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

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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 \( \chi^2 \) 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 \( \chi^2 \).

Resultados: Se encontraron correlaciones significativas entre el modelo génico de los cuatro loci 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 loci, con atopia (\( P < 0.01, \) OR=2.09).

Conclusiones: Nuestros resultados indicaron que el modelo génico de cuatro loci 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.

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>
<thead>
<tr>
<th>Table 1. Asthma Predictive Indexa</th>
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<tbody>
<tr>
<td><strong>Major Criteria</strong></td>
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<tr>
<td>1. Parental asthmab</td>
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<td>2. Eczemae</td>
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</table>

aLoose 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 prediction of asthma: early frequent wheezing plus at least 1 of 2 major criteria or 2 of 3 minor criteria.
bHistory of physician-diagnosed asthma.
cPhysician-diagnosed allergic rhinitis, as reported in questionnaires at ages 2 or 3.
dPhysician-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 the 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 \( \chi^2 \) or Fisher exact test was used to compare categorical variables. All the SNPs were examined for Hardy-Weinberg equilibrium using the \( \chi^2 \) 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 \( \chi^2 \) 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>
<thead>
<tr>
<th>Table 2. High- and Low-Risk Genotype of Asthma According to the 4 Genotypes Studied</th>
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<tbody>
<tr>
<td><strong>Groups</strong></td>
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<tr>
<td>---------------------------------------------------------------</td>
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<tr>
<td>Low-risk genotype</td>
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<td>High-risk genotype</td>
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</table>
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 3. Baseline Demographic Characteristics in Preschoolers With High-Risk and Low-Risk Genotypes

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

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].

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Loose API</th>
<th>Stringent API</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive,</td>
<td>Negative,</td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>High-risk group</td>
<td>119 (68.4%)</td>
<td>55 (31.6%)</td>
</tr>
<tr>
<td>Low-risk group</td>
<td>101 (47.9%)</td>
<td>110 (52.1%)</td>
</tr>
</tbody>
</table>

$P$ Value 5.12E-5 1.22E-10

OR (95% CI) 2.36 (1.55-3.58) 4.08 (2.63-6-3.33)

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

### Table 5. Association Between Genotype Risk Group and Atopy

<table>
<thead>
<tr>
<th>Group</th>
<th>Positive, No. (%)</th>
<th>Negative, No. (%)</th>
<th>$P$ Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-risk group</td>
<td>79 (71.8%)</td>
<td>31 (28.2%)</td>
<td>.006</td>
<td>2.09 (1.22-3.55)</td>
</tr>
<tr>
<td>Low-risk group</td>
<td>77 (55%)</td>
<td>63 (45%)</td>
<td></td>
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</table>

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 allergenic 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, \( P < 0.0001 \), OR=4.08; loose API, \( P < 0.0001 \), 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 Th2 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 \( IL4RA \) demonstrated a significant association with plasma total IgE [27]. Moreover, \( \beta_{2} \) 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**

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References


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