Gene-gene interactions for asthma and plasma total IgE concentration in Chinese children

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Background: Asthma is a complex disease resulting from interactions between multiple genes and environmental factors. Study of gene-gene interactions could provide insight into asthma pathophysiology.
Objective: We investigated the interaction among 12 different loci in 8 candidate genes and asthma and increased plasma total IgE concentrations in 240 Chinese asthmatic subjects and 140 control subjects.
Methods: Genotyping was performed by means of RFLP analysis. Multifactor dimensionality reduction and logistic regression were used to analyze gene-gene interactions.
Results: A significant interaction was found between R130Q in the IL-13 gene (*IL13*) and I50V in the IL-4 receptor α gene (*IL4RA*) on the risk of asthma, with a cross-validation consistency of 10 of 10 and a prediction error of 33.7% (P = .014). The odds ratio of the high-risk to low-risk group was 2.6 (95% CI, 1.4-5.0; P = .004). For increased plasma total IgE concentration, the best 2-locus model consisted of R130Q in *IL13* and C-431T in the thymus and activation-regulated chemokine gene (*TARC*). This model showed a maximum cross-validation consistency of 10 and a minimum prediction error of 36.1% (P = .022). The odds ratio of the high-risk to low-risk group was 3.9 (95% CI, 2.0-7.7; P = .0001). Logistic regression revealed significant interactions between *IL13* and *IL4RA* for asthma (P = .042) and *IL13* and *TARC* for increased total IgE concentration (P = .012).
Conclusions: Our data suggest significant interactions between *IL13* and *IL4RA* for asthma and *IL13* and *TARC* for increased plasma total IgE concentrations in Chinese children.
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Key words: *Asthma, candidate gene, gene-gene interactions, IgE, polymorphism*

Abbreviations used
ADRB2: β_2-adrenoceptor gene
CTLA4: Cytotoxic T lymphocyte antigen 4 gene
CV: Cross-validation
FCERIB: β-Subunit of high-affinity IgE receptor gene
IL4RA: IL-4 receptor-α gene
MBL: Mannose-binding lectin
MDR: Multifactor dimensionality reduction
OR: Odds ratio
TARC: Thymus and activation-regulated chemokine gene

Asthma is one of the most common chronic respiratory diseases. It is a complex genetic disease resulting from interactions between multiple genes and environmental factors.[1-3] Genes have been reported to be associated with asthma in a number of studies.[4-6] However, thus far, not a single factor or gene was found to have a strong and independent effect on asthma.[7,8] Therefore searching for susceptibility genes for asthma requires a thorough understanding of gene-gene and gene-environment interactions. Traditionally, gene-gene interactions in complex diseases have been examined by means of logistic regression, multilocus linkage disequilibrium, and Hardy-Weinberg equilibrium tests, all of which have limitations in their general application.[9] The identification and characterization of gene-gene interactions have been limited by the lack of powerful statistical methods and large sample size.[9] Recently, Hahn et al[10] developed a software called multifactor dimensionality reduction (MDR) for detecting and characterizing high-order gene-to-gene interactions in case-control and discordant sib-pair studies with relatively small samples. MDR has been used in identifying gene-gene interactions in hypertension,[11] type 2 diabetes mellitus,[12] atrial fibrillation,[13] and myocardial infarction.[14]

In this study we genotyped 12 polymorphic markers in 8 candidate genes thought to be associated with asthma, atopy, or both in Chinese asthmatic patients and control subjects (Table I). Gene-gene interactions were examined with MDR. These genetic loci were R130Q of the IL-13 gene (*IL13*)[15,16]; C-159T of the *CD14* gene[17,18]; C-431T of the thymus and activation-regulated chemokine gene (*TARC*)[19,20]; −550 H/L, −221 X/Y, and codon 54 A/B of the mannose-binding lectin gene (*MBL*)[21,22]; +49 A/G of the cytotoxic T lymphocyte antigen 4 gene (*CTLA4*)[23]; I50V of the IL-4 receptor α gene (*IL4RA*)[24-26]; *Rsa*I_in2 and *Rsa*I_ex7 of the β-subunit of the high-affinity IgE receptor gene (*FCERIB*)[27]; and R16G and E27Q of the β_2-adrenoceptor gene (*ADRB2*).[28]

METHODS

Study population

This study recruited unrelated ethnic Chinese children aged 5 to 18 years with asthma diagnosed according to the American Thoracic

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TABLE I. Candidate genes and variants analyzed in this study

Chromosome	Candidate gene	Polymorphism	Association
5q31-33	*IL13*	R130Q	IgE, asthma
5q31	*CD14*	C-159T	IgE
16q13	*TARC*	C-431T	Atopy, asthma
10q11.2-q21	*MBL*	Codon-54 A/B; −221 X/Y; −550 H/L	Asthma
2q33	*CTLA4*	+49 A/G	Atopy, asthma
16p12	*IL4RA*	I50V	Atopy, IgE, atopic asthma
11q13	*FCERIB*	*Rsa*I_in2; *Rsa*I_ex7	Atopy, IgE, BHR
5q32-34	*ADRB2*	R16G; E27Q	IgE, BHR, treatment response, nocturnal asthma

BHR, Bronchial hyperresponsiveness.

Society guidelines[29] from the pediatric outpatient clinics of a university teaching hospital in Hong Kong. Both parents of these subjects were ethnic Chinese. Age- and sex-matched control subjects were selected among children attending the hospital for minor nonrespiratory and nonallergic complaints, and they did not require any drug treatment at the time of this study. All subjects were free from any self-reported symptoms of infection for 4 weeks before the study. Subjects or their parents provided written consent, and the Clinical Research Ethics Committee of our university approved this study.

Evaluation of atopy and lung function

Plasma total IgE concentration and specific IgE antibodies to 5 locally relevant aeroallergens were measured. Total IgE results were compared with our local upper limits of reference values to determine whether the levels were increased. Children with at least one positive allergen-specific IgE test response were classified as atopic.[16] Asthmatic patients also underwent spirometric assessment to measure their FEV_1 and forced vital capacity (see this article's additional text in the Online Repository at www.jacionline.org for details).

Genotyping

PCR and RFLP were used to genotype the 12 polymorphisms, as listed in Table I. The genotyping conditions for PCR and RFLP are summarized in Table E1 in the Online Repository at www.jacionline.org. The results of these RFLP assays were validated by means of direct sequencing of the polymorphisms with Big Dye Terminator Cycle sequencing kits with an ABI-310 autosequencer (Applied Biosystems, Foster City, Calif) of 30 randomly selected samples.

Statistical analysis

The results on plasma total IgE concentration and allergen sensitization as risk factors for the development of asthma between different groups were compared by using the Student *t* test, the χ^2 test, or the Fisher exact test as appropriate. Allele frequencies were estimated by using the gene-counting method, and the χ^2 test was used to examine the Hardy-Weinberg equilibrium. Association of asthma and plasma total IgE concentration with 12 loci was made with the Pearson χ^2 test.

MDR (version 0.3.1; Computational Genetics Laboratory, Dartmouth Medical School, Hanover, NH; http://www.epistasis.org) was used to determine high and low risk on the basis of the work of Moore et al.[10,30] This method determines one genetic model, either single or multilocus, that most successfully predicts class or phenotype from several loci, environmental factors, or both that can be divided into high-risk and low-risk combinations. Detailed explanations on this statistical method are provided in the additional text in the Online Repository at www.jacionline.org. Briefly, cross-validation (CV) consistency and prediction error were calculated for each combination of a pool of genetic polymorphisms. When CV consistency is maximal for one model and prediction error is minimal for another, statistical parsimony is used to choose the best model. We determined statistical significance by comparing the average prediction error from the observed data with the distribution of average prediction errors under the null hypothesis of no associations derived empirically from 1000 permutations. The null hypothesis was rejected when the *P* value derived from the permutation test was .05 or less.

In the present analysis, high-risk genotypes for asthma and increase in total IgE concentration were defined as if the ratio of the percentage of patients to control subjects was greater than the threshold of 1.0 with a difference in percentage by more than 2%, whereas low-risk groups were defined as if the threshold was not exceeded with a difference in percentage by more than 2%. A difference of 2% was introduced in this analysis to increase the power of high- and low-risk classification. Logistic regression analysis and χ^2 tests were performed to confirm the results from MDR analyses. A *P* value of less than .05 was considered statistically significant.

RESULTS

Study population

Two hundred forty asthmatic children and 140 control subjects were enrolled in the study. The mean (SD) age of asthmatic patients was 10.0 (3.9) years, and 64% of them were male. The clinical and laboratory characteristics of these subjects are shown in Table E2 in the Online Repository at www.jacionline.org.

Asthma, plasma total IgE concentration, and individual polymorphisms

All 12 polymorphisms examined in this study followed Hardy-Weinberg equilibrium. The allele frequencies of all the studied genes are presented in Table E3 in the Online Repository at www.jacionline.org, and that for *IL13* R130Q differed significantly between patients and control subjects ($P = .043$). Table II summarizes the distribution of different genotypes with regard to asthma diagnosis and increased plasma total IgE concentration. None of the polymorphisms was found to be significantly associated with asthma. For the increase in plasma total IgE concentration, only R130Q in *IL13* and *Rsa*I_ex7 in *FCERIB* showed significant associations ($P = .003$ and .048, respectively).

MDR analysis

Table III shows the results of CV consistency and prediction error obtained from MDR analysis of the data set of patients and control subjects. One 2-locus model had a minimum prediction error of 33.7% ($P = .014$) and a maximum CV consistency of 10 of 10. This 2-locus model consisted of the R130Q polymorphism in the coding region of *IL13* and I50V in the coding region of *IL4RA* (Fig 1). Because the percentage values of patients and control subjects in the genotype combinations of *IL4RA* 50I/V

TABLE II. Association of gene polymorphisms with asthma and increased plasma total IgE concentration

Polymorphism	Asthmatic patients	Control subjects	*P* value*	Subjects with increased total IgE	Subjects with normal total IgE	*P* value*
R130Q	n = 238	n = 126		n = 225	n = 139	
RR	79	54		73	60	
RQ	117	57		106	68	
QQ	42	15	.128	46	11	.003
C-159T	n = 237	n = 128		n = 226	n = 139	
CC	49	25		50	24	
CT	114	68		108	74	
TT	74	35	.640	68	41	.467
C-431T	n = 235	n = 134		n = 225	n = 144	
CC	90	62		85	67	
CT	122	65		121	66	
TT	23	7	.157	19	11	.247
Codon-54 A/B	n = 236	n = 138		n = 227	n = 147	
AA	7	4		7	4	
AB	65	39		55	49	
BB	164	95	.989	165	94	.159
−221 X/Y	n = 233	n = 136		n = 223	n = 146	
XX	16	7		14	9	
XY	72	46		72	46	
YY	145	83	.720	137	91	.985
−550 H/L	n = 237	n = 138		n = 227	n = 148	
LL	82	46		77	51	
LH	112	72		109	75	
HH	43	20	.559	41	22	.711
+49 A/G	n = 216	n = 133		n = 207	n = 142	
AA	33	17		33	17	
AG	92	59		85	66	
GG	91	57	.807	89	59	.463
I50V	n = 232	n = 131		n = 221	n = 142	
II	64	37		57	44	
IV	123	67		119	71	
VV	45	27	.936	45	27	.559
*Rsa*I_in2	n = 207	n = 132		n = 203	n = 136	
RR	10	6		12	4	
Rr	62	45		59	48	
Rr	135	81	.726	132	84	.265
*Rsa*I_ex7	n = 228	n = 133		n = 220	n = 141	
RR	213	125		201	137	
Rr	15	8		19	4	
Rr	0	0	.832	0	0	.048†
R16G	n = 231	n = 136		n = 221	n = 146	
RR	80	35		78	37	
RG	104	71		99	76	
GG	47	30	.201	44	33	.132
E27Q	n = 230	n = 136		n = 220	n = 146	
EE	15	14		12	17	
EQ	37	18		33	22	
QQ	178	104	.367	175	107	.096

*Compared with Pearson χ^2 test.
†Compared with χ^2 test with Yates correction.

with *IL13* 130R/R and 130R/Q and *IL4RA* 50V/V with *IL13* 130Q/Q were quite similar, these combinations were not classified into either high- or low-risk groups (unclassified). On the other hand, we were able to classify the combinations of *IL4RA* 50I/I with *IL13* 130R/R and *IL4RA* 50V/V with *IL13* 130R/R as the low-risk group, and all others as the high-risk groups. In this model the odds ratio (OR) for the high-risk to the low-risk group was 2.6 (95% CI, 1.4-5.0; $P = .004$).

Table IV shows the multilocus interaction model for increased plasma total IgE concentration by means of the MDR method. The best 2-locus model consisted of R130Q in the *IL13* gene and C-431T in the *TARC* gene. This model had a maximum CV consistency of 10 and

TABLE III. Multilocus interaction model for asthma by means of the MDR method

Loci number and combination	CV consistency	Prediction error (%)
2 locus: R130Q, I50V	10	33.67*
3 locus: R130Q, −221 X/Y, I50V	7	37.74
4 locus: R130Q, C-159T, −221 X/Y, I50V	4	38.19
5 locus: R130Q, C-159T, −550 H/L, I50V, *Rsa*I_in2	6	41.70
6 locus: R130Q, C-159T, −550 H/L, +49 A/G, I50V, *Rsa*I_in2	8	47.27

*P = .014 on the basis of 1000 permutations.

TABLE IV. Multilocus interaction model for the increase in plasma total IgE concentration by means of the MDR method

Loci number and combination	CV consistency	Prediction error (%)
2 locus: R130Q, C-431T	10	36.07*
3 locus: C-159T, +49 A/G, *Rsa*I_in2	2	48.38
4 locus: R130Q, C-159T, C-431T, I50V	5	43.24
5 locus: R130Q, C-159T, C-431T, +49 A/G, I50V	4	46.81
6 locus: C-159T, C-431T, −550 H/L, I50V, *Rsa*I_in2, R16G	3	47.47

*P = .022 on the basis of 1000 permutations.

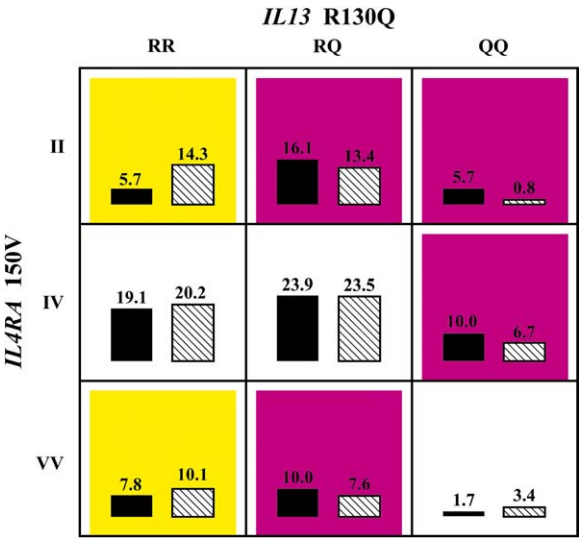


FIG 1. Best 2-locus model for asthma in the Chinese children derived from analyses of 12 variants. High-risk genotypes are in pink, and low-risk genotypes are in yellow. The percentages of asthmatic patients *(left black bar in boxes)* and control subjects *(right hatched bar in boxes)* are shown for each 2-locus genotype combination. All the *white boxes* are unclassified.

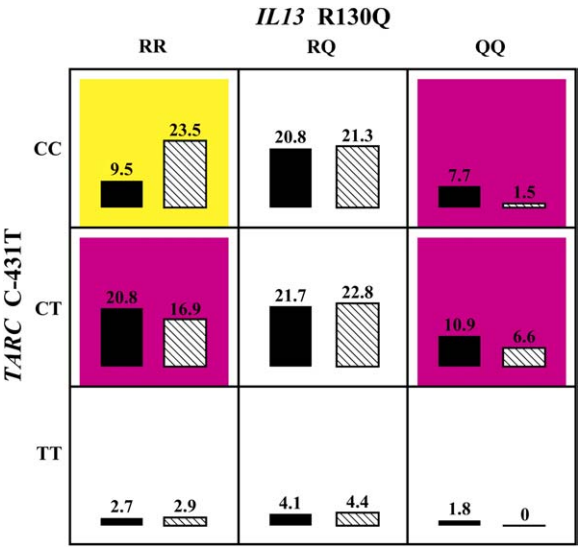


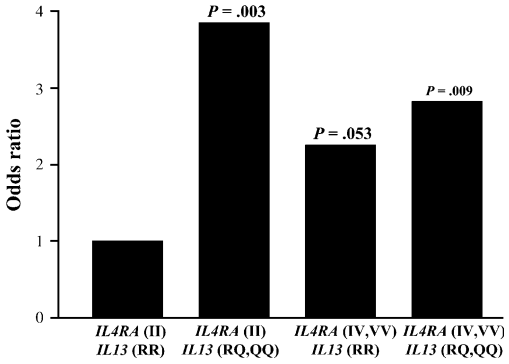
FIG 2. Best model for increased plasma total IgE concentration derived from 12 variants. High-risk genotypes are in pink, and low-risk genotypes are in yellow. The percentages of subjects with increases in total IgE concentration *(left black bar in boxes)* and subjects with normal total IgE concentrations *(right hatched bar in boxes)* are shown for each combination. All the *white boxes* are unclassified.

a minimum prediction error of 36.1%. The combinations of *IL13* 130R/R with *TARC* −431C/T and *IL13* 130Q/Q with *TARC* −431C/C and −431C/T were shown to be the high-risk groups. By contract, *IL13* 130R/R with *TARC* −431C/C was the low-risk group (Fig 2). The OR for the high-risk to low-risk groups was 3.9 (95% CI, 2.0-7.7; P = 0.0001). R130Q alone was also significantly associated with the increase in plasma total IgE concentration (P = .003, Table II). The OR of 130Q/Q to 130R/R in increased plasma total IgE concentration was 3.4 (95% CI, 1.6-7.2; P = .001).

Logistic regression analyses

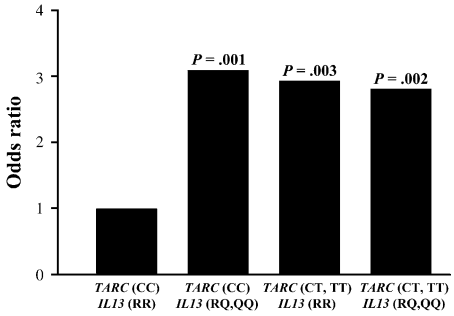
A significant interaction between R130Q in *IL13* and I50V in *IL4RA* on the risk of asthma was also found by means of logistic regression analysis (P = .042), adjusting for age and sex as covariates. Individuals carrying the minor alleles of *IL13* (Q) and *IL4RA* (V) had a significant 3.1 (95% CI, 1.1-8.9) higher risk of having asthma than those homozygous for both wild types. Fig 3 shows the relation between asthma and different combinations of *IL13* and *IL4RA* genotypes.

For increase in plasma total IgE concentration, logistic regression analysis found a significant interaction between R130Q in *IL13* and C-431T in *TARC* (P = .012), with adjustments for age and sex. Those with minor alleles of *IL13* (Q) and *TARC* (T) had a 3.2 (95% CI, 1.3-8.2) higher risk of increasing total IgE concentration than those homozygous for both wild types. Fig 4 shows the relation between increased total IgE concentration and different combinations of *IL13* and *TARC* genotypes.



No. of asthmatics	13	50	62	105
No. of controls	17	17	36	49

FIG 3. Interactions between *IL13* and *IL4RA* genotypes for asthma diagnosis. *Bars* indicate the ORs between the different combinations of genotypes for *IL13* (R130Q) and *IL4RA* (I50V). The nonrisk genotype for each gene was used as the reference OR.



Subjects with elevated total IgE	21	63	52	85
Subjects with normal total IgE	32	31	27	46

FIG 4. Interactions between *IL13* and *TARC* genotypes for increased plasma total IgE concentration. *Bars* indicate the ORs between the different combinations of genotypes for *IL13* (R130Q) and *TARC* (C-431T). The nonrisk genotype for each gene was used as the reference OR.

DISCUSSION

As analyzed with both MDR and logistic regression, this study suggests that the susceptibility to asthma in Chinese children involves a gene-gene interaction between *IL13* and *IL4RA*. Increased plasma total IgE concentration appears to be mediated by an interaction between *IL13* and *TARC*. The combinations of alleles at a variety of candidate loci might be more important than the variants that are found at any single locus in conferring the risk of having asthma. This finding of epistasis is consistent with similar results in the Dutch population.[26] We have previously reported that the R130Q mutant of *IL13* was associated with total IgE and allergen-specific IgE but not with physician-diagnosed asthma.[16] *IL4RA* has previously been associated with numerous atopic conditions,[24,25] and our group first reported significant associations between *TARC* C-431T and atopy, aeroallergen sensitization, and peripheral eosinophilia in Chinese children.[20] In the present study we found a significant gene-gene interaction between 2 loci out of a total of 12, demonstrating the sensitivity of the MDR method in detecting polygenic interactions among many different loci in multiple genes.

This study adopted a different analytic approach compared with another study that also reported significant gene-gene interaction between *IL13* and *IL4RA*.[26] In our study with MDR analysis, we did not set any *a priori* assumption on whether there was interaction between any specific pair of genes or polymorphic markers. On the other hand, the latter report performed gene-gene interaction analysis only for the 2 polymorphisms with the strongest evidence for association with asthma genotype (ie, *IL4RA* S478P and *IL13* −1111 C/T). Although the same gene-gene interaction was observed, our study included another 2 polymorphisms that were reported to be associated with total and allergen-specific IgE in Chinese children (ie, *IL4RA* I50V and *IL13* R130Q).[16,31] For patients with increased plasma total IgE concentrations, this study found significant interaction between *IL13* R130Q and *TARC* C-431T. Individually, only the *IL13* marker was associated with the phenotypes of asthma and increased total IgE concentration in our Chinese children. The above findings support a central role for IL-13 in the pathogenesis of asthma and atopy. In addition, the fact that our study reproduced the same interaction between *IL13* and *IL4RA* as reported in the previous publication[26] supports the occurrence of this gene-gene interaction.

The nature of gene-gene interaction between R130Q in *IL13* and I50V in *IL4RA* for asthma susceptibility is not clear. When the MDR method was previously applied to breast cancer,[30] there was a consistent trend of high-risk or low-risk cells across a series of rows or columns, which indicated that a particular locus had a main effect. We could not find such a trend in our best 2-locus model. Because asthma does not have a typical pattern of Mendelian inheritance that can be explained by a linear model,[2] this lack of a definite trend might not be surprising. Rather, this is an indicator of epistasis; that is, the influence of each genotype on asthma risk appears to be dependent on the genotypes at each of the other loci. Sorting out the functional basis of the interactions in this 2-locus model remains an interpretive challenge.

The binding of IL-13 or IL-4 to the IL-4 receptor induces the initial response for T_H2 lymphocyte polarization. Both IL-13 and IL-4 are produced by T_H2 cells and are capable of inducing immunoglobulin class switching of B cells to produce IgE after allergen exposure. These cytokines share a common receptor component, IL-4Rα.[32,33] The Q130 polymorphism of *IL13* had a lower affinity with the IL-13 receptor α_2 chain, a decoy receptor, causing less clearance. This variant also demonstrated an enhanced stability in both human and mouse plasma.[34] Another study showed that the combination of *IL4RA* V50 and R551 variants resulted in the expression of an IL-4Rα with enhanced sensitivity to IL-4.[35] The coexistence of both functional polymorphisms probably interacts

to augment this IL-13/IL-4Rα signaling. It is therefore possible that different polymorphisms in *IL4RA* and *IL13* contribute to the complex regulation of atopy or asthma phenotypes.

For the increase in plasma total IgE concentration, the best 2-locus model was an interaction between R130Q in *IL13* and C-431T in the *TARC* promoter. TARC is a key chemokine for attracting T_H2 lymphocytes into the site of allergic inflammation.[36,37] Our recent studies suggested that plasma TARC concentration was significantly associated with chronic stable asthma[38] and the severity of asthma exacerbation in children.[39] Increased TARC production was also associated with a state of systemic allergic inflammatory response. The resulting T_H2 activation could lead to the increase in IgE production and recruitment of eosinophils from bone marrow under actions of the important T_H2 cytokines IL-4 and IL-5, respectively.[40] Tsunemi et al[41] found, by using the transient-transfection assay, that *TARC* C-431T was functional, and several studies reported that −431T was associated with increased plasma TARC concentration.[19,20] Our recent report suggested a significant association between the *TARC* C-431T polymorphism with the susceptibility to atopy but not with increased total IgE concentration.[20] In the present study the interaction between R130Q in *IL13* and C-431T in *TARC* in increased plasma total IgE concentration was significant ($P = .022$). The OR of the high-risk to low-risk genotypes was 3.9. However, the risk was similar to the effect with the presence of R130Q alone (OR, 3.4). It has been suggested that a multilocus approach should be used to analyze quantitative traits of a complex disorder. Xu et al[42] reported that total serum IgE concentration was regulated by 2 unlinked loci on 5q. Two-locus segregation and linkage analysis might provide a significant additional amount of statistical power compared with studies of discrete disease phenotypes.

Small sample size is a well-recognized problem in genetic studies for complex human diseases. The use of MDR analysis in this study would minimize this limitation. It is a method for detecting and characterizing high-order gene-gene and gene-environment interactions in case-control and discordant sib-pair studies with relatively small samples.[30] Previous studies had successfully used this statistical method to delineate gene-gene interactions for a number of complex multifactorial traits, with a total sample size that varied between 177 and 686.[11-14,30] With MDR, multilocus genotypes are pooled into high-risk and low-risk groups, effectively reducing the genotype predictors from *n* dimensions to 1 dimension. The new, 1-dimensional multilocus-genotype variable is evaluated for its ability to classify and predict disease status through CV and permutation testing.[10,14,30,43] The MDR approach is nonparametric (ie, no hypothesis about the value of a statistical parameter is made) and model free (ie, assumes no particular inheritance model). Using simulated data, Ritchie et al[30,43] showed that MDR has high power to identify gene-gene interactions in the presence of 5% genotyping error, 5% missing data, or a combination of both.

In summary, by implementing the MDR method, this study demonstrated significant gene-gene interactions between *IL13* and *IL4RA* on the risk of having asthma and between *IL13* and *TARC* on the increase in plasma total IgE concentration. These results are consistent with those obtained from logistic regression analyses. The susceptibility to asthma is the result of multiple genetic and environmental factors. Genetic studies of asthma will continue to provide insight into the pathophysiologic mechanisms of the disease. This could lead to new and more effective therapeutic interventions, new diagnostic methods for presymptomatic diagnosis, and development of strategies for disease prevention in susceptible individuals.

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