

A polymorphism in the coding region of interleukin-13 gene is associated with atopy but not asthma in Chinese children

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Summary

Background Interleukin (IL)-13 is an important cytokine secreted from type 2 helper T lymphocytes. It is essential for modulating IgE synthesis by human B cells. Previous studies showed that polymorphisms in the IL-13 gene were associated with serum total IgE or allergic asthma. The relationship of this marker with sensitization to individual aeroallergens has not been evaluated.

Objective We tested whether a polymorphism in the coding region of the IL-13 gene is associated with asthma and atopy in asthmatic children in Hong Kong.

Methods We used restriction fragment length polymorphism to detect R130Q genotype in Chinese children with asthma and control subjects. Serum total IgE was measured by microparticle immunoassay and specific IgE to common aeroallergens was measured using fluorescent enzyme immunoassay. Pulmonary function studies were performed using spirometry.

Results One hundred and fifty-seven patients and 54 control children were recruited. Their mean serum total IgE concentrations were 994 kIU/L and 473 kIU/L, respectively ($P < 0.0001$). Atopy as defined by ≥ 1 positive RAST was found in 141 patients and 32 control children. The GlnGln form of the R130Q polymorphism in the IL-13 gene was associated with serum total IgE ($P = 0.005$) as well as specific IgE to Der p 1 ($P = 0.021$), mixed cockroaches ($P = 0.03$) and dog ($P = 0.003$) but not with physician-diagnosed asthma ($P = 0.621$). In addition, the R130Q polymorphism did not correlate with subjective or objective indicators of asthma severity in our patients.

Conclusion Our results suggest that the R130Q polymorphism of the IL-13 gene is associated with elevated serum total and allergen-specific IgE but not asthma in Chinese children.

Keywords: aeroallergens, asthma, Chinese, immunoglobulin E, IL-13, R130Q polymorphism

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Introduction

The occurrence of asthma and wheezing illnesses has been increasing in children and young adults world-wide [1]. The prevalence of self-reported wheeze varies from 3.1%

to 13.2% among Chinese-speaking children in countries from south-east Asia [2]. In the recent International Study of Asthma and Allergy in Childhood, 11% of 4665 schoolchildren in Hong Kong suffered from asthma [3]. Atopy is characterized by elevated serum total and allergen-specific IgE concentrations, and has been found to be a major component of asthma [4]. Asthma and atopy are related conditions caused by a complex interaction of

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genetic factors and environmental influences. Using candidate gene approaches, many susceptibility loci for asthma and atopy have been described [5–8]. These genes are responsible not only for causing asthma but also regulating its severity. Alternatively, genome-wide searches suggested that asthma could be mapped to chromosomes 5q31–33, 12q15–24 and 11q13 in the Caucasian as well as Japanese populations [9–13].

Genetic analyses also showed that the regulation of total IgE concentration is linked to a region on chromosome 5q31–33 that encodes a cluster of cytokine genes [9,12–14]. Several interleukins (IL), including IL-4 and IL-13, map to this area and have biological functions that are involved in the regulation of IgE synthesis [15,16]. IL-13 is secreted by both type 1 and type 2 helper T lymphocytes as well as CD8⁺ cells [17]. Jung et al. found that IL-13 is produced by naive T cells that do not express IL-4, and that it may play an important role in the initiation of isotype switching to IgE in human B cells [18]. A recent study in transgenic mice provided additional evidence for a role of IL-13 in regulating IgE synthesis [19]. Grunig *et al.* suggested that IL-13 may be a crucial determinant of bronchial hyper-responsiveness (BHR) in allergic asthma, and this effect is independent of IL-4 [20].

A novel and functional IL-13 promoter C to T polymorphism was recently identified to be associated with allergic asthma and altered regulation of IL-13 production [21]. In another study, Graves *et al.* reported seven allelic variants in the IL-13 gene, and the Gln form of the R130Q polymorphism was shown to be strongly associated with increased serum IgE levels in three different Caucasian asthmatic populations [22]. Such association was found mainly in those patients non-reactive to local aeroallergens on skin prick tests. Nonetheless, the investigators did not investigate the relationships between this marker and asthma phenotype as well as sensitization to individual inhalant allergens. Heinzmann *et al.* reported that this polymorphism was more prevalent in Japanese and British asthmatics as compared with population controls [23]. The aim of this study was to investigate the correlation between R130Q polymorphism in the coding region (CR) of the IL-13 gene and asthma phenotypes, as well as serum total and allergen-specific IgE concentrations in Chinese children with asthma in Hong Kong.

Materials and methods

Study population

This study involved children aged 5 years or above, with asthma as diagnosed according to the criteria proposed by the American Thoracic Society [24], who were followed in the general paediatric outpatient clinic of a university

teaching hospital. These asthmatic patients were recruited only when their diagnosis was made before 15 years of age. Age-matched control subjects were selected among children attending the hospital for non-allergic and non-immunological diseases. Both parents of our patients as well as control subjects had to be ethnic Chinese. The occurrence of allergic diseases in control subjects was excluded using a detailed questionnaire. All subjects should also be free from infection for 4 weeks before study. Subjects or their parents gave informed written consent and this study was approved by the Clinical Research Ethics Committee of our university.

Data collection

The demographic data, as well as the severity of asthma in our patients, were assessed by a standardized questionnaire based on the Disease Severity Score (DSS, 6–30 points) [25]. Briefly, this scoring system assessed the frequency of acute asthma exacerbation and occurrence of cough, wheezing or chest tightness in the daytime and at night during the 6 months prior to evaluation. The presence of exercise-related dyspnoea and details of anti-asthma treatment were also considered. The presence of common environmental triggers as well as personal and family history of other allergic diseases were also recorded.

Total and allergen-specific IgE

Peripheral venous blood was collected from patients and control subjects for the measurement of serum total IgE concentration by microparticle immunoassay (IMx analyser, Abbott Laboratories, Abbott Park, IL, USA) and specific IgE antibodies to Der p 1, cat, dog, mixed cockroaches and mixed moulds by fluorescent enzyme immunoassay (AutoCAP system, Pharmacia Diagnostics AB, Uppsala, Sweden). The grading of specific IgE into radioallergosorbent test (RAST) classes 1–6 was made according to manufacturer's instructions, with specific IgE concentration ≥ 0.35 kIU/L being positive. Serum total IgE levels were presented following logarithmic transformation ($\text{IgE}_{\log}$). Children were defined as atopic if they had at least one positive result on testing for allergen-specific IgE.

Spirometry

All asthmatic patients underwent spirometric assessment (COMPACT II, Vitalograph, Buckingham, UK) to determine their asthma severity according to the American Thoracic Society standards [26]. FEV₁, FVC, FEV₁/FVC and PEFR measured in our patients were compared with the local age- and sex-matched predicted values [27]. The subjects were categorized into severity groups by using

FEV₁% predicted (mild, FEV₁ > 80%; moderate, FEV₁ 60–80% and severe, FEV₁ < 60%), as described previously [28].

PCR assay for R130Q polymorphism in the IL-13 gene

Peripheral blood was collected from study participants, and germline DNA extracted using High Pure Viral Nucleic Acid Kit (Boehringer Mannheim, GmbH, Mannheim, Germany). Subjects were genotyped for R130Q polymorphism by polymerase chain reaction (PCR) amplification of the CR of the IL-13 gene using the following primers: 5'-CTT CCG TGA GGA CTG AAT GAG ACG GTC-3' and 5'-GCA AAT AAT GAG CTT TCG AAG TTT CAG TGG A-3' according to published methods [22]. Briefly, PCR reactions were carried out in a total volume of 25 µL containing approximately 60 ng of genomic DNA, 10 mmol/L Tris-hydrochloric acid buffer (pH 8.3), 4.0 mmol/L magnesium chloride, 200 µmol/L of each deoxynucleotide triphosphate, 50 ng of each primer, and 0.6 unit of Taq DNA polymerase (GibcoBRL, Life Technologies, Rockville, MD, USA). Samples were denatured at 95 °C for 2 min followed by 38 cycles at 94 °C for 30 s, 61 °C hybridization temperature for 1 minute and 72 °C for 1 minute, and then a final extension for 8 min at 72 °C. Ten microlitres of the 236-bp PCR fragment generated was then digested by addition of 5 units of *Nla*IV (New England Biolabs, Hitchin, Herts, UK) and incubated overnight at 37 °C. *Nla*IV digests 32-bp from the 3'-end of the fragment when the G nucleotide (Arg130) is present to produce a 178-bp fragment. The digested products were visualized following electrophoresis on 5% (w/v) agarose gel containing ethidium bromide.

The above restriction enzyme digestion was performed in 96-well plates. Eighty-eight samples were digested together with another eight PCR products of known genotypes as positive control in every reaction. In addition, 10 samples from each of the genotypes (i.e. homozygote R130, homozygote Q130 and heterozygote) were sequenced to confirm the genotyping results obtained by restriction enzyme digestion. DNA sequencing of PCR products was performed by BigDye Terminator Cycle sequencing kits with an ABI-310 autosequencer (Applied Biosystems, Foster City, CA, USA). All genotypes from restriction enzyme digestion were confirmed.

Statistical analysis

The clinical characteristics and results of allergen sensitization between different groups of subjects were compared using the student *t*-test, χ^2 statistics or Fisher exact test as appropriate. The results on serum total IgE and allergen sensitization as risk factors for the development of asthma were assessed by the χ^2 statistics. Standard analysis of

variance (ANOVA) test for quantitative traits was used to compare means of serum total and allergen-specific IgE (RAST class), spirometric variables and clinical indicators of asthma severity between different groups according to R130Q genotypes. All comparisons were made two-sided. *P*-values less than 0.05 were considered significant.

Results

Demographic data

One hundred and fifty-seven asthmatic children and 54 non-allergic control subjects were enrolled between November 1999 and August 2000. The mean (SD) ages at evaluation in patients and control children were 10.3 (3.6) years and 11.4 (3.8) years, respectively (*P* = 0.062), with 62% of patients and 59% of controls being males (*P* = 0.743). The clinical characteristics of our asthmatic patients are summarized in Table 1. On average, our patients had 2.0 asthmatic attacks within 6 months prior to

Table 1. The demographic data and indicators of asthma control in our 157 patients

Characteristics	Results*
Background information	
Age at evaluation (years)	10.3 ± 3.6
Sex (male : female)	97 : 60
Number with allergic rhinitis (%)	128 (82)
Number with eczema (%)	60 (38)
Clinical indicators of asthma severity†	
Number of asthma exacerbations	2.0 ± 1.7
Number of hospitalizations related to asthma	0.3 ± 0.7
Number of unscheduled outpatient visits	1.6 ± 2.1
Number of emergency room visits	0.4 ± 0.8
Nocturnal cough per month (days)	4.9 ± 6.5
Number of days of school absence	1.6 ± 2.8
Disease Severity Score	13.2 ± 3.6
Weekly use of inhaled bronchodilator (dose)	1.6 ± 2.9
Use of inhaled corticosteroid (%)	93 (59)
Spirometric assessment	
FEV ₁ (% predicted)	99 ± 19
Severity of asthma according to FEV ₁ (% of all patients; mild: moderate: severe)	83 : 16 : 1
FVC (% predicted)	120 ± 25
FEV ₁ to FVC ratio (%)	77 ± 11
PEFR (% predicted)	73 ± 19

FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; PEFR = peak expiratory flow rate.

*Expressed as means ± standard deviations unless otherwise stated. †Values recorded within 6 months prior to evaluation.

Table 2. The pattern of allergen sensitization as determined by serum specific IgE assays

Characteristics	Asthmatics	Controls
Serum logarithmic total IgE (IgE _{log})*	2.63 ± 0.59†	2.12 ± 0.75
Presence of <i>in vitro</i> IgE by RAST test		
Der p 1 (%)	137 (88)	32 (59)
Mixed cockroaches (%)	47 (30)	12 (22)
Cat (%)	24 (15)	2 (4)
Dog (%)	4 (3)	0
Mixed moulds (%)	8 (5)	0

*Expressed as means ± standard deviations. † $P = 0.00002$ between two groups by student *t*-test.

assessment, whereas they suffered from night cough for 4.9 days every month. The mean DSS in our patients was 13.2. Based on spirometric assessment of the FEV₁, 130 of the 157 asthmatic children had mild airflow limitation, whereas only one patient could be classified as having severe asthma.

Atopic status and spirometric assessment

Tables 2 and 3 describe the serum total and allergen-specific IgE measurements. The IgE_{log} in our patients was significantly higher than in non-allergic controls ($P < 0.0001$). Atopy as defined by the presence of ≥ one specific IgE to inhalants occurred in 141 (90%) patients and 32 (59%) age- and sex-matched control children (odds ratio 6.06, $P < 0.0001$). The three major environmental allergens in our hospital-based cohort of asthmatic children were house dust mite (HDM, 88%), mixed cockroaches (30%) and cat (15%). The same patterns of allergic

Table 3. Aeroallergen sensitization as risk factor for physician-diagnosed asthma compared with age- and sex-matched controls

Characteristics	Odds ratios (95% CI)	<i>P</i> -values
Atopy (≥ 1 positive specific IgE)	6.06 (2.69–13.75)	0.0000005
Specific IgE to individual aeroallergens		
Der p 1	4.71 (2.17–10.28)	0.000009
Mixed cockroaches	1.50 (0.69–3.31)	0.276
Cat	4.69 (1.02–29.81)	0.025
Dog*	NA	0.574
Mixed moulds*	NA	0.117

CI = confidence interval; NA = not available. *Odds ratios cannot be calculated by χ^2 as one of the cells is zero.

sensitization were found in the control group. Sensitization to HDM and cat were significantly more common in asthmatic children as compared with the control children (Table 3).

Association of asthma and atopy with IL-13 R130Q genotypes

The overall allele frequencies for Gln130 and Arg130 in our cohort were 0.41 (172/422) and 0.59 (250/422), respectively. There was no significant association between the R130Q polymorphism of the IL-13 gene and the diagnosis of asthma as shown in Table 4. There was no significant correlation between this polymorphism and the measured FEV₁ in our patients (104.6% vs. 96.8% vs. 98.3% for genotypes GlnGln, GlnArg and ArgArg, respectively, $P = 0.188$). This gene marker was also not associated with any other spirometric variables or clinical indicators of asthma severity outlined in Table 1 (results not shown). Table 5 summarizes the association between serum total and allergen-specific IgE levels and the R130Q genotypes. The IgE_{log} was significantly higher in asthmatic patients as well as all subjects with the GlnGln genotype ($P = 0.018$ and 0.005 , respectively). In addition, this genotype was significantly associated with sensitization to Der p 1, dog and mixed cockroaches.

Discussion

Allergic asthma is characterized by BHR to a variety of specific and non-specific stimuli, chronic pulmonary eosinophilia, elevated serum IgE and excessive airway mucus production [29]. The pathogenesis of asthma is thought to be mediated by CD4⁺ T lymphocytes that produce a type 2 cytokine profile [15]. Wills-Krap *et al.* showed that IL-13, being one of these cytokines, induced the pathophysiological features of asthma in a manner that was independent of IL-4, IgE and eosinophils [30]. IL-13 also plays a pivotal role in the regulation of IgE synthesis, especially in those with allergic asthma [15,16,31]. Several polymorphisms in the IL-13 gene, located in chromosome 5q31–33, have recently been described.

The first of these IL-13 polymorphisms was a functional one located in the promoter region of the gene and was found to be associated with allergic asthma [21]. Nonetheless, the authors did not study the relationship of this marker with serum total or allergen-specific IgE levels, which are key predispositions to asthma in children [32]. Graves *et al.* found that the Arg130Gln (R130Q) polymorphism, also resulting in a change from glutamine to arginine residues, located in the CR of IL-13 gene consistently correlated with serum total IgE concentration in three white populations living in divergent social and

Table 4. Association between asthma phenotypes and R130Q genotypes in the IL-13 gene

Phenotypes of asthma	R130Q genotypes				P-values†
	n	GlnGln (%)	ArgGln (%)	ArgArg (%)	
All physician-diagnosed asthma	157	29 (18)	74 (47)	54 (34)	0.621
with control children	54	7 (13)	26 (48)	21 (39)	
Atopic asthma*	141	28 (20)	65 (46)	48 (34)	0.406
with non-atopic asthma	16	1 (6)	9 (56)	6 (38)	
with non-atopic controls	22	2 (9)	9 (41)	11 (50)	
with all control children	54	7 (13)	26 (48)	21 (39)	

*Atopy defined as ≥ 1 positive result for allergen-specific IgE. †Comparisons made by Pearson χ^2 test.

geographical conditions [22]. Another study also showed similar results between this polymorphism and asthma, atopy and higher serum IL-13 level in British and Japanese subjects [23]. We show that this R130Q polymorphism is associated with serum total IgE, but not asthma phenotype, in the Chinese population. The present study also suggests, for the first time, that this gene marker is linked to serum concentrations of specific IgE to HDM, dog and cockroaches. Together with other association studies involving different ethnic groups, our findings support the crucial role of IL-13 in the regulation of serum IgE level and atopy.

Several previous studies suggested that there were significant interethnic differences in the allele frequencies of asthma predisposition genotypes in Asians compared with the Caucasian population [6,8,33]. With regard to the R130Q polymorphism, the Gln130 form associated with atopy could be detected in two-fifths of our Chinese subjects. This figure was intermediate between the reported

allele frequencies of 0.58 in the Japanese and 0.23–0.32 in several Caucasian populations [22,23]. Because this Gln130 form occurs commonly in subjects from diverse ethnic backgrounds, the R130Q polymorphism in the IL-13 gene can be potentially useful in the detection of subjects at risk of having elevated serum IgE or atopy. Further population studies are needed to investigate this gene marker.

Unoki *et al.* recently reported significant association between T924C polymorphism in the thromboxane A2 receptor gene and asthma in Japanese adults (≥ 18 years) but not children [34]. This observation illustrates the importance of choosing the appropriate target patients for asthma genetic studies. Epidemiological surveys suggested different risk factors for asthma in children compared with adults [35], and environmental factors such as cigarette smoking and occupational chemical exposure, in addition to atopy, may exert significant influences on the pathogenesis

Table 5. Association between R130Q genotypes in the IL-13 gene and serum total and aeroallergen-specific IgE levels

Phenotypes of atopy	Subjects	R130Q genotypes*				P-values†
		n	GlnGln	ArgGln	ArgArg	
Serum logarithmic total IgE (IgE _{log})	Patients	157	2.91 $\pm$ 0.52	2.56 $\pm$ 0.53	2.57 $\pm$ 0.66	0.018
	Controls	54	2.32 $\pm$ 0.70	2.33 $\pm$ 0.74	1.80 $\pm$ 0.70	0.038
	All subjects	211	2.79 $\pm$ 0.60	2.50 $\pm$ 0.60	2.35 $\pm$ 0.75	0.005
Serum allergen-specific IgE (by RAST classes)‡	All subjects	211	4.7 $\pm$ 1.9	3.9 $\pm$ 2.2	3.5 $\pm$ 2.3	0.021
Der p 1	Patients	157	1.1 $\pm$ 1.3	0.4 $\pm$ 0.7	0.6 $\pm$ 1.0	0.003
Mixed cockroaches	All subjects	211	0.9 $\pm$ 1.2	0.4 $\pm$ 0.8	0.5 $\pm$ 0.9	0.030
Dog	Patients	157	0.2 $\pm$ 0.7	0	0.0 $\pm$ 0.1	0.005
Dog	All subjects	211	0.2 $\pm$ 0.6	0	0.0 $\pm$ 0.1	0.003

*Results expressed as means $\pm$ standard deviations for different R130Q genotypes. †Compared using ANOVA. ‡Analyses performed for asthmatic patients and control children separately as well as for all study participants; only statistically significant results were listed.

of asthma in adults. In the latter group, it may thus be more difficult to confirm the effects of genetic factors on asthma and atopy. The present study focused on the association between a cohort of children with early onset asthma and atopy (mean age 10.3 years) and could possibly minimize the confounding effects of environmental factors on asthma and atopy in our subjects.

One major drawback of this study is that our asthmatic patients and control children were selected from a hospital cohort and this approach generally identified a more severe group of patients compared with the general population as shown by several epidemiological studies [1,10,36]. Leung *et al.* showed in a study involving 1062 schoolchildren in Hong Kong that 58% of subjects were atopic as defined using skin prick tests [36]. In contrast, atopy was found in 90% of asthmatic patients and 59% of age-matched controls in the present study. Nonetheless, the clinical and spirometric indicators of asthma severity in our asthmatic children, as outlined in Table 1, suggested that they also suffered from mild to moderate asthma. Less than one-fifth of these patients had $\leq 80\%$ FEV₁ compared with local reference values. Their mean disease severity score, validated as a useful tool in assessing the severity of asthma [25], was only 13.2 (maximum 30). Judging from these criteria, our hospital-based cohort of asthmatic patients is similar in asthma severity to the general population. Although our findings may not be generalizable to the general population, this study suggests that the R130Q polymorphism in the CR of the IL-13 gene may be an important predictor of atopy in Chinese children. Larger population-based studies are necessary to confirm the association of this genetic marker with asthma or atopy in Chinese subjects.

On the other hand, we can also argue that the power of the present case-control study to identify significant association between R130Q polymorphism and asthma might have been weakened by the fact that the majority of our patients suffered from mild to moderate asthma. This point is supported by Weir *et al.*, who showed that the severity of asthma in their recruited cohort significantly affected results on the association studies for asthma predisposition genotypes [8]. It is essential for future researches to include larger patient samples in order to demonstrate the effects of the R130Q polymorphism in the IL-13 gene on asthma and atopy phenotypes in our population.

Although an increasing number of genetic markers are linked to various phenotypic expressions of asthma and atopy [37], inherited factors alone cannot explain the marked differences in the prevalence of allergic diseases in population-based studies [1,2,38]. Among the proposed environmental factors related to asthma pathogenesis, allergen sensitization has been the most widely implicated

in susceptible persons. Exposure to HDM and cat dander was found to be associated with the development [39], exacerbation [40] and severity [41] of asthma symptoms. Our results support that sensitization to these two indoor allergens is an important risk factor for asthma in Chinese children.

In conclusion, this hospital-based study confirms that sensitization to HDM and cat is an important risk factor for childhood asthma in Hong Kong. The R130Q polymorphism in the IL-13 gene is associated with elevated serum total IgE phenotype as well as sensitization to HDM, dog and cockroaches, but not the development or severity of asthma, in our Chinese children.

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