

Four-Locus Gene Interaction Between *IL13*, *IL4*, *FCER1B*, and *ADRB2* for Asthma in Chinese Han Children

Li Hua, MD,¹ Xian-Bo Zuo, PhD,² Yi-Xiao Bao, MD, PhD,^{1*} Quan-Hua Liu, MD,¹ Jing-Yang Li, MD,¹ Jie Lv, MD,¹ Ding-Zhu Fang, MD, PhD,¹ Qian Lin, MD,¹ Jun Bao, MD, PhD,¹ and Ruo-Xu Ji, MD¹

Summary. Rationale: *IL13*, *IL4*, *IL4RA*, *FCER1B*, and *ADRB2* are important inflammatory genes associated with immunoglobulin E levels. This study attempts to determine whether there are gene–gene interactions in the five genes among asthmatic children of Chinese Han nationality. Methods: Nine single-nucleotide polymorphisms (SNPs) in the five genes were genotyped in 1,000 asthmatic children and 1,000 healthy controls using TaqMan real-time quantitative polymerase chain reaction. Multifactor–dimensionality reduction method was applied for the analysis. Results: A four-way gene–gene interaction model consisting of *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108 was chosen as the optimal one for determining asthma susceptibility (testing balanced accuracy = 0.6089, cross-validation consistency = 10/10, $P = 6.98 \times 10^{-5}$). Each of the four SNPs was identified to have an independent association with childhood asthma (G allele of rs20541, odds ratio (OR) = 1.24, $P = 1.23 \times 10^{-3}$; T allele of rs2243250, OR = 1.25, $P = 3.81 \times 10^{-3}$; A allele of rs1042713, OR = 1.29, $P = 6.75 \times 10^{-5}$; G allele of rs569108, OR = 1.27, $P = 3.86 \times 10^{-3}$). Individuals homozygous for the risk alleles at all the four loci (rs20541 GG, rs2243250 TT, rs1042713 AA, and rs569108 GG) had a significantly higher risk of asthma compared with those without any risk homozygotes (OR = 13.55, $P = 4.28 \times 10^{-3}$), and also greater than those with less than four risk homozygotes (OR = 10.09, $P = 6.51 \times 10^{-3}$). Conclusions: Our results suggest that *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108, four SNPs with significant sole effect on asthma, interact to confer a higher risk for the disease in Chinese Han children. **Pediatr Pulmonol.** 2016;51:364–371. © 2015 Wiley Periodicals, Inc.

Key words: asthma; gene–gene interactions; *IL13*; multifactor-dimensionality reduction; single-nucleotide polymorphism.

Funding source: Scientific Research Fund of Xin Hua Hospital, Number: 12QYJ03; Scientific Research Fund for Selection and Cultivation of Outstanding Young Teachers in Colleges and Universities of Shanghai, Number: jdy10116; Key Technologies R&D Program of Science and Technology Commission of Shanghai Municipality, Number: 10DZ1951000; Program of Science and Technology Commission of Shanghai Municipality, Number: 14411964800; General Program of National Natural Science Foundation of China, Number: 30972750.

INTRODUCTION

Asthma is a common chronic inflammatory disease of the airways, characterized by airway hyper-responsiveness and intermittent airflow obstruction. Its incidence is on the rise among children, which brings heavy burden to the whole society and results in huge medical expenditure around the world. Accumulating evidence has shown that asthma is caused by a complex interaction of genetic and environmental factors.^{1,2} The overall genetic contribution is suggested to be around 50–60%.³ Numerous genes may control the development of asthma, each having a small and possibly synergistic effect on the phenotype.

Ober and Hoffjan⁴ reviewed 118 genes with association to asthma or atopy, among which 54 genes have been replicated in 2–5 independent samples, 15 genes in 6–10

¹Department of Pediatric Pulmonology, Xin Hua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

²Key Laboratory of Dermatology, Anhui Medical University, Ministry of Education, Hefei, Anhui, China.

Conflict of interest: None

*Correspondence to: Yi-Xiao Bao, MD, PhD, Department of Pediatric Pulmonology, Xin Hua Hospital, Shanghai Jiao Tong University School of Medicine, 1665 Kongjiang Road, Shanghai 200092, China. E-mail: smilebao@sjtu.edu.cn

Received 21 May 2015; Revised 13 August 2015; Accepted 14 September 2015.

DOI 10.1002/ppul.23322

Published online 27 November 2015 in Wiley Online Library (wileyonlinelibrary.com).

independent samples, and 10 genes in >10 independent samples. The 25 genes associated with asthma- or atopy-related phenotypes in six or more independent samples are considered to be true susceptibility genes. The elite group of genes replicated in >10 studies include *IL13*, *IL4*, *IL4RA*, *FCER1B*, and *ADRB2*. The majority of asthma is associated with atopy, characterized by enhanced T-cell secretion of T helper 2 (Th2) cytokines. *IL13*, *IL4*, and *IL4RA* genes encode the cytokines which play a crucial role in the widely used immunological model with an altered balance between Th1 and Th2 immune responses.⁵ A number of single-nucleotide polymorphisms (SNPs) in the *IL13*, *IL4*, and *IL4RA* genes have been shown to be associated with elevated immunoglobulin E (IgE) levels,^{6–8} as well as the development of atopy and asthma.^{9–11} *FCER1B* gene codes high-affinity IgE receptor-beta chain (FCER1B). IgE-dependent activation of mast cells and basophils through FCER1 is involved in the pathogenesis of allergen-induced immune responses in atopic diseases and FCER1B is thought to function as an amplifier of FCER1 expression and signaling.¹² Variants of the *FCER1B* gene have been reported to be correlated with atopic asthma¹³ and high total serum IgE levels.¹⁴ Beta2-adrenergic receptor (ADRB2) mediates the actions of catechol amines which modulate the smooth muscle of the respiratory tree to keep airway patent. The *ADRB2* gene contains several common genetic variations which were associated with asthmatic symptoms¹⁵ and bronchodilator response.¹⁶ All these five genes, *IL13*, *IL4*, *IL4RA*, *FCER1B*, and *ADRB2*, are important inflammatory genes associated with IgE levels.

Our study attempts to determine whether there are gene–gene interactions between genetic variants in the aforementioned five genes among asthmatic children of Chinese Han nationality. Multifactor-dimensionality reduction (MDR), which has been introduced by Ritchie et al. as a nonparametric and genetically model-free alternative to logistic regression, is a powerful tool for detecting high-order gene–gene interactions in a small population.^{17,18} In this study, nine loci in the five genes proved to be related with asthma were genotyped in Chinese Han population, and gene–gene interactions were examined with MDR.

MATERIALS AND METHODS

Study Participants

A total of 2,000 unrelated Chinese Han individuals were enrolled in the study: 1,000 asthmatic children and an equal number of healthy controls. Table 1 shows demographic characteristics of the study subjects. Written informed consent was obtained from all the adult participants and the parents of each minor participant. The study was approved by the Ethics

TABLE 1—Demographic Characteristics of the Study Participants

	Asthmatics	Controls
Number	1000	1000
Male (%)	503 (50.30%)	503 (50.30%)
Female (%)	497 (49.70%)	497 (49.70%)
Mean (range) age (years)	4.90 (3–12) ¹	23.32 (18–25) ¹
Atopy (%) ²	807 (80.70%)	NA

NA, not available.

¹Controls were older than asthmatics, which allowed a longer period for exclusion of asthma.

²Positive allergy skin prick tests.

Committee of Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Ethics Approval Number: XHEC-D-2008-012) and conducted according to the principles in the Declaration of Helsinki. All patients were recruited from the Asthma Clinic of Xin Hua Hospital. Each of them had at least one active asthma symptom. Asthma was defined by the following two criteria: two or more episodes of wheezing and shortness of breath during the past year; reversibility of wheezing and dyspnea, either spontaneously or by bronchodilator treatment as defined by the guidelines of the American Thoracic Society.¹⁹ The diagnosis of asthma in participants was confirmed clinically by a pediatrician who had treated the child for more than two years. Because wheezing is often associated with viral respiratory infection in young children, we excluded children younger than three years of age. Among the patients, 80.70% had positive allergy skin prick tests. The control participants were healthy students from several universities at Shanghai. The inclusion criteria for the control group were as follows: no symptoms or history of asthma, no symptoms or history of other pulmonary diseases, no symptoms or history of allergy, and no first-degree relatives with a history of asthma or atopy.

Selection of Genes and SNPs

Ober reviewed 118 genes with association to asthma or atopy, among which 25 have been replicated in six or more studies and thus are believed to be the susceptible genes most likely associated with asthma and atopy.⁴ From the 25 genes, we selected five key inflammatory genes associated with IgE levels, which have been replicated in more than 10 studies. These included genes for (i) cytokines: interleukin 13 (*IL13*), *IL4*, *IL4* receptor-alpha chain (*IL4RA*); (ii) mediators of atopy and airway inflammation: *ADRB2*, *FCER1B*. Within these genes, nine functional SNPs^{6–11,13–16,20–25} were chosen for analysis, which had minor allele frequencies greater than 10%. Table 2 shows the list of SNPs analyzed in this study.

TABLE 2—Candidate Genes and Single-Nucleotide Polymorphisms Analyzed in This Study

Gene	SNP	rs Number	Chromosome position	Location	Association
<i>IL13</i>	−1112C>T	rs1800925	5:132657117	Promoter	Asthma ^{6,9} , atopy ¹⁰
<i>IL13</i>	+1923C>T	rs1295686	5:132660151	Intron 3	Asthma ²⁰
<i>IL13</i>	R110Q	rs20541	5:132660272	Exon 4	Asthma ^{7,9} , atopy ¹⁰ , IgE ^{6–8}
<i>IL4</i>	−590C>T	rs2243250	5:132673462	Promoter	Asthma ^{11,21} , IgE and atopy ²²
<i>ADRB2</i>	R16G	rs1042713	5:148826877	Exon1	Asthma ¹⁵ , bronchodilator response ¹⁶
<i>FCER1B</i>	−109C>T	rs1441586	11:60088555	Promoter	Asthma ²³ , IgE ¹⁴
<i>FCER1B</i>	E237G	rs569108	11:60095631	Exon7	Asthma ^{13,23,24} , IgE ²⁴
<i>IL4RA</i>	I75V	rs1805010	16:27344882	Exon 5	Asthma ¹⁰
<i>IL4RA</i>	Q551R	rs1801275	16:27363079	Exon 12	Atopic asthma ²⁵

SNP, single-nucleotide polymorphism; rs, reference SNP.

Genotyping

Genomic DNA was isolated from an oral mucosal swab using TIANamp Swab DNA Kit (TIANGEN Biotech, Beijing, China). TaqMan real-time quantitative polymerase chain reaction^{26,27} (ABI 9700, Applied Biosystems, Foster City, CA) was applied to detect the genotypes of the nine SNP loci (*IL13* rs1800925, rs1295686, rs20541, *IL4* rs2243250, *ADRB2* rs1042713, *FCER1B* rs1441586, rs569108, *IL4RA* rs1805010, and rs1801275). The reaction was performed in a 384-well plate format. Each well contained FAM-labeled and VIC-labeled probes. The total volume of 10 μ L included 10 ng of genomic DNA, 5 μ L of TaqMan[®] Universal Master Mix (Applied Biosystems, Foster City, CA), and 0.2 μ L of TaqMan[®] SNP Genotyping Assay Mix. Cycling conditions included one cycle at 95°C for 10 min, 40 cycles at 95°C for 15 sec, and at 60°C for 60 sec. To avoid contamination, negative controls were included in each PCR reaction. After PCR, the results from each sample were automatically determined by measuring allele-specific final fluorescence in an ABI Prism 9700HT detection system, using the SDS 2.2 software for allele discrimination (Applied Biosystem). Typings were validated by blind retesting of 10% of the total samples, and the concordance rate reached 99.5%. The call rate for each SNP was greater than 99%.

Statistical Analysis

All SNPs were examined for Hardy–Weinberg equilibrium in control subjects using the χ^2 test. Association between asthma and each of the nine loci was made with the Pearson's χ^2 statistics. In addition to the allelic test of association, the genetic models (additive model, dominant model, and recessive model) were tested for the SNPs using logistic regression analysis, controlled for age, and gender. The association *P* value, odds ratio (OR), and 95% confidence intervals (95% CIs) were estimated with PLINK 1.07 (<http://pngu.mgh.harvard.edu/~purcell/plink/>). Two-tailed *P* values of 0.0055 or less after

Bonferroni Multiple Testing correction were considered statistically significant.

Gene–gene interactions were analyzed with MDR (V2.0 Beta 2), which can reduce high dimensional data to one dimension with two classes. First, the sample dataset is divided into 10 parts of equal size for 10-fold cross-validation (nine parts for training and one part for testing). Secondly, an exhaustive search of a set of *n* SNPs is performed. For example, for three loci with three genotypes each, there are 27 possible combinations. Finally, the case/control ratio is calculated for each combination, which is assigned as “high risk” (>1) or “low risk” (<1). MDR models are evaluated on the testing balanced accuracy (TBA), the cross-validation consistency (CVC) and the statistical significance. TBA assesses how often the subjects are correctly classified with respect to their case or control status, while CVC indicates the consistency with which individuals are classified. Heuristically, a satisfactory TBA score is above 0.55. The best model should be the combination of loci that maximizes CVC and TBA. The statistical significance of the final best model was determined using 10,000 permutation testing. In addition, results from MDR analysis were confirmed by χ^2 tests. The statistically significant level is a *P* value of less than 0.05. For the SNPs located within a 20 kb region, linkage disequilibrium (LD) statistics *D'* and *r*² were calculated among controls. The detection power of the sample in this study was 0.81 for asthma, if the minor allele frequency was 0.25 and its OR for asthma reached 1.20.

RESULTS

Association Between Childhood Asthma and Individual SNPs

All nine SNPs examined were in Hardy–Weinberg equilibrium (*P* > 0.05). As shown in Table 3, SNPs *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713n, and *FCER1B* rs569108 were solely associated with asthma. The G allele of rs20541 (OR = 1.24, *P* = 1.23E−03),

TABLE 3—Genetic Effects of Individual SNPs on Asthma

SNP	Group	Risk allele	Risk allele frequency	P value ¹ OR (95%CI)	Genotype frequency AA/AB/BB	Additive ² P value ³ OR (95%CI)	Dominant ² P value ³ OR (95%CI)	Recessive ² P value ³ OR (95%CI)
<i>IL13</i> rs1800925	Asthma Control	T	352 (17.60%) 349 (17.45%)	9.01E-01 1.01 (0.86-1.19)	CC/CT/TT 683 (68.30%)/282 (28.20%)/35 (3.50%) 685 (68.50%)/281 (28.10%)/34 (3.40%)	8.97E-01 1.02 (0.80-1.29)	9.23E-01 1.01 (0.84-1.22)	9.03E-01 1.03 (0.64-1.67)
<i>IL13</i> rs1295686	Asthma Control	T	731 (36.55%) 689 (34.45%)	1.65E-01 1.10 (0.96-1.25)	CC/CT/TT 397 (39.70%)/475 (47.50%)/128 (12.80%) 434 (43.40%)/443 (44.30%)/123 (12.30%)	3.71E-01 1.07 (0.93-1.23)	9.33E-02 1.17 (0.97-1.39)	7.36E-01 1.05 (0.80-1.36)
<i>IL13</i> rs20541	Asthma Control	G	1363 (68.15%) 1266 (63.30%)	1.23E-03 1.24 (1.09-1.41)	GG/GA/AA 470 (47.00%)/423 (42.30%)/107 (10.70%) 390 (39.00%)/486 (48.60%)/124 (12.40%)	2.47E-02 0.85 (0.73-0.98)	3.07E-04 0.72 (0.60-0.86)	2.35E-01 0.85 (0.64-1.11)
<i>IL4</i> rs2243250	Asthma Control	T	1595 (79.75%) 1519 (75.95%)	3.81E-03 1.25 (1.07-1.45)	TT/TC/CC 650 (65.00%)/295 (29.50%)/55 (5.50%) 590 (59.00%)/339 (33.90%)/71 (7.10%)	6.16E-02 0.84 (0.70-1.01)	5.75E-03 0.77 (0.65-0.93)	1.42 E-01 0.76 (0.53-1.10)
<i>ADRB2</i> rs1042713	Asthma Control	A	1240 (62.00%) 1116 (55.80%)	6.75E-05 1.29 (1.14-1.47)	AA/AG/GG 396 (39.60%)/448 (44.80%)/156 (15.60%) 308 (30.80%)/500 (50.00%)/192 (19.20%)	5.01E-04 0.79 (0.70-0.90)	3.92E-05 0.68 (0.56-0.82)	3.40E-02 0.78 (0.62-0.98)
<i>FCER1B</i> rs1441586	Asthma Control	C	732 (36.60%) 718 (35.90%)	6.45E-01 1.03 (0.91-1.17)	TT/TC/CC 416 (41.60%)/436 (43.60%)/148 (14.80%) 406 (40.60%)/470 (47.00%)/124 (12.40%)	2.77E-01 1.08 (0.94-1.24)	6.50E-01 0.96 (0.80-1.15)	1.18 E-01 1.23 (0.95-1.59)
<i>FCER1B</i> rs569108	Asthma Control	G	406 (20.30%) 335 (16.75%)	3.86E-03 1.27 (1.08-1.49)	AA/AG/GG 659 (65.90%)/276 (27.60%)/65 (6.50%) 688 (68.80%)/289 (28.90%)/23 (2.30%)	1.36E-05 1.72 (1.35-2.19)	1.67E-01 1.14 (0.95-1.38)	1.16E-05 2.95 (1.82-4.79)
<i>IL4RA</i> rs1805010	Asthma Control	A	963 (48.15%) 948 (47.40%)	6.35E-01 1.03 (0.91-1.17)	GG/GA/AA 269 (26.90%)/499 (49.90%)/232 (23.20%) 291 (29.10%)/470 (47.00%)/239 (23.90%)	6.96E-01 1.03 (0.91-1.16)	2.73E-01 1.12 (0.92-1.36)	7.12E-01 0.96 (0.78-1.18)
<i>IL4RA</i> rs1801275	Asthma Control	G	340 (17.00%) 335 (16.75%)	8.33E-01 1.02 (0.86-1.20)	AA/AG/GG 675 (67.50%)/310 (31.00%)/15 (1.50%) 685 (68.50%)/295 (29.50%)/20 (2.00%)	4.30E-01 0.87 (0.62-1.22)	6.32E-01 1.05 (0.87-1.26)	3.96E-01 0.75 (0.38-1.47)

SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

¹P values for Pearson's χ^2 tests.²Additive model (AA vs. AB vs. BB), dominant model (AA vs. AB+BB), and recessive model (AA+AB vs. BB), where A is the major allele and B is the minor allele.³P values for logistic analysis controlling for age and gender.

T allele of rs2243250 ($OR = 1.25$, $P = 3.81E-03$), A allele of rs1042713 ($OR = 1.29$, $P = 6.75E-05$), and G allele of rs569108 ($OR = 1.27$, $P = 3.86E-03$) were significantly increased in asthmatic compared with non-asthmatic subjects. The most significant association with asthma was found under dominant model for SNPs rs20541 ($P = 3.07E-04$) and rs1042713 ($P = 3.92E-05$), while under recessive model for rs569108 ($P = 1.16E-05$). Significant differences between asthma and control groups were not found among the other five loci ($P > 0.0055$, after Bonferroni Multiple Testing correction).

High-Order Gene–Gene Interactions Using MDR Approach

Results of gene–gene interactions explored among all nine investigated SNPs were summarized in Table 4. Totally, there were three models exhibiting a maximum CVC of 10/10. Based on the TBA and permutation P values, significant multi-loci interactions were found in the three models: *ADRB2* rs1042713 and *FCER1B* rs569108 ($P = 9.34E-04$); *IL13* rs20541, *IL4* rs2243250, and *ADRB2* rs1042713 ($P = 3.12E-04$); *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108 ($P = 6.98E-05$). Among them, the four-way interaction model, which showed the highest TBA and lowest 10,000 permutations P value, was regarded as the optimal model. As SNPs R110Q in the *IL13* gene and $-590C > T$ in the *IL4* gene are located within a 20 kb region, LD was measured ($D' = 0.1934$, $r^2 = 0.0069$), indicating a low LD between the two SNPs.

Interactions Between *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108 Genotypes for Asthma

Table 5 shows a significant interaction between rs20541, rs2243250, rs1042713, and rs569108 on the risk of asthma. Individuals homozygous for the risk alleles at all the four SNP loci (*IL13* rs20541 GG, *IL4* rs2243250 TT, *ADRB2* rs1042713 AA, and *FCER1B* rs569108 GG) had a significantly higher risk of asthma compared with those without any risk homozygotes (OR (95% CI) = 13.55 (1.71–107.38), $P = 4.28E-03$), and also greater than those with less than four risk homozygotes (OR (95% CI) = 10.09 (1.29 – 78.98), $P = 6.51E-03$).

DISCUSSION

Asthma is a chronic inflammatory condition of the lung airways resulting in episodic airflow obstruction. Airways inflammation has been widely demonstrated in all forms of asthma and is of great importance in the pathogenesis of this disease. Since the majority of childhood asthma is

associated with atopy, we selected *IL13*, *IL4*, *IL4RA*, *FCER1B*, and *ADRB2*, five key allergic inflammatory genes, which have been replicated in more than ten studies,⁴ to elucidate potential interactions between them on childhood asthma. Of the models tested using an MDR approach, the four-way gene–gene interaction model consisting of *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108 was chosen as the optimal one for determining asthma susceptibility based on its TBA, CVC and 10,000 permutations P value. Each of the four SNPs was identified to have an independent association with childhood asthma. Carriers of all four risk homozygotes (*IL13* rs20541 GG, *IL4* rs2243250 TT, *ADRB2* rs1042713 AA, and *FCER1B* rs569108 GG) were estimated to be at more than 10-fold higher risk for asthma. This four-way interaction model is reported for the first time in Chinese Han population.

Development of IgE-mediated inflammatory responses is the central component of allergic asthma. The switching of immunoglobulin production from immunoglobulin class M to E in activated B lymphocytes, the key biological feature of atopic diseases, is largely influenced by the activation of the *IL4/IL13* pathway.²⁸ *IL4* and *IL13* are immuno-regulatory cytokines produced by activated Th2 cells and have many structural and functional similarities. They share a common signaling pathway in binding to a heterodimeric receptor of *IL4RA* and *IL13RA1*, and both can switch B cells to produce IgE.²⁹ Asthma-associated IgE mechanisms are regulated by *ADRB2* on human B cells. Stimulation of *ADRB2* on a B cell responding to allergen results in an increase in IgE by a unique signaling pathway, independently of class switch recombination. IgE is secreted from B cells into circulation, where it binds to *FCER1* on the surfaces of effector cells of allergic reactions, such as mast cells and basophils.³⁰ *FCER1B* combines with an IgE-binding alpha-subunit and a gamma-subunit dimer to compose an *FCER1* tetramer complex, which is essential for IgE-mediated allergic responses. *FCER1B* functions as an amplifier of *FCER1* expression and signaling, which enhances mast cell and basophil activation and up-regulates IgE-mediated inflammatory responses.¹² Considering those biological functions, the *IL4*, *IL13*, *ADRB2*, and *FCER1B* genes are all involved in IgE-mediated inflammatory pathway from different aspects. Interestingly, significant interactions between these four genes were observed in our study. Four SNPs *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108 constituted the best statistical interaction model. Each of the polymorphisms has been associated with differences in expression of the corresponding gene,^{31–35} as well as increased IgE levels.^{6,22,24} We supposed that these SNPs interact with each other through IgE-mediated inflammatory pathway in the pathogenesis of asthma. Further studies are needed to

TABLE 4—Summary of Gene–Gene Interaction for Asthma by MDR Analysis

Interacting SNPs	TBA	CVC	10,000 permutations <i>P</i> value
<i>ADRB2</i> rs1042713 × <i>FCER1B</i> rs569108	0.5865	10/10	9.34E–04
<i>IL13</i> rs20541 × <i>IL4</i> rs2243250 × <i>ADRB2</i> rs1042713	0.5936	10/10	3.12E–04
<i>IL13</i> rs20541 × <i>IL4</i> rs2243250 × <i>ADRB2</i> rs1042713 × <i>FCER1B</i> rs569108	0.6089	10/10	6.98E–05

MDR, multifactor-dimensionality reduction; SNP, single-nucleotide polymorphism; TBA, Testing-balanced accuracy; CVC, Cross-validation consistency.

establish biological interaction between the variants in functional assays in vitro and in vivo.

Our study confirmed the independent role of rs20541, rs2243250, rs1042713, and rs569108 on risk of asthma, consistent with previous reports.^{9,15,21,23} Each of these SNPs has been reported as being related to raised IgE levels^{6,22,24} and *ADRB2* rs1042713 has been identified as being associated with bronchodilator response in asthmatic children.¹⁶ Using MDR, T. F. Leung et al. found significant interactions among *IL13* rs20541, *ADRB2* rs1042713, and one SNP in gene *STAT* for influencing lung function change in Chinese asthmatic children.³⁶ A three-way gene–gene interaction has been elucidated between three genes *GSTP1*, *INSIG2*, and *IL4RA* on the risk of lifetime asthma in Chinese schoolchildren, with a TBA of 57.83% and CVC 10/10.³⁷ In this study, we found four-locus genetic interactions among *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108, which conferred a significantly higher risk for childhood asthma than any single genetic polymorphism. The model showed a higher TBA of 60.89% with an equal CVC of 10 out of 10. These four SNPs may be associated with key clinical parameters of asthma, such as IgE levels and lung function, which should be tested in our future studies.

Another limitation of this study is lack of replication. Further validation studies of the four-locus model in other Chinese Han populations or other races are required.

Our study showed that the combination of homozygosity in the four SNPs was associated with a much higher risk for asthma, however it only occurred in 1% of asthmatics versus 0.1% of controls. The total asthma incidence of childhood asthma aged 0–14 year old in cities in China was

3.02% and prevalence in 2009 and 2010 was 2.32%.³⁸ The overall risk for asthma is influenced by numerous genes in different biological pathways that interact with environmental exposures.³⁷ Therefore, when we move forward to identify constellations of interacting genes that convey risk for asthma, environmental context should also be taken into account as much as possible.

The four-way statistical interaction model in this study may be useful for identifying children who are genetically at high risk of developing asthma. After the future biological studies, as well as validation studies and gene-environment investigations, it could be applied and translated into specific prevention and management strategies for childhood asthma.

In addition, further studies are needed to investigate whether this gene model is useful in predicting adult asthma, or allergic versus non-allergic asthma. A limitation of this study is that allergy was not tested in the control group. Although skin prick tests were conducted in the asthmatics and 80.70% had positive results, we can not determine whether this four-way gene model is useful for identifying atopic asthma by investigating the atopic asthmatics versus the controls. Allergy skin prick tests need be conducted among the controls in the future studies. Based on this, we can examine whether this gene combination could be used to identify allergic subjects with or without asthma.

In conclusion, the data presented in our study provide evidence for a four-locus genetic interaction model in determining the risk of childhood asthma in Chinese Han population. Each of the four loci has an independent effect on the development of asthma, and the combined effects

TABLE 5—Interactions Between *IL13* rs20541, *IL4* rs2243250, *ADRB2* rs1042713, and *FCER1B* rs569108 Genotypes for Asthma

Number of homozygotes for the risk alleles of the four SNPs	Phenotype		<i>P</i> value ¹	OR (95%CI)
	Asthma, n (%)	Control, n (%)		
0	110 (11.00%)	149 (14.90%)		1
1	352 (35.20%)	457 (45.70%)	7.69E–01	1.04 (0.79–1.38)
2	395 (39.50%)	329 (32.90%)	8.38E–04	1.62 (1.22–2.17)
3	133 (13.30%)	64 (6.40%)	1.10E–07	2.82 (1.91–4.14)
4	10 (1.00%)	1 (0.10%)	4.28E–03	13.55 (1.71–107.38)

OR, odds ratio.

¹*P* values for χ^2 tests.

are significantly stronger. The multi-loci interaction may indicate biological interaction between the four SNPs in the IgE-mediated inflammatory pathway involved in the pathogenesis of asthma. Although biological studies, as well as validation studies and gene-environment investigations, are needed in future, this four-locus model may be translated into a specific tool for predicting and screening childhood asthma.

ACKNOWLEDGMENTS

We are grateful to Yueqing Zhang for his early contributions and input into the study. We thank Jing Huo and Fei Fan for their assistance with sample collection. We also heartily thank all the study participants and parents of the minor participants.

REFERENCES

- Kauffmann F, Demenais F. Gene-environment interactions in asthma and allergic diseases: challenges and perspectives. *J Allergy Clin Immunol* 2012;130:1229–1240. quiz 1241–1242.
- von ME. Gene-environment interactions in asthma. *J Allergy Clin Immunol* 2009;123:3–11. quiz 12–13.
- Holloway JW, Jongepier H, Beghé B, Koppelman GH, Holgate ST, Postma DS. The genetics of asthma. *Eur Respir Monogr* 2003;8:26–56.
- Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. *Genes Immun* 2006;7:95–100.
- Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy parasites, and the hygiene hypothesis. *Science* (80-) 2002;296:490–494.
- Hunninghake GM, Soto-Quiros ME, Avila L, Su J, Murphy A, Demeo DL, Ly NP, Liang C, Sylvia JS, Klanderman BJ, et al. Polymorphisms in IL13, total IgE, eosinophilia, and asthma exacerbations in childhood. *J Allergy Clin Immunol* 2007;120:84–90.
- Bottema RW, Nolte IM, Howard TD, Koppelman GH, Dubois AE, de Meer G, Kerkhof M, Bleecker ER, Meyers DA, Postma DS. Interleukin 13 and interleukin 4 receptor-alpha polymorphisms in rhinitis and asthma. *Int Arch Allergy Immunol* 2010;153:259–267.
- Bottema RW, Reijmerink NE, Kerkhof M, Koppelman GH, Stelma FF, Gerritsen J, Thijs C, Brunekreef B, van Schayck CP, Postma DS. Interleukin 13, CD14, pet and tobacco smoke influence atopy in three Dutch cohorts: the allergenic study. *Eur Respir J* 2008;32:593–602.
- Black S, Teixeira AS, Loh AX, Vinall L, Holloway JW, Hardy R, Swallow DM. Contribution of functional variation in the IL13 gene to allergy, hay fever and asthma in the NSHD longitudinal 1946 birth cohort. *Allergy* 2009;64:1172–1178.
- Beghe B, Hall IP, Parker SG, Moffatt MF, Wardlaw A, Connolly MJ, Fabbri LM, Ruse C, Sayers I. Polymorphisms in IL13 pathway genes in asthma and chronic obstructive pulmonary disease. *Allergy* 2010;65:474–481.
- Baye TM, Butsch KM, Biagini MJM, Martin LJ, Lindsey M, Patterson TL, He H, Ericksen MB, Gupta J, Tsoras AM, et al. Differences in candidate gene association between European ancestry and African American asthmatic children. *PLoS ONE* 2011;6:e16522.
- Kraft S, Rana S, Jouvin MH, Kinet JP. The role of the Fc epsilon RI beta-chain in allergic diseases. *Int Arch Allergy Immunol* 2004;135:62–72.
- Hill MR, Cookson WO. A new variant of the beta subunit of the high-affinity receptor for immunoglobulin E (Fc epsilon RI-beta E237G): associations with measures of atopy and bronchial hyper-responsiveness. *Hum Mol Genet* 1996;5:959–962.
- Hizawa N, Yamaguchi E, Jinushi E, Kawakami Y. A common FCER1B gene promoter polymorphism influences total serum IgE levels in a Japanese population. *Am J Respir Crit Care Med* 2000;161:906–909.
- Hall IP, Blakey JD, Al BKA, Wheatley A, Sayers I, Pembrey ME, Ring SM, McArdle WL, Strachan DP. Beta2-adrenoceptor polymorphisms and asthma from childhood to middle age in the British 1958 birth cohort: a genetic association study. *Lancet* 2006;368:771–779.
- Basu K, Palmer CN, Tavendale R, Lipworth BJ, Mukhopadhyay S. Adrenergic beta(2)-receptor genotype predisposes to exacerbations in steroid-treated asthmatic patients taking frequent albuterol or salmeterol. *J Allergy Clin Immunol* 2009;124:1188–1194.e3.
- Ritchie MD, Hahn LW, Moore JH. Power of multifactor dimensionality reduction for detecting gene-gene interactions in the presence of genotyping error, missing data, phenocopy, and genetic heterogeneity. *Genet Epidemiol* 2003;24:150–157.
- Hahn LW, Ritchie MD, Moore JH. Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. *Bioinformatics* 2003;19:376–382.
- American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225–244.
- Liu Y, Liu T, Nie W, Lai G, Xiu Q. Interleukin-13 +1923C/T polymorphism is associated with asthma risk: a meta-analysis. *Biomed Res Int* 2013;2013:394316.
- Liu S, Li T, Liu J. Interleukin-4 rs2243250 polymorphism is associated with asthma among Caucasians and related to atopic asthma. *Cytokine* 2012;59:364–369.
- de Guia RM, Ramos JD. The -590C/TIL4 single-nucleotide polymorphism as a genetic factor of atopic allergy. *Int J Mol Epidemiol Genet* 2010;1:67–73.
- Yang HJ, Zheng L, Zhang XF, Yang M, Huang X. Association of the MS4A2 gene promoter C-109T or the 7th exon E237G polymorphisms with asthma risk: a meta-analysis. *Clin Biochem* 2014;47:605–611.
- Shirakawa T, Mao XQ, Sasaki S, Enomoto T, Kawai M, Morimoto K, Hopkin J. Association between atopic asthma and a coding variant of Fc epsilon RI beta in a Japanese population. *Hum Mol Genet* 1996;5:1129–1130.
- Loza MJ, Chang BL. Association between Q551R IL4R genetic variants and atopic asthma risk demonstrated by meta-analysis. *J Allergy Clin Immunol* 2007;120:578–585.
- Wilhelm J, Pingoud A. Real-time polymerase chain reaction. *ChemBiochem* 2003;4:1120–1128.
- Gibson NJ. The use of real-time PCR methods in DNA sequence variation analysis. *Clin Chim Acta* 2006;363:32–47.
- Holt PG. Parasites, atopy, and the hygiene hypothesis: resolution of a paradox. *Lancet* 2000;356:1699–1701.
- Kelly-Welch AE, Hanson EM, Boothby MR, Keegan AD. Interleukin-4 and interleukin-13 signaling connections maps. *Science* (80-) 2003;300:1527–1528.
- Levine SJ, Wenzel SE. Narrative review: the role of Th2 immune pathway modulation in the treatment of severe asthma and its phenotypes. *Ann Intern Med* 2010;152:232–237.
- Vladich FD, Brazille SM, Stern D, Peck ML, Ghittoni R, Vercelli D. IL-13 R130Q, a common variant associated with allergy and asthma, enhances effector mechanisms essential for human allergic inflammation. *J Clin Invest* 2005;115:747–754.
- Arima K, Umeshita-Suyama R, Sakata Y, Akaiwa M, Mao XQ, Enomoto T, Dake Y, Shimazu S, Yamashita T, Sugawara N, et al. Upregulation of IL-13 concentration in vivo by the IL13 variant

- associated with bronchial asthma. *J Allergy Clin Immunol* 2002; 109:980–987.
33. Rosenwasser LJ, Klemm DJ, Dresback JK, Inamura H, Mascali JJ, Klennert M, Borish L. Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. *Clin Exp Allergy* 1995;25:74–78. discussion 95–96.
34. Drysdale CM, McGraw DW, Stack CB, Stephens JC, Judson RS, Nandabalan K, Arnold K, Ruano G, Liggett SB. Complex promoter and coding region beta 2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. *Proc Natl Acad Sci USA* 2000;97:10483–10488.
35. Kim YK, Oh SY, Oh HB, Chun SY, Cho SH, Koh YY, Min KU, Kim YY. Coding single nucleotide polymorphism in the high-affinity immunoglobulin E receptor b chain (FcεRI-β) gene is associated with immunoglobulin E receptor-mediated histamine release from basophils. *Clin Exp Allergy* 2002;32: 751–755.
36. Leung TF, Chan IH, Wong GW, Li CY, Tang NL, Yung E, Lam CW. Association between candidate genes and lung function growth in Chinese asthmatic children. *Clin Exp Allergy* 2007;37: 1480–1486.
37. Su MW, Tung KY, Liang PH, Tsai CH, Kuo N, Lee YL. Gene-gene and gene-environmental interactions of childhood asthma: a multifactor dimension reduction approach. *PLoS ONE* 2012;7: e30694.
38. The National Cooperative Group on Childhood Asthma, Institute of Environmental Health and Related Product Safety, Chinese Center For Disease Control and prevention. Third nationwide survey of childhood asthma in urban areas of China. *Chin J Pediatr* 2013;51:729–735.