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IL1B Promoter Haplotypes Are Associated with Spirometric Indices and Plasma Total IgE in Chinese Children

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Abstract Interleukin (IL)-1 gene family encodes for pleiotropic pro-inflammatory and anti-inflammatory cytokines. Genome screens mapped asthma phenotypes to chromosome 2q12-21 where IL-1 gene cluster is located. This study investigated the relation between asthma traits and polymorphisms at positions -31 and -511 of IL-1β gene (*IL1B*) in Chinese children. Plasma total IgE and allergen-specific IgE concentrations were measured by immunoassays. *IL1B* promoter genotypes were characterised by restriction fragment length polymorphism. One hundred and fifty-eight patients and 56 controls were recruited. Significant inter-ethnic variations in allele frequencies of *IL1B* were found between Chinese and other populations. Neither *IL1B* polymorphisms was associated with asthma. However, patients homozygous for *IL1B* -31C had lower FEV_1 (p=0.03) and FVC (p=0.008). More subjects with *IL1B* -31C/-511T haplotype had increased plasma total IgE (OR 1.61, 95%CI 1.02-2.54; p=0.04) and decreased FEV_1 (OR 1.78, 95%CI 1.06-3.02; p=0.03) and FVC (OR 1.87, 95%CI 1.09-3.22; p=0.02). In conclusion, *IL1B* promoter polymorphisms are associated with poorer lung function and increased plasma total IgE concentration in Chinese children.

Key words Asthma; Atopy; Chinese; Interleukin-1β gene; Spirometry

Introduction

Asthma is characterised by chronic airway inflammation caused by a complex interaction between genetic and environmental factors. A key element in the inflammatory response of the asthmatic airways is the production of pro-inflammatory cytokines, notably interleukin (IL)-1β, and to a lesser extent, tumour necrosis factor-α.[1] The human airway smooth muscle releases IL-1β, which, together with IL-5 from type 2 helper T lymphocytes, modulates bronchial hyperresponsiveness (BHR).[2] On the other hand, IL-1Ra possesses anti-inflammatory properties, and inhibits *in vivo* BHR to histamine and airway inflammation in sensitised animals.[2] The imbalance between pro- and anti-inflammatory cytokines, especially IL-1β and IL-1Ra, might thus be an important determinant of asthma.[3,4] Genome-wide searches mapped asthma and atopy to a number of chromosomal regions,[5-10] including chromosome 2q12-21 where the genes encoding IL-1α (*IL1A*), IL-1β (*IL1B*) and IL-1 receptor antagonist (IL-1Ra, *IL1RN*) were located.[11] These genetic and functional characteristics of IL-1 support the candidacy of IL-1 cluster as an asthma susceptibility locus. Using candidate gene approach, *IL1RN* polymorphisms were associated with various asthma and atopy phenotypes.[3,12,13] Interestingly, a gender-specific effect was seen between *IL1B* -511 polymorphism and

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asthma susceptibility in Finnish adults.[13] On the other hand, the effects of different polymorphic sites in *IL1B* on asthma severity and atopy have not been studied. The aim of this study is to investigate the correlation between *IL1B* polymorphisms at positions -31 and -511 and physician-diagnosed asthma, atopy and spirometric parameters of asthma severity in Chinese children.

Patients and Methods

Study Population

This case-control study recruited children aged 5 years or above, with asthma as diagnosed according to the criteria proposed by the American Thoracic Society[14] and onset of disease before 15 years of age, who were followed in the general paediatric clinic of a university teaching hospital in Hong Kong. Both parents were ethnic Chinese by self-reporting. In brief, these patients had typical asthma symptoms that were relieved by bronchodilators and the presence of reversibility and/or hyperreactivity on spirometry. Controls were selected among children attending the hospital for non-allergic and non-immunologic diseases. Subjects or their parents gave informed written consent, and the Clinical Research Ethics Committee of our university approved this study.

Plasma IgE Measurements

Plasma total IgE concentration was measured by micro-particle immunoassay (IMx analyser, Abbott Laboratories, Abbott Park, IL), and results were expressed following logarithmic transformation (IgE_{log}). Specific IgE antibodies to locally prevalent allergens[15,16] *D. pteronyssinus*, cat, dog, mixed cockroaches and mixed molds were measured by fluorescent enzyme immunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden), with concentration ≥0.35 kIU/l being positive. Atopy was defined as the presence of at least one type of allergen-specific IgE.

Spirometry

All asthmatic patients underwent spirometry (COMPACT II, Vitalograph, Buckingham, England) to measure their lung functions.

PCR Assays for IL1B Polymorphisms

Genomic DNA was extracted from peripheral venous blood using High Pure Viral Nucleic Acid Kit (Boehringer Mannheim, Indianapolis, IN). *IL1B* polymorphisms at -31 and -511 were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism.[3,17,18] Table 1 describes the PCR primers and assay conditions used in this study. In particular, we created a *Hae* III restriction site at position -31 (1903 bp of the complete DNA sequence) in *IL1B* promoter with the use of a pair of novel mismatched primers: 5'-CTC CTA CTT CTG CTT TTG AAG GC-3' and 5'-GAG CAA TGA AGA TTG GCT GA-3' (GenBank accession number X04500).[19,20] The final PCR products were digested using appropriate restriction enzymes (New England Biolabs, Hitchin, Herts, UK) and visualised on agarose gel containing ethidium bromide.

Restriction enzyme digestions were performed each time in a 96-well plate containing 88 samples and 8 positive controls with PCR products of known genotypes. Ten random samples from each *IL1B* genotype were also sequenced using BigDye Terminator Cycle sequencing kits and ABI-310 autosequencer (Applied Biosystems, Foster City, CA). All genotypes from restriction enzyme digestion were confirmed.

Table 1 PCR primers, restriction enzymes, assay conditions, PCR products and allele frequencies for the *IL1B* polymorphisms

Poly-morphism	PCR primers	$MgCl_2$ (mmol/L)	PCR hybridisation temperature (°C)	Restriction enzymes (units)	Alleles	Size of fragments (bp)	Allele frequency*
IL1B -31	*Forward*:						
	5'-CTC CTA CTT CTG CTT TTG AAG GC-3'	0.75	65.0	*Hae* III (5)	T	124	0.49
	Reverse:						
	5'-GAG CAA TGA AGA TTG GCT GA-3'				C	22 and 102	0.51
IL1B -511	*Forward*:						
	5'-GCC TGA ACC CTG CAT ACC GT-3'	0.5	65.0	*Ava* I (5)	C	88 and 67	0.48
	Reverse:						
	5'-GCC AAT AGC CCT CCC TGT CT-3'				T	155	0.52

*Pooled frequencies for all 214 subjects in this study.

Statistical Analysis

The clinical phenotypes of asthma and atopy between patients and control subjects were compared using χ^2 or Fisher Exact test. The distributions of different genotypes in the two polymorphic markers between asthmatics and controls were compared using χ^2. The clinical, laboratory and spirometric variables between various subject or genotype groups were compared by Student *t* test or ANOVA as appropriate. Linkage disequilibrium between the two polymorphic markers was assessed by Haploview (Daly Lab, Cambridge, MA). Two-locus haplotype frequencies for *IL1B* -31 and -511 were maximum likelihood estimates (EH program, Laboratory of Statistical Genetics at Rockefeller University, New York, NY). All comparisons were made two-sided. A p-value <0.05 was considered to be significant.

Results

Clinical Characteristics

One hundred fifty-eight asthmatic children and 56 non-allergic control subjects were recruited. Table 2 summarises the clinical characteristics of our asthmatic patients. Their mean±SD ages at evaluation were 10.2±3.6 years and 11.1±4.0 years respectively (p=0.121), with 61% of patients and 59% of controls being males (p=0.746).

Plasma Total and Allergen-specific IgE Concentrations

The mean±SD of IgE_{log} in kIU/l in asthmatic patients was significantly higher than in non-allergic controls (2.62 ±0.60 *vs* 2.12±0.74, p<0.0001). Table 3 summarises the pattern of allergic sensitisation. Atopy was found in 141 (90%) of patients and 33 (59%) of control children.

Table 2 Patient characteristics and indicators of their asthma control

Characteristics	Results*
Demographics	
Age at evaluation (years)	10.2 ± 3.6
Sex (male : female)	97 : 61
Clinical indicators of asthma severity†	
Number of asthma attacks	2.0 ± 1.7
Number of hospitalisation related to asthma	0.3 ± 0.7
Number of unscheduled outpatient visit	1.6 ± 2.1
Number of patients using inhaled corticosteroid (%)	94 (59)
Spirometric assessment	
FEV_1 (% predicted)	98 ± 19
FVC (% predicted)	119 ± 22
FEV_1/FVC ratio (%)	78 ± 11

FEV_1, forced expiratory volume in one second; FVC, forced vital capacity.
*Expressed as means ± standard deviations unless otherwise stated.
†Values recorded within 6 months prior to evaluation.

Table 3 The pattern of allergic sensitisation to inhalant allergens in our subjects*

Characteristics	Patients* n (%)	Controls n (%)	OR (95% CI)
Elevated plasma total IgE concentration**	127 (80)	28 (50)	4.23 (2.09-8.63)†
Positive allergen-specific IgE			
D. pteronyssinus (%)	138 (88)	33 (59)	5.06 (2.33-11.04)†
Mixed cockroaches (%)	45 (29)	12 (21)	1.47 (0.68-3.25)
Cat (%)	23 (15)	2 (4)	4.63 (1.01-29.49)‡
Dog (%)	4 (3)	0	NA
Mixed molds (%)	9 (6)	0	NA
Atopy (≥1 positive specific IgE)	141 (90)	33 (59)	6.14 (2.75-13.82)†

CI, confidence interval; n, number of subjects; NA, not available; OR, Odds ratio.
*Plasma samples not available from one patient for allergen-specific IgE.
**Compared with the in-house upper limits of age-matched references (120 kIU/l for 5-year-old; 160 kIU/l for 6- and 7-year-old; 180 kIU/l for ≥8-year-old).
†p<0.001; ‡p<0.05.

Sensitisation to *D. pteronyssinus* and cat were significant risk factors for asthma diagnosis in our subjects.

IL1B Polymorphisms and Asthma or Atopy Phenotypes

The allele frequencies of *IL1B* polymorphic markers are listed in Table 1, and the allele-genotype distributions of polymorphic markers -31 and -511 followed Hardy-Weinberg equilibrium (p=0.395 and 0.409, respectively). *IL1B* -31 and -511 were in strong linkage disequilibrium (D'=0.971; r^2=0.891), with -31T being linked to -511C and vice versa. There was no significant association between the diagnosis of asthma, whether classified as atopic or nonatopic, and polymorphisms in *IL1B* (results not shown). Neither were these polymorphisms associated with plasma total IgE concentration or the presence of any allergen-specific IgE in this study (results not shown).

IL1B Polymorphisms and Spirometric Variables

Table 4 summarises the relationship between spirometric variables in our patients and *IL1B* polymorphisms. There was significant correlation between *IL1B* -31 polymorphism and the measured FEV_1 (104% *vs* 99% *vs* 93% for wild-type, heterozygous and mutant respectively, p=0.03) and FVC (127% *vs* 118% *vs* 113%, p=0.008).

Association between Clinical Phenotypes and IL1B Haplotypes

Table 5 summarises the results of haplotype analysis for *IL1B* -31 and -511 polymorphisms. Significantly more subjects with mutant alleles at both loci (-31C/-511T) had increased plasma total IgE concentrations (OR 1.61, 95%CI 1.02-2.54; p=0.04) and reduced FEV_1 (OR 1.78, 95%CI 1.06-3.02; p=0.03) and FVC (OR 1.87, 95%CI 1.09-3.22; p=0.02) as compared to those with wild-type alleles (-31T/-511C). However, *IL1B* promoter haplotype was not associated with a diagnosis of asthma or atopy.

Discussion

The present study shows that asthmatic patients homozygous for -31C in *IL1B* promoter have poorer lung function (FEV_1 and FVC) as compared to other genotypes. On the other hand, *IL1B* -511 is not associated with asthma phenotypes, plasma IgE or lung function in our Chinese children. Haplotype analysis confirms the association between *IL1B* -31/-511 and spirometric variables. This *IL1B* promoter haplotype is also associated with plasma total IgE concentration, with subjects having -31C/-511T have increased total IgE.

The pathogenesis of asthma is mediated by $CD4^+$ T lymphocytes that produce a type 2 cytokine profile.[1,21] The surge in these interleukins, in the presence of pro-inflammatory cytokines such as IL-1β, IL-6 and tumour necrosis factor-α, results in airway inflammation and BHR.[1,2] The imbalance in pro-inflammatory cytokine IL-1β and anti-inflammatory IL-1Ra could be seen in patients with severe asthma.[4] Genome-wide screens have also mapped asthma to chromosome 2q12-21 containing the IL-1 gene cluster.[5,7,9] Polymorphisms in this gene cluster were associated with asthma susceptibility in the Caucasian population.[12,13] One of the objectives of this study is thus to investigate the usefulness of *IL1B* polymorphisms in predicting asthma severity in Chinese children. To the best of our knowledge, this study shows for the first time that *IL1B* polymorphism is linked to FEV_1 and FVC (i.e. asthma severity). Haplotype analysis of *IL1B* T-31C and C-511T confirmed this association. Our results also reveal that the haplotype with mutant alleles at both -31 and -511 is associated with increased plasma total IgE concentrations whereas genotype analyses for each of these polymorphic sites were negative. Haplotype analysis is thus a more powerful method than single nucleotide polymorphism in identifying susceptibility loci for asthma phenotypes.

Table 4 Relationship between *IL1B* polymorphisms and spirometric variables

Polymorphism	Genotypes	n	%FEV_1	p-values*	%FVC	p-values*
IL1B -31	Wild-type (T/T)	43	104 ± 25	0.030	127 ± 25	0.008
	Heterozygous	71	99 ± 16		118 ± 21	
	Mutant (C/C)	44	93 ± 17		113 ± 18	
IL1B -511	Wild-type (C/C)	40	102 ± 24	0.245	126 ± 25	0.076
	Heterozygous	71	99 ± 15		118 ± 21	
	Mutant (T/T)	47	95 ± 19		115 ± 20	

n: number of subjects with that genotype.

*Analysed by ANOVA or student *t* test as appropriate.

Table 5 Association between *IL1B* -31 and -511 haplotypes and different asthma or atopy phenotypes

Clinical phenotype	*IL1B* haplotype	Haplotype frequencies in subject groups		OR (95% CI)	p-values with Yate's correction
Physician-diagnosed		Asthma (n=158)	Control (n=56)		
asthma	-31T/-511C	0.47	0.46	1.00	
	-31T/-511T	0.03	0.01	2.79 (0.34-60.92)	0.55
	-31C/-511C	0.01	0.01	0.70 (0.05-19.87)	0.71
	-31C/-511T	0.50	0.52	0.94 (0.60-1.50)	0.89
Atopic status		Atopy (n=174)	No atopy (n=39)		
	-31T/-511C	0.46	0.51	1.00	
	-31T/-511T	0.02	0.01	2.05 (0.25-44.92)	0.80
	-31C/-511C	0.01	0.01	0.51 (0.04-14.65)	0.87
	-31C/-511T	0.51	0.46	1.23 (0.73-2.08)	0.48
Plasma total IgE levels		Increased* (n=155)	Normal (n=59)		
	-31T/-511C	0.44	0.56	1.00	
	-31T/-511T	0.03	0.01	3.91 (0.48-85.09)	0.32
	-31C/-511C	0.01	0.01	0.98 (0.07-27.76)	0.55
	-31C/-511T	0.53	0.42	1.61 (1.02-2.54)	**0.04**
FEV_1		Lower 40%† (n=63)	Upper 40% (n=63)		
	-31T/-511C	0.42	0.54	1.00	
	-31T/-511T	0.01	0.05	0.20 (0.01-1.74)	0.22
	-31C/-511C	0.01	0.01	1.19 (0.0-44.65)	0.55
	-31C/-511T	0.57	0.41	1.78 (1.06-3.02)	**0.03**
		Lower 40%† (n=63)	Upper 40% (n=63)		
FVC	-31T/-511C	0.40	0.54	1.00	
	-31T/-511T	0.02	0.05	0.46 (0.06-2.68)	0.56
	-31C/-511C	0.02	0	NA	0.92
	-31C/-511T	0.57	0.41	1.87 (1.09-3.22)	**0.02**

CI, confidence interval; NA, not applicable; OR, Odds ratio.

*Compared with the in-house upper limits of age-matched references.

†Analysed between patients with spirometric variables (FEV_1 or FVC) lying in the lower 40% and the upper 40% of all measured values.

Our asthmatic patients carrying the C allele at *IL1B* -31 had significantly lower FEV_1 compared to those with T allele. Interestingly, this restriction site at *IL1B* -31 is located at the TATA box that is essential for normal gene transcription.[19] Thus, this single-base substitution probably results in diminished *IL1B* transcription. Because IL-1β is pro-inflammatory,[1,2] this mutation would theoretically decrease airway inflammation and lead to better performance on spirometric measures that contradicts results obtained in this study. One possible explanation is that *IL1B* T-31C and C-511T, or other polymorphisms in close linkage, may alter the production of both IL-1β and IL-1Ra. As reported also by Tillie-Leblond et al., these changes can upset the balance between IL-1β and IL-1Ra that in turn serves as an important mechanism in the pathogenesis of asthma.[4] The ability of leukocytes to produce IL-1β and IL-1Ra from subjects having different *IL1B* -31 (and -511) polymorphisms is not tested in the present study. Further studies should try to characterise any functional consequence associated with T→C substitution at position -31 of *IL1B*, and to delineate the relationship between *IL1B* haplotypes and plasma concentrations of IL-1β and IL-1Ra.

DPP10, encoding a homolog of dipeptidyl peptidases that cleave terminal dipeptides from cytokines and chemokines, has recently been identified as a novel gene influencing asthma.[22] In this study, Allen and colleagues initially investigated the association between asthma and polymorphisms in the IL-1 gene cluster. Although such association could not be detected, they observed that asthma susceptibility in these subjects was highly significantly associated with the microsatellite *D2S308* in the

neighbouring region. Using positional cloning on a comprehensive, high-density, single-nucleotide polymorphism linkage disequilibrium map, the authors identified *DPP10* as a novel asthma candidate gene. Interestingly, this gene is also located on chromosome 2q14, about 800 kb distal to the IL-1 cluster, which harbors various promising candidate genes that mediate inflammatory responses.[11,23-25] Thus, it is possible that our observed associations between asthma-related traits and *IL1B* polymorphisms may be due to the linkage with *DPP10* or other candidate genes in this region. Future studies should extend our findings by including the genotyping of other polymorphic markers in the adjacent region of *IL1B* and its gene cluster.

Our research group as well as others reported significant inter-ethnic variations in the allele frequencies of a number of asthma candidate genes in Chinese as compared with Caucasians or even Japanese.[26-32] With regard to *IL1B* polymorphisms (Table 1), the frequency of -511C was detected in 48% of our subjects that is similar to those in Japanese and Caucasian[3,18,20] whereas -31T was present in 49% of our Chinese children and 38% of Caucasians.[20] Thus, results of Caucasian genetic studies may not be generalisable to the oriental population. Population-based studies are needed to investigate the allele frequencies and clinical importance of our *IL1B* markers in Chinese subjects.

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