond). 2017;131:1723-35.
26. Chu Y, Hua L, Liu Q, Bao Y. A common variant associated with asthma, interleukin 13R130Q, promotes the production of IgE. Int J Immunogenet. 2012;39:308-13.
27. Chan IHS, Tang NLS, Leung TF, Huang W, Lam YY, Li CY, et al. Study of gene-gene interactions for endophenotypic quantitative traits in Chinese asthmatic children. Allergy. 2008;63:1031-9.
28. Kim SH, Jeong HH, Cho BY, Kim M, Lee HY, Lee J, et al. Association of four-locus gene interaction with aspirin-intolerant asthma in Korean asthmatics. J Clin Immunol. 2008;28:336-42.
29. de Guia RM, Echavez MD, Gaw EL, Gomez MR, Lopez KA, Mendoza RC, et al. Multifactor-dimensionality reduction reveals interaction of important gene variants involved in allergy. Int J Immunogenet. 2015;42:182-9.
30. Burgess S, Thompson SG. Use of allele scores as instrumental variables for Mendelian randomization. Int J Epidemiol. 2013;42:1134-44.
31. Kawai VK, Levinson RT, Adefurin A, Kurnik D, Collier SP, Conway D, et al. A genetic risk score that includes common type 2 diabetes risk variants is associated with gestational diabetes. Clin Endocrinol (Oxf). 2017;87:149-55.

32. Knowles JW, Zarafshar S, Pavlovic A, Goldstein BA, Tsai S, Li J, et al. Impact of a genetic risk score for coronary artery disease on reducing cardiovascular risk: a pilot randomized controlled study. Front Cardiovasc Med. 2017;4:53.
33. Arabkhazaeli A, Ahmadizar F, Leusink M, Arets HGM, Raaijmakers JAM, Uiterwaal CSPM, et al. The association between a genetic risk score for allergy and the risk of developing allergies in childhood-Results of the WHISTLER cohort. Pediatr Allergy Immunol. 2018;29:72-7.
34. Bolat E, Arikoglu T, Sungur MA, Batmazd SB, Kuyucu S. Prevalence and risk factors for wheezing and allergic diseases in preschool children: A perspective from the Mediterranean coast of Turkey. Allergologia Et Immunopathologia. 2017;45:362-8.

Manuscript received March 1, 2018; accepted for publication May 8, 2018.

Yixiao Bao

Department of Pediatric Pulmonology
Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine, 1665 Kongjiang Road
Shanghai, China
E-mail: asthma_group_child@163.com