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Significant Association of Chitinase 3-like 1 Genotypes to Asthma Risk in Taiwan

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Abstract. *Background/Aim: Chitinase 3-like 1 (CHI3L1) is overexpressed in asthma, and negatively associated with forced expiratory volume in the first second. This study aimed at evaluating whether CHI3L1 genotypes affect asthma risk. Materials and Methods: The blood samples of 198 asthma patients and 453 control subjects were collected, and the genotypic patterns of CHI3L1 -131C/G (rs4950928) and -247G/A (rs1262491437) were examined. Results: The percentages of CG and GG at CHI3L1 -131C/G were 32.8% and 7.6% among the asthma cases, respectively, significantly higher than the 23.8% and 3.1% among the non-asthmatic healthy subjects (p for trend=0.0009). The allelic frequency distribution analysis showed that the G allele at CHI3L1 -131C/G conferred a significantly higher asthma risk than the wild-type C allele (p<0.0001). The genotypic and allelic frequency analyses for CHI3L1 -247G/A did not show any significant difference. Conclusion: The G allele at CHI3L1 -131C/G serves as a biomarker in determining personal susceptibility to asthma in Taiwan.*

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Asthma has a complex etiology, including chronic airway inflammation, bronchial hyper-responsiveness, and various degrees of airway obstruction (1). The most recent study has indicated that the global incidence of asthma has been 43.12 million new cases/year (0.56%), while global prevalence and mortality accounted for 272.68 million cases (3.57%) and 0.49 million deaths (0.006%), respectively, in 2017 (2). The number is suggested to be increased to 400 million by 2025 and is a threat to our health (3). Although the exact etiology of asthma is still unrevealed, it is believed to be caused by specific interaction between genomic and environmental factors (4, 5).

The *Chitinase 3-like 1* (*CHI3L1*) gene is located on chromosome 1q32.1 (6) and encodes YKL-40 (7). As early as 2008, a genome-wide association study (GWAS) has identified *CHI3L1* as an asthma susceptibility gene with the evidence that its genotype may associate with airway hyper-responsiveness and decline in lung function in a Caucasian population (8). YKL-40 has been found to be pro-inflammatory and is released by activated human macrophage (9), vascular smooth muscle cells (10), and neutrophils (11). YKL-40 protein is involved in the Th2 cell mediated inflammatory pathway, tissue remodeling and fibrosis (6, 12) and may associate with the etiology of asthma. The incidence of asthma may vary in different countries and ethnicities. Since polymorphic sites in the promoter region may closely determine the expression levels of *CHI3L1*, we aimed to examine the association of the *CHI3L1* -131C/G (rs4950928) and -247G/A (rs1262491437) variants with adult asthma in Taiwan.

Materials and Methods

Collection of case and control groups. A total of 198 patients with asthma were recruited at the China Medical University Hospital

Table I. *Distributions of age and gender among the 198 asthma patients and the 453 matched controls.*

Characteristics	Controls (n=453)		Patients (n=198)		*p*-Value[a]
	n	%	n	%	
Age (years)					
25~40	285	63.4%	133	67.2%	
>40	168	36.6%	65	32.8%	0.3286
Gender					
Male	190	41.9%	83	41.9%	
Female	263	58.1%	115	58.1%	0.9956
Smoking habits					
Yes	48	10.6%	37	18.7%	
No	405	89.4%	161	81.3%	0.0048*

[a]Based on Chi-square test; *$p<0.05$, Statistically identified as significant.

Table II. *Distribution of CHI3L1 -131C/G genotypes among asthma patients and controls.*

-131C/G	Controls		Patients		OR (95%CI)	*p*-Value[a]
	n	%	n	%		
Genotype						
CC	331	73.1%	118	59.6%	1.00 (reference)	
CG	108	23.8%	65	32.8%	1.69 (1.16-2.45)	0.0056*
GG	14	3.1%	15	7.6%	3.00 (1.41-6.41)	0.0030*
P_{trend}						0.0009*
Carrier analysis						
CC+CG	439	96.9%	183	92.4%	1.00 (reference)	
GG	14	3.1%	15	7.6%	2.57 (1.22-5.43)	0.0107*
CC	331	73.1%	118	59.6%	1.00 (reference)	
CG+GG	122	26.9%	80	40.4%	1.84 (1.29-2.61)	0.0006*

[a]Based on chi-square test without Yates' correction; *$p<0.05$, Statistically identified as significant; OR: odds ratio; CI: confidence interval.

under the supervision of the Human Research Committees of China Medical University hospital (CMUH106-REC1-004). Five ml of venous blood sample from each participant were collected and used for DNA extraction and further genotyping.

CHI3L1 genotyping methodologies. The genomic DNA was extracted from each participant's peripheral blood leukocytes within 24 h, carefully quantitated, diluted and stored at −80˚C until further processed (13-16). In the current study, the genotypes at *CHI3L1* -131C/G (rs4950928) and -247G/A (rs1262491437) were determined for all the investigated subjects *via* polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using the BioRad Mycycler machine (BioRad, Hercules, CA, USA). All PCR reactions were performed in a total volume of 25 μl containing 5 μl 10×Buffer, 2 μl template DNA, 1 μl (20 μM) upstream primer, 1 μl (20 μM) downstream primer, 0.5 μl Taq DNA polymerase, 2 μl dNTP, and 13.5 μl deionized sterile water. PCR procedures were as follows: 95˚C initial denaturation for 5 min; followed by 40 cycles of denaturing at 95˚C for 30 s, annealing at 55˚C for 30 s and extension for 30 s at 72˚C; final extension at 72˚C for 10 min. Then the genotypes were identified after the 3% DNA agarose gel electrophoresis.

Statistical analysis. The Student's *t*-test was applied for the comparison of age index between the asthma and the control groups. Pearson's Chi-square or Fisher's exact test (as any number was less than 5) was applied to compare the distributions of the numbers among the subgroups. The Hardy-Weinberg equilibriums were checked by chi-square goodness-of-fit test ($p>0.05$) using gene frequencies of the healthy individuals in the control group. The associations between *CHI3L1* genotypes and asthma risk were estimated by calculating the odds ratios (ORs) and their 95% confidence intervals (95%CIs) from logistic regression analysis. Statistically, any difference between any two groups compared with $p<0.05$ was considered as significant.

Results

Subject demographics. Baseline characteristics of the 198 cases and 453 non-asthmatic controls are presented in Table I. The case group consisted of 83 males and 115 females while 190 males and 263 females were in the control group. Since we matched all the controls and cases by age and gender criteria,

Table III. *Distribution of CHI3L1 -131C/G alleles among asthma patients and controls.*

	Controls	%	Patients	%	OR (95%CI)	*p*-Value[a]
-131C/G						
Allele C	770	85.0%	301	76.0%	1.00 (reference)	
Allele G	136	15.0%	95	24.0%	1.79 (1.33-2.40)	<0.0001*

[a]Based on chi-square test; *$p<0.05$, Statistically identified as significant; OR: odds ratio; CI: confidence interval.

Table IV. *Distribution of CHI3L1 -247G/A genotypes among asthma patients and controls.*

-247G/A	Controls		Patients		OR (95%CI)	*p*-Value[a]
	n	%	n	%		
Genotype						
GG	231	51.0%	99	50.0%	1.00 (reference)	
AG	186	41.1%	79	39.9%	0.99 (0.70-1.41)	0.9602
AA	36	7.9%	20	10.1%	1.30 (0.71-2.35)	0.3919
P_{trend}						0.6651
Carrier analysis						
GG+AG	417	92.1%	178	89.9%	1.00 (reference)	
AA	36	7.9%	20	10.1%	1.30 (0.71-2.35)	0.3672
GG	231	51.0%	99	50.0%	1.00 (reference)	
AG+AA	222	49.0%	99	50.0%	1.04 (0.75-1.45)	0.8156

[a]Based on chi-square test without Yates' correction; OR: odds ratio; CI: confidence interval.

Table V. *Distribution of CHI3L1 -247G/A alleles among asthma patients and controls.*

	Controls	%	Patients	%	OR (95%CI)	*p*-Value[a]
-247G/A						
Allele G	648	71.5%	277	70.0%	1.00 (reference)	
Allele A	258	28.5%	119	30.0%	1.08 (0.83-1.40)	0.5646

[a]Based on chi-square test; OR: odds ratio; CI: confidence interval.

there was no difference between the case and control groups regarding age and gender (p=0.2972 and 0.9956, respectively). Noticeably, a higher number of smokers were in the asthma group than in the control group (p=0.0048) (Table I).

Analysis of CHI3L1 promoter SNPs. All subjects were successfully genotyped and no deviation from Hardy-Weinberg equilibrium was observed in *CHI3L1* -131C/G or 247G/A ($p>0.05$). The distributions of the *CHI3L1* -131C/G genotypes among the 198 asthma cases and the 453 non-asthmatic controls are presented and compared in Table II. The results show that the genotype *CHI3L1* -131C/G are differently distributed between the asthma patient and healthy control groups (p for trend=0.0009). In detail, the *CHI3L1* -131C/G heterozygous variant CG and the homozygous variant GG were associated with elevated asthma risk, compared with the wild-type CC genotype (OR=1.69 and 3.00, 95%CI=1.16-2.45 and 1.41-6.41, respectively). The dominant and recessive models also showed statistical significances (OR=2.57 and 1.84, 95%CI=1.22-5.43 and 1.29-2.61, respectively).

To confirm the novel findings in Table II, the allelic frequency distribution for *CHI3L1* -131C/G was analyzed and the results are presented in Table III. The results confirmed that the variant G allele at *CHI3L1* -131C/G was associated with a relatively increased risk of asthma compared to the wild-type C allele (OR=1.79, 95%CI=1.33-2.40). On the other hand, neither the AG nor AA genotype at *CHI3L1* -247G/A were associated with asthma risk among the Taiwanese (Table IV). Also, analysis of allelic frequency distribution showed no significant difference regarding the polymorphism *CHI3L1* -247G/A (Table V).

Discussion

In the current study, the *CHI3L1* genotypes were found to contribute to asthma risk in a representative population in Taiwan. It has been reported that people having the wild-type genotype CC at the promoter *CHI3L1* -131C/G polymorphic site, overexpressed the protein and had elevated serum YKL-40 levels compared with CG or GG genotypes (8). However, Li and his colleagues have demonstrated that an association between *CHI3L1* -131G and childhood asthma was found (17). While, Ober and his colleagues reported that -131C was associated with childhood asthma in another study (8). Moreover, another group found no significant association between the genetic polymorphism in the *CHI3L1* -131C/G and asthma risk (18, 19). There are genetic differences among populations and the etiology of childhood and adult asthma may differ from each other.

Accumulating evidence has indicated that *CHI3L1* gene polymorphisms might contribute to the etiology of adult asthma. Kjaergaard and his colleagues have performed an excellent study, investigating the genotypes of 9000 individuals. They have identified 59 polymorphic sites in *CHI3L1* gene, and fifteen of them were associated with the expression levels of plasma YKL-40 (20). We found that the *CHI3L1* -131C/G polymorphism, which is located in the promoter region of *CHI3L1* gene, was significantly associated to asthma risk. Several studies have investigated the contribution of this polymorphism to asthma risk, but the conclusions are controversial. For instance, Ober and his colleagues have identified that the C allele in *CHI3L1* -131C/G is the risk allele for asthma, whereas Rathcke and his colleagues have reported that the G allele was associated with elevated asthma risk in a Caucasian population (8, 21). Our data showed that the G allele was associated with increased asthma risk in Taiwan population (Table II). Therefore, additional studies are necessary to reveal the role of *CHI3L1* -131C/G for asthma. Tsai and his colleagues have examined the association of the *CHI3L1* with asthma. They examined ten tagSNPs (rs903358, rs7542294, rs946259, rs880633, rs12128727, rs1538372, rs10399805, rs10399931, rs6691378, and rs946261) and found rs1538372 and rs10399931 to be significantly associated with asthma risk in a Taiwan population (22).

Serum YKL-40 levels are not a specific diagnostic tool, since they are elevated in several diseases such as multiple sclerosis, rheumatoid arthritis, osteoarthritis, infectious diseases, cardiovascular disease, and cancer. YKL-40 may not serve as a good diagnostic biomarker for asthma, but it has been reported to be associated with poor prognosis (6) and overall mortality risk (23). The correlation of serum YKL-40 levels and *CHI3L1* genotypes has yet to be validated.

In conclusion, the results may provide novel evidence showing that the variant G allele of *CHI3L1* -131C/G may play an important role in determining the susceptibility to asthma among Taiwanese.

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Conflicts of Interest

All the Authors have declared no conflicts of interest in relation to this study.

Authors' Contributions

Research design: Chen CL and Wang SC; patient and questionnaire summaries: Chen CL, Shen TC and Hsia TC; experimental work: Chang WS and Tsai CW; statistical analysis: Wang SC, Lin C and Chang WS; article writing: Tsai CW and Bau DT; review and revision: Tsai CW and Bau DT.

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