

Original article

The polymorphisms of interleukin 17A (*IL17A*) gene and its association with pediatric asthma in Taiwanese population

Background: The interleukin 17A (*IL17A*) gene, located on chromosome 6p and linked to asthma phenotype, is a highly potential candidate gene conferring asthma susceptibility. The purpose of this study was to investigate the genetic association between single nucleotide polymorphisms (SNPs) of *IL17A* and asthma in Taiwanese children.

Methods: We selected and performed genotyping on nine SNPs that encompass the genomic region of *IL17A* in Taiwanese children with or without asthma. A total of 1939 subjects containing 1027 subjects in testing group and 931 subjects in validation group were recruited in this study.

Results: After Bonferroni correction, SNP rs8193036 was found to have a weak association ($P = 0.0074 \times 9 = 0.066$) in genotype frequency test. This association was confirmed by validation group. Logistic regression adjusted allergy comorbidity and gender showed a slightly weaker association.

Conclusions: The results indicated an independent role of *IL17A* promoter polymorphism rs8193036 in the association with pediatric asthma in Taiwanese population.

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Chronic asthma is a complex disease affecting nearly 300 million individuals worldwide (1). The rising incidence of asthma and atopic disorders over the past decades attests to the importance of environmental and lifestyle factors in disease risk assessment (2–4). Strong genetic components associated with asthma were supported by family and twin studies (5, 6), and many genes have been identified or suspected to be involved in the pathogenesis of asthma (7). In Taiwan, there are slight differences in the reported symptoms of allergic diseases; nevertheless, the prevalence of allergic diseases is rising (8–10).

Interleukin 17A (*IL17A* or known as *IL-17*), the key cytokine of T_H17 cells, is known to induce pro-inflammatory cytokines, the hallmarks of acute inflammatory processes (11). Experimental models suggested that T_H17 cells may be important for neutrophilic inflammation in acute airway inflammation (12–14). In obstructive airway diseases, such as bronchial asthma and chronic obstructive pulmonary disease, accumulation of neutrophils in the airways has been a major characteristic (15, 16). Elevation plasma *IL17A* level associated with asthma severity (17) suggests the potential role in airway remodeling. The accumulated evidence suggests that *IL17A* may play an important pathologic role in the development of allergies and asthma (18). However, there are no reports

on the association of *IL17A* gene polymorphisms with asthma.

Chromosome 6p of which the genomic region has been reported to be linked to asthma and asthma-related phenotypes in multiple genome scans (19–21). *IL17A* is located on 6p12.1 the genomic region of which was reported to be associated with different types of asthma (21) but not on 6p21–23 region revealed by linkage studies on asthma (19, 20). Previously, researchers reported that *IL17A* is a good candidate gene for studying genetic susceptibility of asthma. In our study, we investigated the association between selected single nucleotide polymorphisms (SNPs) of *IL17A* and asthma and validated the results in another independent subject group. The results strongly indicate that polymorphism in *IL17A* promoter region is associated with pediatric bronchial asthma in Taiwanese population.

Materials and methods

Sample composition and clinical evaluation

Our study population consists of asthmatic children with their age ranging from 5 to 12 years. The study protocol was approved by the Ethical and Clinical Trial Committee of National Cheng-Kung

University Hospital and Mackay Memorial Hospital. Informed consent form was required from all participants or their guardians after they answered a modified British Medical Society respiratory questionnaire, which is the same as that of the European Community Respiratory Health Survey. These surveys have the similar validity as ISAAC pertinent to the diagnosis and assessment of asthma (22, 23). Pulmonary function was tested using standard methods including spirometry before and after the administration of two puffs of inhaled salbutamol (200 µg/puff). The definition of asthma must meet the following criteria: (i) history of having wheezing and experiencing short of breath during or without concurrent respiratory infections, (ii) chronic coughing for more than 1 month and being diagnosed by physician of the presence of wheezing episode(s) and (iii) bronchodilator test has confirmed the positive response of increased 15% of Forced Expiratory Volume in the first second (FEV1). Nonasthma controls were defined as neither with asthma history as above criteria (i) nor diagnosed as criteria (ii). Other evaluations included skin prick tests for responsiveness to six common aeroallergens, a differential blood count (including total eosinophil count), and levels of total serum immunoglobulin E (IgE), as well as IgE specific to house dust and mixed pollens using Unicap system (Pharmacia Diagnostic, Uppsala, Sweden). A positive skin test was defined as the presence of ≥ 1 reaction with a wheal diameter ≥ 5 mm. Total serum IgE was measured by solid-phase immunoassay (Pharmacia IgE EIA; Pharmacia Diagnostics). Nonallergy subjects were defined as having a total serum IgE < 200 and with negative skin test. The subjects group 1 (1027 subjects) were recruited from National Cheng-Kung University Hospital in the periods of 2002–2004. The 931 subjects in the validation group (group 2) were recruited from MacKay Memorial Hospital and National Cheng-Kung University Hospital during 2005–2006. All study subjects are Han-Taiwanese and living in Taiwan.

DNA preparation

Genomic DNAs were extracted from blood samples of the study subjects using QIAamp DNA Blood kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The extracted genomic DNAs were analyzed by agarose gel electrophoresis, quantified by spectrophotometer, and stored at -80°C until use.

SNP selection and genotyping

The tagging SNPs (tSNPs) of *IL17A* genomic region and upstream 1500 base pairs were selected according to Seattle SNPs website (<http://pga.mbt.washington.edu/education.html>). The Seattle SNPs database showed 12 polymorphisms [minor allele frequency (MAF) ≥ 0] in our target region. According to Han-Chinese Beijing data, nine tSNPs were selected (minimum $R^2 = 0.8$) from the 12 polymorphisms.

All SNP genotypings were performed using the Taqman[®] SNP genotyping assays (ABI: Applied Biosystems Inc., Foster City, CA, USA). The primers and probes of selected SNPs were from ABI assay on demand kit. Reactions were carried out according to the manufacturer's protocol. The probe fluorescence signal detection was performed using the ABI Prism 7900 Real-Time PCR System.

Statistical analysis

Quality of the genotype data was evaluated by the Hardy-Weinberg equilibrium (HWE) proportion tests. Inter-marker linkage disequilibrium (LD) measures, r^2 and D' , were estimated and haplotype blocks defined using the Haploview program ([http://www.broad.](http://www.broad.mit.edu/mpg/haploview/)

[mit.edu/mpg/haploview/](http://www.broad.mit.edu/mpg/haploview/)). All single-point association analyses were carried out using the SAS/genetics package (SAS Inc. Cary, NC, USA). Single nucleotide polymorphism(s) showing significant association ($P \leq 0.05$) in the tests were further evaluated using logistic regressions adjusted with allergy in odds ratio (OR) analysis.

Results

Characteristics of study subjects

One thousand twenty-seven DNA samples extracted from 546 nonasthma children and 481 asthmatic children were collected as group 1 and genotypings were performed on all selected SNPs. Group 2 subjects (including 729 asthmatic children and 202 controls), as validation group, were recruited from two different hospitals and only asthma status and total serum IgE by fulfilling earlier described criteria were evaluated. In two study groups, the asthma subjects had a higher level of total serum IgE than nonasthma subjects [comparing $\ln(\text{IgE})$, t -test, $P < 0.0001$]. According to allergy phenotype, 1027 subjects in group 1 were further divided into four subgroups, 380 allergic asthma, 101 nonallergic asthma, 250 allergy subjects without asthma, and 296 nonallergy and nonasthma subjects. In group 1, the proportion of allergy was significantly greater in asthma group than in the nonasthma group [380/481 vs 250/546; $P < 0.0001$; OR = 4.45; 95% confidence interval (CI) 3.38, 5.89].

The mean of ages of initial diagnosis from asthma children and control was 8.11 ± 2.97 and 8.37 ± 2.45 , respectively. There was no significant difference between the two groups ($P = 0.214$, t -test). The ratio of male/female gender from asthma children and control was 321/160 and 254/289, respectively. There was a significant difference in gender between the two groups ($P < 0.0001$, chi-squared test).

The SNP rs8193036 in *IL17A* promoter region associated with asthma

Except rs1974226, genotype distributions of other polymorphic SNPs did not deviate from the HWE either in asthma subjects or in nonasthma subjects. Linkage disequilibrium of the polymorphic SNPs of the *IL17A* genes is listed in Fig. 1. One haplo-block including five SNPs was identified at the *IL17A* gene.

Strengths of associations and genotype frequencies of all polymorphic SNPs with asthma are summarized in Table 1. One SNP rs8193036 was significantly associated with asthma ($P = 0.0074$, genotype test). Although there is no single SNP displaying statistically significant difference after Bonferroni correction ($P < 0.05/9 = 0.0055$), the SNP rs8193036 showed marginally significant association with asthma ($P = 0.0074 \times 9 = 0.067$) after Bonferroni correction.

To confirm and validate the results described above, genotyping on the association SNPs for the group 2

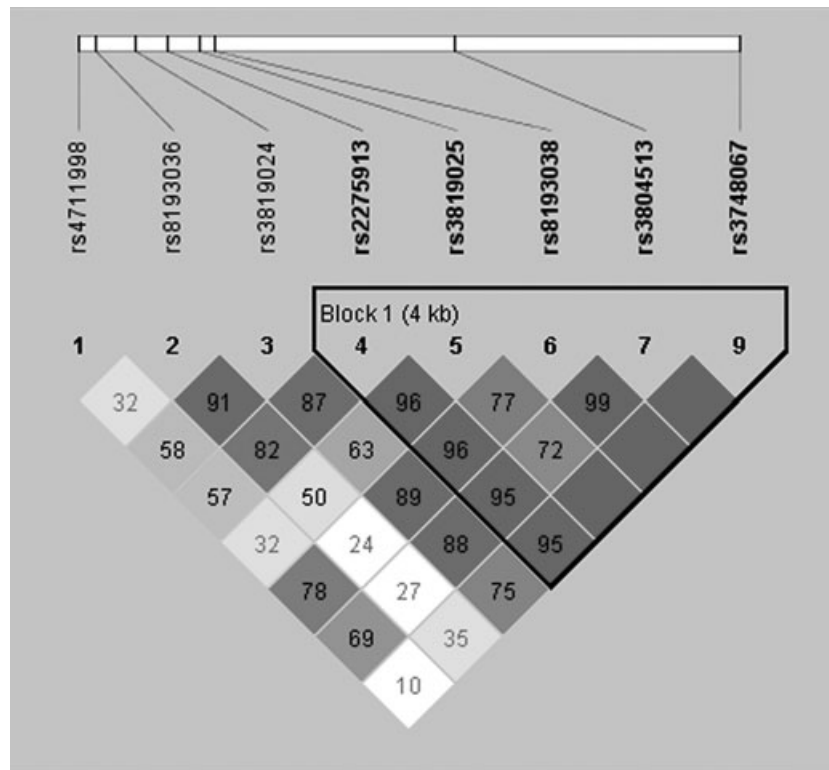


Figure 1. Linkage disequilibrium plot in D' demonstrating adjacent strength between SNP-pairs at the IL17A gene. D' values are multiplied by 100, e.g. 77 in the square at the bottom means a D' of 0.77. Square without a number has a value of 100 that equals to a D' of 1.

Table 1. Chi-squared test result of genotyping for IL17A testing SNPs

SNP ID	Location	Allele 1/2	Case 11/12/22	Control 11/12/22	<i>P</i> -value
rs4711998	-832*	A/G	275/173/30	290/210/33	0.58
rs8193036	-692*	C/T	285/151/36	273/220/43	0.0074
rs3819024	-399*	A/G	103/223/143	115/263/138	0.41
rs2275913	-152*	A/G	110/234/129	122/251/141	0.98
rs3819025	Intron_1	A/G	18/132/328	15/154/363	0.67
rs8193038	Intron_1	A/G	379/95/6	429/106/2	0.30
rs3804513	Intron_2	A/T	370/103/6	412/123/3	0.45
rs1974226	3' UTR	A/G	11/48/412	9/50/466	0.73
rs3748067	3' UTR	A/G	381/92/6	404/118/5	0.43

SNPs, single nucleotide polymorphisms, UTR, untranslation region.

*The distance from start position of IL-17A mRNA (NM_002190.2).

subjects was performed. Genotype distributions of rs8193036 in group 2 did not deviate from the HWE either in asthma subjects or in nonasthma subjects. It showed that SNP rs8193036 displayed statistical significance in group 2. The validation also showed that rs8193036 is statistically significant in the comparison with total subjects (Table 2). The OR analysis showed that the risk genotype of rs8193036 was CC and was consistent in group 1, group 2 and total subjects (Table 3). In group 1 subjects, effects of rs8193036 on asthma were further adjusted for the influence of allergy comorbidity and gender by logistic regression. As shown

Table 2. Genotype frequencies, proportions and statistical analyses of two independent subject groups for SNPs rs8193036

Genotype	Group 1		Group 2		Total	
	Case	Control	Case	Control	Case	Control
CC (%)	285 (60)	273 (51)	455 (62)	107 (53)	740 (62)	380 (51)
CT (%)	151 (32)	220 (41)	238 (33)	78 (39)	389 (32)	298 (40)
TT (%)	36 (8)	43 (8)	36 (5)	17 (8)	72 (6)	60 (8)
χ^2	9.6868		7.3560		23.5023	
<i>P</i> -value	0.0074		0.0253		<0.0001	

SNPs, single nucleotide polymorphisms.

in Table 3, the strength of associations was slightly diluted by allergy comorbidity and gender. Group 2 subjects and total subjects were not further adjusted for allergy and gender because group 2 subjects were recruited from two different hospitals, which did not use consistent allergy evaluation and gender information was not recorded completely.

Discussion

In the current study, we investigated the association between *IL17A* polymorphisms and pediatric asthma. We found that association between asthma phenotype and

Table 3. Odds ratio analyses of two independent subject groups for SNPs rs8193036

Genotype	Group 1		Group 2		Total	
	Case	Control	Case	Control	Case	Control
CC	285	273	455	107	740	380
CT + TT (ref.)	187	263	274	95	461	358
OR (95% CI)	1.47 (1.14, 1.89)		1.47 (1.08, 2.02)		1.55 (1.29, 1.87)	
P-value	0.0026		0.0152		<0.0001	
OR (95% CI) adjust with allergy*	1.36 (1.04, 1.77)					
OR (95% CI) adjust with sex*	1.36 (1.05, 1.76)					

SNPs, single nucleotide polymorphisms; case, asthma subjects; control, nonasthma subjects; OR, odds ratio; CI, confidence interval.

*SNP was coded as a categorical variable, 1 for CC, and 0 for others.

IL17A polymorphism rs8193036 was significant before Bonferroni correction in group 1 subjects. After Bonferroni correction, it revealed that rs8193036 is marginally significantly associated with asthma. The association between asthma phenotype and rs8193036 was further validated using group 2 subjects and still showed a significant association. The results of this study indicated a possibility that the polymorphisms of *IL17A* gene may confer risk for pediatric asthma in Taiwanese population.

The SNP rs8193036 is located at position -692 from the starting site of mRNA and -737 from the start codon of *IL17A*. Another *IL17A* promoter SNP rs2275913 is located at position of -197 from the start codon and associated with ulcerative colitis (24). The SNP 3804513, in *IL17A* intron 2, has been reported to be associated with radiographic progression in Japanese patients with early rheumatoid arthritis (25). No nonsynonymous SNP of *IL17A* was reported in NCBI SNP database suggesting that regulatory polymorphism(s) of *IL17A* may play a role in pathophysiological processes of related diseases.

IL17A is significantly expressed in sputum samples from patients with asthma compared with control subjects (17, 26–28) and its level in sputum of patients with asthma correlated negatively with the provocative concentration of methacholine causing a 20% fall in FEV1 (27). *IL17A* is able to induce the expression of two mucin genes in bronchial epithelial cells (29), and an increased expression of *IL17A* is associated with enhanced mucin gene expression *in vivo* (30). The above observations suggest that *IL17A* expression level and the

factors that influence *IL17A* expression are strong candidates for asthma susceptibility factors.

In group 1, the SNP rs8193036 only showed a marginally significant association with asthma ($P = 0.067$) after Bonferroni correction. The power to detect significant association was calculated by Power for Association With Errors (PAWE; <http://linkage.rockefeller.edu/pawe/>) (31, 32). For example, rs8193036 in group 1, given 472 cases and 536 controls, adjust P -value = 0.066 and data without error, the power for genotypic test was 0.65. Under the same condition of ratio and allele frequencies in case and control, 726 case subjects and 820 controls are necessary to detect $P = 0.05$ and power = 0.8. The SNP rs8193036 in group 2, given 729 cases and 202 controls, $P = 0.0253$, and data without error, the power for genotypic test was 0.59. The calculation data suggested that our sample size in group 1 and group 2 may not be large enough to detect an association for *IL17A* effect on asthma. In total subjects including 1201 cases and 738 controls, $P < 0.0001$, the power for genotypic test was 0.96. The sample size pooling two study groups should be large enough to detect the association for rs8193036 and asthma.

From the results in this study, it may be suggested that *IL17A* is a candidate gene that confers the genetic susceptibility for pediatric asthma in Taiwanese population. Furthermore, the results provided a genetic basis indicating that the expression regulation was involved in asthma pathological mechanism. To understand further the functions and mechanisms of the associated SNPs in regulating *IL17A* expression demands further investigations.

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