Polymorphisms of *PHF11* and *DPP10* Are Associated with Asthma and Related Traits in a Chinese Population

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Key Words

Asthma • *DPP10* • Genetics • *PHF11* • Single-nucleotide polymorphism

Abstract

Background: Initial studies by positional cloning have identified the genes encoding the plant homeodomain zinc finger protein 11 (*PHF11*) and dipeptidyl-peptidase 10 (*DPP10*) as asthma susceptibility genes. The variants in the two genes have been associated with asthma in several populations of European or Latin American origin. **Objective:** The aim of this study was to assess the common *PHF11* and *DPP10* polymorphisms for associations to asthma and asthma-related traits in a Han Chinese population. **Methods:** We genotyped six polymorphic markers in *PHF11* and five polymorphic markers in *DPP10* in a Han Chinese case-control cohort consisting of 408 asthma patients and 288 unrelated disease-free controls recruited from the Northern region of China. We analyzed the association between these markers and asthma as well as a number of intermediate, asthma-related traits. Linkage disequilibrium and haplotype patterns were also evaluated. **Results:** Significant associations were identified between two markers in *PHF11* (rs1046295 and rs16659) and asthma susceptibility (odds ratio, OR = 1.32, 95% con-

fidence interval, CI = 1.06–1.65, $p = 0.0096$, for rs1046295, and OR = 1.41, 95% CI = 1.12–1.75, $p = 0.0026$, for rs16659). A strong association was observed between an SNP in *DPP10* (rs10208402) and log_e-transformed total IgE ($p = 0.0003$) and the percentage of peripheral blood eosinophils ($p = 0.0023$). A weak association between rs1430090 in *DPP10* and forced expiratory volume in 1 s was also observed ($p = 0.048$). Haplotype analysis revealed two protective haplotypes in *PHF11* against asthma. **Conclusion:** The results provide supporting evidence for genetic variants in *PHF11* and *DPP10* genes underlying asthma susceptibility and asthma-related quantitative traits in a Han Chinese population.

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Introduction

Asthma is one of the few chronic diseases occurring in both developed and developing countries. It is increasing in prevalence and one of the most common disorders

J.G., Y.L. and Y.Z. contributed equally to this work. This study has been accepted as a poster presentation at the ATS International Conference 2009 (San Diego).

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0025-7931/10/0791-0017\$26.00/0

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encountered in clinical medicine in both children and adults. The genetic variants and environmental risk factors contribute almost equally to its course [1]. Since the first whole genome-wide linkage screening for asthma and allergy was completed in 1996 [2], several loci influencing asthma susceptibility or asthma-related traits have been identified by positional cloning and whole genome association [3–8]. The polymorphisms of the PHD finger protein 11 (*PHF11*) gene and the dipeptidyl-peptidase 10 (*DPP10*) gene were found to have associations with asthma and related traits by positional cloning [4, 5]. The functions of the two genes are not fully understood. *PHF11* on chromosome 13q14 is suggested to genetically impact asthma via serum total IgE regulation and altered Th1/Th2 cytokine release [4, 9]; *DPP10*, which is located on chromosome 2q14, is strongly associated with asthma susceptibility, possibly through regulating the activities of chemokines and cytokines [5].

Genetic risk factors for asthma have often differed among different populations [10]. This may be caused by phenotypic heterogeneity, different ethnicity and linkage disequilibrium (LD) patterns. Replication of the results in other populations provides strong evidence for associations, particularly for complex disease genes. In order to determine if polymorphisms in *PHF11* and *DPP10* also affect other populations, we tested six markers from chromosome 13 that covered *PHF11* and five markers from chromosome 2 that covered *DPP10* in a case-control study in a Han Chinese population.

Patients and Methods

Subjects and Phenotype Definition

We recruited 408 asthma patients and 288 controls with Han ethnicity from the Northern region of China. All subjects were unrelated. Asthma cases were recruited at the Pulmonary Clinic, Peking Union Medical College Hospital. Ethnicity-matched controls were selected from non-asthmatic, non-atopic, healthy individuals. The study protocol was approved by the Human Research Ethics Committee of the Peking Union Medical College Hospital, and all subjects gave written informed consent to participate in the study.

Measurement of serum total IgE was performed by applying the UniCAP System (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions.

Atopy was defined as ≥ 100 IU/ml of serum total IgE [11, 12]. All cases had current asthma symptoms including wheezing, cough, awakening at night and shortness of breath. Asthmatics with forced expiratory volume in 1 s (FEV_1) $\geq 70\%$ of predicted underwent the methacholine challenge test, and positive bronchial hyperresponsiveness was defined by a 20% fall in FEV_1 at inhaled methacholine concentrations ≤ 12.8 μmol . Patients with $FEV_1 < 70\%$ of predicted underwent an airway reversibility test,

with positive airway reversibility defined by both 12% improvement in FEV_1 and an increase ≥ 200 ml in the absolute FEV_1 value after β_2 -agonist inhalation. Asthma diagnoses were made using the criteria defined by the American Thoracic Society [13].

Genotyping

DNA was collected from peripheral blood leukocytes by standard phenol-chloroform extraction. A total of eleven markers were genotyped. Single nucleotide polymorphisms (SNPs) were genotyped by means of restriction fragment length polymorphism polymerase chain reaction (PCR). Marker rs16659 is a 15-bp insertion/deletion polymorphism and was genotyped by direct loading of PCR products onto 4% agarose gel (see table 1 for detailed PCR information). In general, PCR cycle conditions were as follows. In total, 35 cycles were performed, each cycle consisted of (i) 60 s at 94°C, followed by (ii) 60 s at 50–60°C and then (iii) 30 s at 72°C. After PCR, products were subject to a 4-hour enzymatic digestion including 2–4 units of enzyme per reaction. The reactions were then loaded onto 3–4% agarose gel for genotype scoring.

Statistical Analysis

All genotype and phenotype data were analyzed using PLINK. Genotype and allele frequencies in cases and controls were compared by contingency table analysis. Genotype relative risks were calculated according to the statistical method described by Lathrop [14]. This method compares case genotype frequencies with expected control genotype frequencies under the assumption of the Hardy-Weinberg equilibrium, and is more powerful than standard contingency table analysis.

Quantitative traits were tested using a variance component approach implemented in the statistical package Merlin [15]. Quantile normalization was applied to all quantitative traits prior to analysis (with the exception of total serum IgE which had already been subject to a log transformation). Missing genotypes were inferred using the –infer option available in Merlin. LD and haplotypes were assessed using the open source program Haploview (version 4.0) [16]. All markers were included in these analyses on a gene-by-gene basis, although only haplotypes present in $\geq 1\%$ of the population were considered for associations to asthma. Empirical p values were determined by permutation ($n = 1,000$) and $p < 0.05$ was regarded as statistically significant.

Results

Characteristics of the Subjects

In total, 696 unrelated subjects of Han ethnicity were included in this study (408 asthma cases and 288 controls). The incidence of asthma was higher in females than in males (58.2 vs. 41.8%). The baseline FEV_1 was significantly lower in asthmatics than normal controls (70.65 ± 24.15 vs. $90.6 \pm 14.92\%$ of predicted, $p < 0.00001$). One hundred and thirty-five (33%) asthma subjects with baseline $FEV_1 \geq 70\%$ of predicted were examined using the airway challenge test; in all of these subjects, positive bronchial hyperresponsiveness was documented. The airway reversibility test was carried out in

Table 1. Primers and PCR conditions for amplification of the *PHF11* and *DPP10* genes

Markers	SNPs	Primers (5' → 3')	Restriction enzymes	T °C	MgCl ₂ mM
rs11619265	A/G	F: AACATAGATGTTCTTACTTAG R: ATCTCTAATAACTGAATGGTA	<i>RsaI</i>	50	2
rs3765526	A/G	F: GTTTTAAAGAAAGGTAAACAATT R: CAAAGAGAATAGGTCTTGTTTC	<i>NfeI</i>	50	2
rs9526569	C/T	F: CTAAGTTTCCACAATTA AAAAG R: GTCCCTTTGTATATCTTTGAG	<i>HindIII</i>	50	2
rs1046295	G/A	F: GCATCTCAACAAAGGTGGC R: CATGACTCTTGGCTTAGGA	<i>BsmAI</i>	55	2
rs16659 (15 bp)	I/D	F: GGAACACCATAATTTTGGTG R: TCTTCAAGTCAACCCTCTTA	–	50	2.5
rs953502	A/G	F: TAACACGTTTCGAAACTGC R: CTTCAATTATAAGGTAAAGCTG	<i>Fnu4HI</i>	50	2
rs7568000	C/T	F: TAGGTCACCTTATTCTCAGT R: CAGAATATCAGCATTGGGAA	<i>AseI</i>	55	2
rs10208402	T/C	F: GCCTCTAAAATGTGCAGATTT R: CACTACATGGGAAGGGTAAAG	<i>MnII</i>	60	2
rs1430090	T/G	F: CTAACCTCTGCCATGGTGAT R: GACGAGACTTCTTTCTACCT	<i>BsrI</i>	60	2
rs17763180	T/C	F: GTTATAGAGTCCAAGTTCAG R: TCCAAGTCTATCCTCCTTGA	<i>PvuII</i>	50	2
rs12151526	A/C	F: AGCACAAGATGCTTTTGCAA R: TCTTGCTACCCTGTTTGAAT	<i>HinfI</i>	50	2

T = Annealing temperature; F = forward; R = reverse; I/D = insertion/deletion.

273 patients (67%). The mean improvement in FEV₁ was 26.36 ± 14.27%. The serum total IgE values in controls were within the normal range. Detailed characteristics of the individuals are listed in table 2.

Association between Polymorphisms in *PHF11* and *DPP10* Genes and Asthma and Related Traits

We selected six polymorphisms in *PHF11* and five polymorphisms in *DPP10* for investigation. The markers in *PHF11* covered 218 kb. Three of these markers (rs3765526, rs9526569 and rs1046295) were either intronic to *PHF11* or located in the 5'-untranslated region (UTR). A proportion of these markers were extensively studied in the initial report on *PHF11* associations [4]. Five markers in and around *DPP10* were selected from 5'-upstream and the introns of splicing variants of exon Ia, IIa and Ic of the gene and covered 114 kb. The marker information and allele genotype frequencies are listed in table 3. All SNPs met the criteria for the Hardy-Weinberg equilibrium in both case and control groups (data not shown).

Single marker analysis for associations showed that two markers in the *PHF11* locus (rs1046295 and rs16659)

Table 2. Characteristics of the asthma patients (n = 408) and controls (n = 288) included in this study

	Asthma	Controls
Mean age, years	41.7 ± 0.3	45.1 ± 0.7
Range	16–76	22–72
Sex, n (%)		
Male	170 (41.8)	158 (55.0)
Female	238 (58.2)	130 (45.0)
log _e IgE, U/l	5.24 ± 1.44	3.64 ± 1.10
Eosinophils, %	7.13 ± 4.91	2.19 ± 0.93
Lung function		
FEV ₁ , %	70.65 ± 24.15	90.6 ± 14.92
FEV ₁ /FVC, %	65.58 ± 14.62	81.53 ± 9.52

were significantly associated with asthma (odds ratio, OR = 1.32, 95% confidence interval, CI = 1.06–1.65, p = 0.0096, for rs1046295, and OR = 1.41, 95% CI = 1.12–1.75, p = 0.0026, for rs16659). Homozygote G/G for rs1046295 and homozygote D/D for rs16659 insertion/deletion polymorphisms (IDPs) were significantly associated with the

Table 3. Polymorphisms in the *DPP10* and *PHF11* genes genotyped in this study

Markers		Location	Gene position	Polymorphisms	MAF	
rs number	name				asthma	controls
DPP10						
rs7568000	543wtc21p	191388		C/T	T = 0.33	T = 0.32
rs10208402	543wtc91p	252279	intron Ia	T/C	T = 0.35	T = 0.37
rs1430090	543wtc122p	259007	intron IIs	A/C	C = 0.39	C = 0.35
rs17763180	543wtc124p	262881	intron IIs	T/C	T = 0.12	T = 0.12
rs12151526	16wtc104p	305609	intron Ic	G/T	T = 0.34	T = 0.36
PHF11						
rs11619265	b1_1	141478		A/G	A = 0.46	A = 0.50
rs3765526	b4_2	178972	intron 5	A/G	A = 0.48	G = 0.49
rs9526569	b5_2	186064	intron 9	C/T	C = 0.20	C = 0.24
rs1046295	b5_3	187062	3'-UTR	G/A	A = 0.45	G = 0.48
rs16659	44593_15bp	191057		I/D	I = 0.45	D = 0.46
rs953502	b38_1	361086		A/G	G = 0.10	G = 0.09

Marker names, locations and positions of the gene were reported previously [4, 5]. MAF = Minor allele frequency. rs16659 is an insertion/deletion polymorphism marker.

Table 4. Association between asthma and polymorphisms of the genomic regions of the *PHF11* and *DPP10* genes

Markers	Allele 1/ allele 2	Allele 2 frequency		Allele 2 vs. allele 1 analysis		Genotype analysis		
		asthma	controls	p	OR (95% CI)	asthma ^a	controls ^a	p
<i>PHF11</i>								
rs11619265	G/A	0.46	0.50	0.25	0.86 (0.66-1.11)	110/149/85	54/101/52	0.29
rs3765526	A/G	0.52	0.49	0.37	0.90 (0.71-1.13)	95/200/83	63/140/69	0.58
rs9526569	T/C	0.20	0.24	0.18	0.83 (0.63-1.09)	258/116/23	165/104/15	0.13
rs1046295	A/G	0.55	0.48	0.0096	1.32 (1.06-1.65)	123/200/83	63/150/75	0.03
rs16659	I/D	0.55	0.46	0.0026	1.41 (1.13-1.75)	112/204/75	56/143/77	0.007
rs953502	A/G	0.10	0.09	0.86	0.95 (0.50-1.78)	317/75/1	182/35/3	0.17
<i>DPP10</i>								
rs7568000	C/T	0.33	0.32	0.84	0.97 (0.72-1.30)	185/175/45	132/122/30	0.96
rs10208402	C/T	0.35	0.37	0.54	0.93 (0.73-1.18)	174/176/53	109/145/33	0.21
rs1430090	A/C	0.39	0.35	0.12	0.83 (0.66-1.05)	144/199/57	113/149/26	0.11
rs17763180	C/T	0.12	0.12	0.81	1.06 (0.67-1.66)	311/79/9	222/60/6	0.47
rs12151526	G/T	0.34	0.36	0.45	0.91 (0.71-1.16)	178/159/54	113/136/35	0.17

Significant values are shown in bold. rs16659 is an insertion/deletion (I/D) polymorphism marker.

^a The three values represent the number of major allele homozygote/heterozygote/minor allele homozygote carriers.

development of asthma ($p = 0.03$ and 0.007 , respectively; table 4).

We further searched for associations between polymorphic markers in *PHF11* and *DPP10* and a number of quantitative asthma-related traits using Merlin. A single marker in *DPP10*, rs10208402, demonstrated highly significant associations with both log-transformed IgE ($p =$

0.00031) and the percentage of eosinophils ($p = 0.0049$). A more moderate association was also observed between marker rs1430090 (also in *DPP10*) and FEV₁ ($p = 0.038$). A single marker in *PHF11* (rs11619265) was significantly associated with the percentage of eosinophils ($p = 0.0078$), and the same marker showed a trend towards significance for log-transformed IgE ($p = 0.083$).

Table 5. Haplotypes of *PHF11*, *DPP10* and asthma

Haplotype		Frequencies			χ^2 test	p value
		total	asthma	controls		
<i>PHF11</i>						
H1	GGTADA	0.322	0.332	0.309	0.736	0.3909
H2	AATGIA	0.170	0.152	0.194	4.14	0.0419
H3	AACGIA	0.144	0.124	0.172	6.248	0.0124
H4	AGTADA	0.072	0.073	0.070	0.073	0.7865
H5	GATGIA	0.069	0.068	0.071	0.036	0.8504
H6	CGTADG	0.036	0.039	0.033	0.361	0.5478
H7	GATADA	0.024	0.030	0.016	2.925	0.0872
H8	GGCGIA	0.021	0.019	0.025	0.569	0.4508
H9	AATGIG	0.021	0.022	0.020	0.058	0.809
H10	AACGIG	0.019	0.016	0.023	0.763	0.3823
<i>DPP10</i>						
H1	CCCCG	0.132	0.138	0.123	0.604	0.437
H2	TCCCC	0.115	0.118	0.110	0.259	0.611
H3	CTCCC	0.098	0.101	0.092	0.314	0.5753
H4	TCACG	0.096	0.095	0.097	0.0070	0.932
H5	CCACT	0.092	0.097	0.085	0.513	0.474
H6	CCACG	0.091	0.087	0.098	0.494	0.4824
H7	CTACG	0.090	0.084	0.097	0.697	0.4038
H8	CTATG	0.077	0.066	0.092	3.105	0.0781
H9	TCACT	0.045	0.042	0.049	0.325	0.5689
H10	CTATT	0.043	0.043	0.042	0.0060	0.94
H11	CCATT	0.041	0.045	0.035	0.82	0.3653
H12	TCATT	0.017	0.014	0.020	0.742	0.3889
H13	TTCCG	0.013	0.017	0.007	2.313	0.1283
H14	TTATT	0.011	0.012	0.010	0.037	0.8483
H15	TTACG	0.011	0.011	0.011	0.0070	0.9348
H16	TTACT	0.011	0.010	0.011	0.013	0.9101

PHF11 marker order: rs1161926-rs3765526-rs9526569-rs1046295-rs16659-rs953502; rs16695 is an insertion/deletion marker. *DPP10* marker order: rs756800-rs10208402-rs1430090-rs17763810-rs12151526. Significant differences in haplotypes are shown in bold.

Haplotype Analyses and LD Tests

Haplotype analyses of all markers gene by gene (including haplotypes present in $\geq 1\%$ of the population genotyped) revealed a moderate association between two *PHF11* haplotypes and asthma (AATGIA, $\chi^2 = 4.14$, $p = 0.0419$, and AACGIA, $\chi^2 = 6.248$, $p = 0.0124$). Only one haplotype remained significant after 1,000 permutations (haplotype = AACGIA, permutation $p = 0.0240$). This haplotype was significantly underrepresented in asthmatics compared with controls, with case and control frequencies of 0.124 and 0.172, respectively. This haplotype therefore appears to confer protection against asthma. No haplotypes in *DPP10* showed a consistent association with asthma (table 5).

Discussion

We performed a case-control study to test the association between polymorphisms in *PHF11* and *DPP10* and asthma, as well as a number of quantitative intermediate traits in 408 patients and 288 controls in a Han Chinese population. Two markers in *PHF11*, rs1046295 and rs16659, were found to be significantly associated with asthma, and a third marker, rs11619265, with peripheral blood eosinophils. Markers in *DPP10* were not significantly associated with asthma, however highly significant associations were observed with both log-transformed serum IgE (rs10208402) and the percentage of peripheral blood eosinophils (rs10208402). A more mod-

erate association was also noted between *DPP10* (rs1430090) and FEV₁. One haplotype in *PHF11* was shown to confer significant protection against the risk of asthma. Together, these results provide support for the hypothesis that polymorphisms in *PHF11* and *DPP10* affect the susceptibility to asthma and/or asthma-related traits.

PHF11, encoding a plant homeodomain finger protein, regulates transcriptional activity with multiple splice variants in T and B cells [4]. A recent study has shown that *PHF11* regulates the expression of Th1-type cytokine genes, and reduced expression of *PHF11* in atopic dermatitis (a disease that commonly presents concomitant with asthma) causes strong Th2 responses characterizing asthma and allergic diseases [9]. In *PHF11*, markers rs3765526 and rs1046295 have been associated with asthma in the general Australian population and are significantly linked to severe asthma in unrelated British individuals [4]. In an extensive study testing positionally cloned asthma genes, several SNPs in *PHF11* were reported to be marginally associated with childhood asthma in European-American and Hispanic families [11].

The positive association between rs1046295 and asthma was confirmed by the initial positional cloning that first implicated *PHF11* in the etiology of asthma. Marker rs1046295 is located in the 3'-UTR of *PHF11* and has also been associated with childhood atopic dermatitis [17]. The G allele of rs1046295 is preferentially transmitted to children with atopic dermatitis and has been associated with decreased *PHF11* RNA in Th1 cells [9]. Marker rs16659 is 4 kb away from rs1046295, positioned within the 5'-UTR of *RCBTB1*, and shows strong LD with rs1046295 ($r^2 = 0.9$, data not shown). *SETDB2* and *RCBTB1* colocalize with *PHF11* within the same LD blocks. They may also be important to the disease, particularly *SETDB2*, which has an expression profile similar to *PHF11* in immune-related cells [4]. In the present study, genetic markers in *PHF11* were closely associated with overall asthma susceptibility but not linked to asthma-related phenotypes, e.g. serum total IgE. This can be due to the fact that for the overall disease risk, phenotypes are influenced by numerous biological pathways that interact with environmental factors [18, 19].

DPP10 encodes a dipeptidyl peptidase, which can cut two peptides from N-termini of certain proinflammatory chemokines, but it is still unknown whether the removal of two peptides activates or deactivates them. *DPP10* is prominently expressed in the brain as well as adrenal glands and trachea. In situ hybridization showed that *DPP10* is expressed in neuronal populations also

known to express Kv4 products, and *DPP10* protein was found to coprecipitate with Kv4.2 channel complexes from brain membranes [20].

In the initial positional cloning study of *DPP10*, marker rs1430090 was strongly associated with asthma. In particular, haplotype rs1430090-D2S308 was positively associated with severe asthma in German children. The marker rs1430090 was also suggested to affect *DPP10* expression by altering the sequence of the known promoter element (CdxA) adjacent to one of the alternative exons [5]. The associations between polymorphisms in *DPP10* and asthma or asthma-related traits were not found in two family-based samples [11]. In our study, marker rs10208402 in *DPP10* was strongly associated with peripheral blood eosinophil (%) and serum total IgE in patients with asthma. The same marker correlated strongly with IgE in Australian and British families [5]. Marker rs1430090 was also associated with FEV₁ in asthma patients.

The discrepancies observed between studies could reflect true differences in the genetic effects of *DPP10* variants or in the patterns of LD between typed and true causative variants [5, 21]. Also, the extent and nature of gene-gene and gene-environment interactions may vary between populations [22]. Environmental exposure, which is crucial for the expression of asthma, may well vary between populations [23]. In addition, differences in the study design may also influence the type of effect observed. In previous studies, the subjects have been children, whereas our study includes only adults. Age is known to have important effects on the expression of asthma. Longitudinal studies have indicated that about 50% of children with asthma will outgrow the symptoms after a number of years [24].

A higher serum total IgE is a robust risk factor for asthma regardless of the specific IgE to allergen [25]. Our study clearly demonstrates that markers within *DPP10* associate with several asthma-related traits. This may be a consequence of allelic heterogeneity whereby distinct alleles yield distinct phenotypic consequences, or an effect of pleiotropy whereby a single allele yields a variety of pathogenic effects. Different asthma-related phenotypes are certainly known to map to different locations within this broad linkage region [8, 26, 27].

The case-control study design adopted in this study is a common strategy in genetic studies of complex diseases such as asthma [18, 28]. The advantage of association studies is that this approach will detect the genes with smaller effects in sample sizes comparable to those used in linkage analyses. Statistically, there is increased power

in studying the equivalent number of individuals in case-control association studies than in family-based studies [18]. However, small population sizes, population stratification, phenotype heterogeneity and varied environmental exposure are often blamed for negative replication of initially positive associations [10, 29]. The current study has some advantages. It was based on a relatively large study cohort including nearly 700 unrelated individuals (408 patients and 288 controls) with Han Chinese ethnicity recruited from the Northern region of China, representing a relatively homogeneous group. The diagnosis of asthma was defined by the present asthmatic symptoms plus an explicit diagnostic test such as bronchial hyper-responsiveness or positive airway reversibility [30]; this study includes adults only, thus, to a greater extent, avoiding the possibility of phenotypic heterogeneity which may cause spurious associations, as evidence shows that childhood and adult asthma differ in several aspects [31, 32].

In conclusion, our findings that genetic variants in *PHF11* and *DPP10* genes partially contribute to asthma susceptibility and asthma-related phenotypes are consis-

tent with the known genetic biology of asthma that several genes at distinct chromosomal regions confer the risk of asthma, having a larger or smaller effect according to environmental risk exposure [27, 33, 34]. However, it should be pointed out that the functional variants in the two loci and the mechanisms by which they mediate the phenotype have yet to be elucidated. Large-scale studies of these genes in other populations, particularly in combination with the underlying disease-causing mechanism(s), are required in order to accurately predict the disease risk conferred by these loci.

Acknowledgment

This work was funded by the New Century Excellent Talents Program from the Chinese Ministry of Education (NCET-06-0156) and a grant from the National Natural Sciences Foundation of China (No. 30470767). We thank Jun Gao and Yang Zhao for their help in collecting the blood samples. We are also indebted to Haijuan He and Ruiqi Wang for their help in serum total IgE measurements.

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