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Association Between Polymorphisms in IL17F and Male Asthma in a Chinese Population

F Qian,1* Q Zhang,2 L Zhou,3 G Ma,1 G Jin,4 Q Huang,5 K Yin3

1Department of Respiratory Medicine, Affiliated Hospital, Jiangsu University, Zhenjiang, Jiangsu, China
2Department of Respiratory Medicine, Affiliated Changzhou No.2 People's Hospital, Nanjing Medical University, Changzhou, China
3Department of Respiratory Medicine, First Affiliated Hospital, Nanjing Medical University, Nanjing, China
4Department of Epidemiology and Biostatistics, Cancer Research Center, Nanjing Medical University, Nanjing, China
5Department of Otolaryngology, Affiliated Hospital, Jiangsu University, Zhenjiang, Jiangsu, China

*These authors contributed equally to the article.

Abstract

Background and Objectives: Interleukin (IL) 17F is a marker of type 17 helper T cells and has a proinflammatory role in asthma. The aim of the present study was to assess the possible association between asthma and polymorphisms in the IL17F gene in a Chinese population.

Methods: A total of 318 asthmatic patients and 352 nonasthmatic controls were recruited. Two single-nucleotide polymorphisms in IL17F (rs763780 and rs13209590) were detected using GenomeLab SNPstream. Logistic regression methods were used to analyze data.

Results: A significantly lower frequency of the C allele in rs763780 was observed in the control group (P = .0148). The rs763780 TC heterozygote was associated with an increased risk of asthma, and a similar trend was observed assuming a codominant genotype effect (adjusted OR, 1.58; 95% CI, 1.06-2.36; P = .0148; adjusted OR, 1.64; 95% CI, 1.10-2.45; P = .0217). Furthermore, a novel finding of the present study was that this association was exclusive to males (adjusted OR, 1.79; 95% CI, 1.06-3.05; P = .0308) and patients with higher immunoglobulin E levels (≥ 1.85 IU/mL) (adjusted OR, 1.88; 95% CI, 1.10-3.22; P = .0213). We also found that the haplotype with minor alleles for rs763780 was associated with an increased risk of asthma (adjusted OR, 1.62; 95% CI, 1.11-2.35; P = .0115).

Conclusion: Our findings indicated that polymorphisms might play a role in susceptibility to asthma.

Key words: Asthma. IgE. Interleukin 17F. Single-nucleotide polymorphism.

Resumen

Introducción y objetivos: La interleucina (IL) 17F es un marcador de las células T 17 cooperadoras y desempeña un papel proinflamatorio en el asma bronquial. La finalidad de este estudio ha sido el determinar la posible asociación entre el padecer asma y diferentes polimorfismos en el gen de IL17F en una población china.

Métodos: Se seleccionó a un total de 318 pacientes asmáticos y a 352 controles sin asma bronquial. Se estudiaron dos polimorfismos de un único nucleótido (SNIP) en IL17F (rs763780 y rs13209590) mediante GenomeLab SNPStream. Los resultados se analizaron utilizando métodos de regresión logística.

Resultados: En el grupo control se observó una frecuencia significativamente más baja del alelo C en rs763780 (P = .0148). El heterocigoto TC de rs763780 se asoció a un riesgo superior de padecer asma y una tendencia similar se observó asumiendo un efecto codominante del genotipo (OR ajustado, 1.58; IC del 95%, 1.06-2.36; P = .0148; OR ajustado, 1.64; IC del 95%, 1.10-2.45; P = .0217). Por otro lado, fue un hallazgo el descubrir que en este estudio, esta asociación se dio exclusivamente en los varones (OR ajustado, 1.79; IC del 95%, 1.06-3.05; P = .0308) y en los pacientes con niveles más elevados de inmunoglobulina E (log10 IgE ≥ 1.85 IU/mL, OR ajustado, 1.88; IC del 95%, 1.10-3.22; P = .0213). Finalmente, se encontró que el haplotipo con los alelos menos comunes para rs763780 se asoció a un mayor riesgo de asma (OR ajustado, 1.62; IC del 95%, 1.11-2.35; P = .0115).

Conclusion: Nuestros hallazgos indican que los polimorfismos pueden desempeñar un papel en la susceptibilidad a desarrollar asma bronquial.

Palabras clave: Asma. IgE. Interleucina 17F. Polimorfismo de un único nucleótido.
Introduction

Asthma is one of the most common chronic inflammatory respiratory disorders and is characterized by airway eosinophilia and elevated immunoglobulin (Ig) E levels. Aberrant T helper (Th) 2 cells are thought to produce a biased immune response in the development of asthma [1], and Th2-mediated immunity is important in some asthma endotypes. However, the current Th2-based inflammation model does not account for the substantial clinical and molecular heterogeneity documented in human asthma [2]. Th17, a new subset of Th cells, is characterized by the production of cytokines from the interleukin (IL) 17 family, namely, IL-17A and IL-17F [3,4]. Increasing evidence has shown that Th17 cells can also participate in the pathogenesis of allergic diseases [5,6], and a major role has been shown for these cells in asthma and in the regulation of feedback for polarization towards distinct T-cell subsets [4,7]. Th17 cells are not only proinflammatory, but have also been shown to induce corticosteroid resistance in murine models [8].

Interest in IL-17F has been growing because of its significance as a marker of Th17. It has been well characterized in vitro and in vivo and has a proinflammatory, inflammatory, with important implications for the therapy of patients with asthma. Epidemiological studies have demonstrated a strong genetic component in the risk of asthma. Our group previously demonstrated that specific polymorphisms have an effect on the risk of asthma in a Chinese population [12]. IL17F is located on chromosome 6p12, a genomic region linked to asthma and asthma-related phenotypes [13]. On the basis of experimental findings and gene expression studies, IL17F is a good positional candidate gene for asthma. However, current data on the association between IL17F polymorphisms and asthma in both Chinese and other populations are inconsistent [14–16]. Therefore, we conducted a case-control study in a Chinese population to evaluate interactions between IL17F genotypes/haplotypes and the risk of developing asthma.

Methods

Study Population

The study population comprised 318 asthmatic patients (aged 14 to 75 years) and 352 nonasthmatic controls (aged 16 to 74 years) (Table 1). Participants were unrelated Han Chinese residing in the city of Nanjing and the surrounding regions. The asthmatic cohort was enrolled from the First Affiliated Hospital, Nanjing Medical University. All participants were questioned to obtain clinical and family histories, as well as data on smoking habits, occupation, and medication. Patients underwent a general physical examination, skin prick test (SPT), and spirometry. Blood analysis and extended laboratory examinations were performed. Asthma was diagnosed and severity classified according to the Global Initiative for Asthma guidelines [17]. Patients were subdivided into 4 groups based on clinical features, as follows: stage 1, intermittent; stage 2, mild persistent; stage 3, moderate persistent; and stage 4, severe persistent.

Healthy volunteers (controls) were recruited from patients’ spouses and the general population. To be included in the study, controls had to be in good health and match the patients in age, gender, and residential region. In addition, they had to have a negative SPT result and normal levels of plasma total IgE.

Table 1. Demographic Characteristics of Asthmatic Patients and Nonasthmatic Controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 318)</th>
<th>Controls (n = 352)</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>39.80 (14.23)</td>
<td>38.26 (13.31)</td>
<td>NS</td>
</tr>
<tr>
<td>Gender, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>183 (57.55)</td>
<td>200 (56.82)</td>
<td>NS</td>
</tr>
<tr>
<td>Male</td>
<td>135 (42.45)</td>
<td>152 (43.18)</td>
<td></td>
</tr>
<tr>
<td>Smoking, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>280 (88.05)</td>
<td>292 (82.88)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoker</td>
<td>38 (11.95)</td>
<td>60 (17.12)</td>
<td></td>
</tr>
<tr>
<td>Atopy, No. (%)</td>
<td>237 (75.00)</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Eosinophils, × 10⁹/L</td>
<td>0.46 (0.71)</td>
<td>0.14 (0.11)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Log₁₀ IgE, IU/mL</td>
<td>1.82 (0.50)</td>
<td>1.19 (0.60)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>FEV₁, %</td>
<td>74.11 (23.66)</td>
<td>94.75 (18.07)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>FEV₁/VC, %</td>
<td>76.18 (12.78)</td>
<td>88.66 (9.43)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Allergic rhinitis, No. (%)</td>
<td>137 (43.08)</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Yes</td>
<td>181 (56.92)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FEV₁, forced expired volume in the first second of expiration; FEV₁/VC, ratio of FEV₁ to forced vital capacity; Ig, immunoglobulin; log₁₀ IgE, log₁₀-transformed immunoglobulin E levels; NS, nonsignificant.

<sup>a</sup> Differences between groups were evaluated using the t test or χ² test, as appropriate.

<sup>b</sup> Differences between groups were evaluated using the t test or χ² test, as appropriate.

The study protocol was approved by the institutional ethics committee. Written informed consent was obtained from all participants.

**Assessment of Clinical Data**

Cumulative smoking was defined as the number of packs of cigarettes smoked per day multiplied by the number of years smoking (pack-years). Participants who had smoked <5 pack-years were defined as nonsmokers; otherwise, they were considered to be smokers [18]. Atopy was determined by SPT with at least 1 positive result for 13 common aeroallergens, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Felis domesticus*, *Canis familiaris*, cockroach, pollen, ragweed, mugwort, molds (*Cladosporium* and *Alternaria*), and animal allergens (cat, dog, and horse). Allergic rhinitis was defined as rhinitis appearing at least twice after exposure to a particular allergen and not related to an infection. SPT, total serum IgE levels, and lung function assessments are described in detail elsewhere [19].

**Genotyping**

DNA was extracted from EDTA-anticoagulated peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer’s protocol. As a preliminary study, we selected and genotyped 2 SNPs (Table 2). Genotyping was performed using the GenomeLab SNPstream genotyping platform (Beckman Coulter) and its accompanying SNPstream software suite at the Chinese National Human Genome Center in Shanghai, China. Polymerase chain reaction (PCR) primers and extension probes with tag sequences were designed using the web-based Autoprimer design tool (http://www.autoprimer.com) and synthesized by SBSgene (SBS Genetech Technology). The sequences of the primers used for PCR are shown in Table 2. SNPstream genotyping was performed as described previously [20]. In brief, the SNPs of interest were amplified in a 12-pair multiplex PCR under universal conditions (5 mM MgCl2, 75 μM dNTPs, 0.1 units AmpliTaq Gold [Applied Biosystems] in a final volume of 5 μL). The PCR was run at 94°C for 1 minute, followed by 34 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute).

The technician was blind to each sample run, and negative controls were included in each 384-well plate. We duplicated 10% of samples to confirm the concordance and accuracy of genotyping. A sample call rate >99% was observed with 100% matching in the replicates.

**Statistical Analysis**

Differences in the distributions of the demographic characteristics, selected variables, and genotypes of the *IL17F* variants between the cases and controls were evaluated using the χ2 test or Fisher exact test, as appropriate. The Hardy-Weinberg equilibrium was tested using the χ2 goodness-of-fit test to compare observed genotype frequencies in asthmatic patients with those of controls. Associations between genotypes and risk of asthma were estimated by computing the ORs and their 95% CIs using logistic regression analyses for crude ORs and adjusted ORs, taking into account age, gender, and smoking status. Statistical significance was set at *P* <.05. The EM algorithm in SAS 9.1.3 PROC HAPLOTYPE was used to infer haplotype frequencies based on the observed genotypes. All statistical analyses were carried out using SAS 9.1.3 (SAS Institute). A 2-tailed *P* value <.05 was considered significant.

**Results**

**Study Population**

Demographic data and clinical characteristics of the study participants are shown in Table 1. No significant differences were observed between the cases and controls in terms of age, gender, or smoking. However, peripheral eosinophil counts, serum IgE, forced expiratory volume in the first second of expiration (FEV1, % predicted), and ratio of FEV1 to forced vital capacity (FVC) showed significant differences in the asthmatic group compared with the control group (*P* <.0001 for all comparisons; Table 1).

**Distribution of Alleles and Genotypes of Polymorphisms in IL17F**

The observed genotype frequencies for rs13209590 and rs763780 in the controls were in Hardy-Weinberg equilibrium (*P* >.30; data not shown). The allele and genotype distributions of the polymorphisms in the cases and controls are shown in the Figure. In the single locus analyses, a significantly lower frequency of the C allele in rs763780 was observed in the control group (*P* = .0142). However, the allele distributions of rs13209590 were not significantly different between the control group and the case group.

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**Table 2. Primers and Probe Sequences of 7488T/C and 9576A/G**

<table>
<thead>
<tr>
<th>SNP</th>
<th>SNP Type</th>
<th>Primer Type-Model</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>9576A/G</td>
<td>A/G</td>
<td>PCRU</td>
<td>CTCCTAAAAATGTAGTGCTGTC</td>
</tr>
<tr>
<td>rs13209590</td>
<td></td>
<td>PCRL</td>
<td>TTACAGGAACAAACAGGATACCG</td>
</tr>
<tr>
<td>7488T/C</td>
<td>T/C</td>
<td>SNPU</td>
<td>GTGATTCTGTACGTGTCGCCCTTT</td>
</tr>
<tr>
<td>rs763780</td>
<td></td>
<td></td>
<td>CAGATTTCTGATCTCTTTTGC</td>
</tr>
</tbody>
</table>

Abbreviation: SNP, single-nucleotide polymorphism.
(P≥.11). Logistic regression analyses revealed that the rs763780 TC heterozygote was associated with a significantly increased risk of asthma (Table 3). When we combined the variant genotypes (rs763780 TC+CC) assuming a codominant genotype, we observed a similar trend to that of the rs763780 TT wild-type homozygote. Furthermore, multivariate analysis of the genotypes adjusted for age, gender, and smoking status confirmed this association (adjusted OR, 1.58 [95% CI, 1.06-2.36; P=.0148]; adjusted OR, 1.64 [95% CI, 1.10-2.45; P=.0217], respectively). However, no association was identified between the genotype of rs13209590 and risk of asthma.

**Haplotype Analysis**

When the 2 loci were combined and tested for haplotype inference using the SAS 9.1.3 PROC HAPLOTYPE program, 3 possible haplotypes were derived from the

| Table 3. Logistic Regression Analysis of Associations Between Polymorphisms and Risk of Asthma |
|---------------------------------------------|---------------------------------------------|----------------|----------------|---------------------------------------------|
| Asthma                                      | Control                                   | Crude Odds Ratio | Adjusted Odds Ratio |
| No. (%)                                     | No. (%)                                   | (95% CI)        | (95% CI)        |
| 9576A/G rs13209590                          |                                            |                |                |
| AA                                          | 233 (73.2)                                | 253 (72.1)      | 1              | 1              |
| AG                                          | 81 (25.5)                                 | 94 (26.8)       | 0.94 (0.66-1.33) | 0.95 (0.67-1.36) |
| GG                                          | 4 (1.2)                                   | 4 (1.1)         | 1.09 (0.27-4.47) | 0.85 (0.19-3.81) |
| AG+GG                                       | 85 (26.7)                                 | 98 (27.9)       | 0.94 (0.67-1.32) | 0.95 (0.67-1.34) |
| 7488T/C rs763780                            |                                            |                |                |
| TT                                          | 244 (76.7)                                | 295 (83.8)      | 1              | 1              |
| TC                                          | 71 (22.3)                                 | 57 (16.2)       | 1.51 (1.02-2.22) | 1.58 (1.06-2.36) |
| CC                                          | 3 (1.0)                                   | 0 (0)           | NS             | NS             |
| TC+CC                                       | 74 (23.3)                                 | 57 (16.2)       | 1.57 (1.07-2.31) | 1.64 (1.10-2.45) |

*Because of genotyping failure, the total number of controls for rs13209590 was 351. Age, gender, smoking status, and atopy were included in the modelto adjust for potential confounders.

**Figure.** Allele distributions in polymorphisms of IL17F.

\[ P=.0142 \] (Fisher exact method for rs763780 in a \( 2 \times 2 \) table)

| Table 4. Haplotypes of the IL17F Gene in Control and Patient Groups |
|-------------------------------------------------|----------------|----------------|----------------|
| Haplotypes of IL17F                           | Allele Frequencies | Adjusted Odds Ratio (95% CI) |
| rs9576A/G rs7488T/C                           | Patients (n = 636) | Controls (n = 704)* |                |
| No. %                                         | No. %             |                |

*Because of genotyping failure, the total number of chromosomes for controls was 702. Age, gender, smoking status, and atopy were included in the model to adjust for potential confounders.

*P* = .0115.
Asthma and IL17FP polymorphisms


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known genotypes (Table 4). Compared with the most common haplotype A-T, the A-C haplotype was associated with an increased risk of asthma (adjusted OR, 1.62; 95% CI, 1.11-2.35). Because the prevalence of carriers of other haplotypes not containing the minor allele at rs763780 was not higher in the cases, this finding only reflects the individual association of rs763780 with asthma.

Association Between rs763780 and Asthma Phenotype

No differences in serum total IgE, FEV₁, or FEV₁/FVC values were observed between participants with different genotypes (data not shown). We also analyzed rs763780 genotypes after stratifying by gender and log₁₀-transformed IgE (< or ≥ 1.85 IU/mL). According to the mean level of log₁₀-transformed IgE (1.85 IU/mL) in the case group, we divided patients into 2 groups. As shown in Table 5, we found that the risk factor of rs763780 TC + CC was more pronounced in male patients (adjusted OR, 1.79; 95% CI, 1.06-3.05; P = .030) and patients with higher serum total IgE levels (≥ 1.85 IU/mL; adjusted OR, 1.88 [95% CI, 1.10-3.22; P = .021]).

Discussion

Our study revealed that the SNP rs763780 was associated with risk of asthma and that this association was exclusive to males and patients with higher IgE levels (≥1.85 IU/mL). The minor allele was more common in the asthma group and was largely present on 1 specific haplotype (A-C) (ie, the difference in allele frequency is the same as the difference in haplotype frequency).

Analysis of associations between polymorphisms in IL17F and asthma or asthma-related phenotypes in American white women [15] and Asian populations [14,16] revealed inconsistent results. Kawaguchi et al [14] found an inverse relationship between rs763780 and the risk of asthma in both men and women. Jin et al [16] failed to find any association between rs763780 and asthma in men and women. Ramsey et al [15] reported no association between rs763780 in IL17F and asthma in white women. Our gender-stratified analysis also confirmed that rs763780 was not associated with female asthma. Therefore, our findings are consistent with those of Ramsey et al. The reason for the gender-specific effect is unclear. Childhood asthma is more common in boys than in girls, while adult-onset asthma typically develops in middle age and is more common in women than in men.

Kawaguchi et al [14] also found that rs763780 TC exerts a protective effect against asthma in the Japanese population, although the differences were not statistically significant. The authors illustrated the exact mechanisms of the SNP in regulating asthma using in vitro functional experiments. We observed that the effect of the IL17F haplotype was due to the specific SNP constituting the haplotype and, therefore, provide no new information.

The importance of IgE-based mechanisms in asthma is supported by the impact of humanized anti-IgE monoclonal antibody treatments for asthma and other allergic diseases [21]. Our study indicates that the minor allele of rs763780 was associated with elevated IgE levels. However, the underlying mechanisms of this association have yet to be determined. Many studies now suggest that IL-17 is associated with IgE levels. Using a murine atopic dermatitis model, Matsushima et al [22] found that malfunction of tumor necrosis factor receptor–associated factor 3 interacting protein 2 caused hyper-IgE-emia and skin inflammation through the IL-17–mediated pathway [22]. IL-17 protein levels in the peripheral blood of affected female mice were 3.3-6.5-fold higher than those of unaffected mice. Conversely, few differences were observed between affected and unaffected male mice [22]. Patients with hyper-IgE syndrome (HIES) associated with atopic dermatitis and increased serum IgE levels and patients with signal transducer and activator of transcription protein (STAT3) mutations with HIES showed significantly lower T<sub>h</sub>17 cell counts than patients with wild-type STAT3 and controls [23].
**Conclusions**

Our findings support the association between polymorphisms in IL17F and the risk of asthma in our sample of Han Chinese patients. To confirm the significance of polymorphisms in IL17F, future studies should be performed in larger samples. More extensive and better-designed studies using appropriate molecular and statistical methods are necessary to further analyze this association.

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Fen-hong Qian

Department of Respiratory Medicine
Affiliated Hospital, Jiangsu University
Zhenjiang
Jiangsu, China
E-mail: zhaqian604@yahoo.com.cn