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RESEARCH ARTICLE

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Association of IL1RL1 rs3771180 and TSLP rs1837253 variants with asthma in the Guangxi Zhuang population in China

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Abstract

Objective: IL-1 receptor-like 1 (IL1RL1) and *thymic stromal lymphopoietin* (TSLP) play important roles in asthma in various ways. *IL1RL1* rs3771180 and *TSLP* rs1837253 single nucleotide polymorphisms (SNPs) are associated with asthma in some European nationals but not in Zhuang people. Accordingly, this study aimed to determine the associations of *IL1RL1* rs3771180 and *TSLP* rs1837253 with asthma in Zhuang people.

Methods: We performed a case-control study to observe the association between the two polymorphisms and asthma in a Guangxi Zhuang cohort consisting of 123 asthmatic patients and 100 healthy controls. These individuals were recruited from the Department of Respiration of the First Affiliated Hospital of Guangxi Medical University. Multiplex PCR assay was used to identify the genotype of rs3771180 and rs1837253. Data were analyzed with SPSS 22.0 and SHEsis.

Results: rs1837253 showed significant differences between asthmatic and control groups in allele comparison (OR = 2.15; 95% CI = 1.27-3.63; $P = 0.004$), as well as in the homozygote (OR = 4.83; 95% CI = 1.47-16.47; $P = 0.012$), heterozygote (OR = 2.69; 95% CI = 1.20-6.00; $P = 0.016$), and dominant (OR = 3.01; 95% CI = 1.39-6.52; $P = 0.005$) genetic models. However, the genotype frequencies of rs3771180 did not obviously differ.

Conclusion: rs1837253 is associated with asthma susceptibility and may increase the risk of asthma in Zhuang people in Guangxi.

KEYWORDS
asthma, IL1RL1, polymorphism, TSLP, Zhuang population

1 | INTRODUCTION

Asthma is a complex disorder characterized by hyper-responsiveness and chronic airway inflammation. The disease is often manifested by wheezing, shortness of breath, chest tightness, coughing, and reversible airflow limitation over time. The prevalence of asthma has significantly increased worldwide and highly contributes to incidence and medical costs. More than 300 million people suffer from asthma, and the disease still claims 346 000 lives every year.[1] Moreover, the prevalence of asthma is believed to be higher in

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low-income countries.[2] Several factors account for asthma, including infection, neuromodulation, and genetics. However, the specific pathogenesis of asthma remains unclear. As a polygene hereditary disease, an increasing number of studies are focusing on genetic factors, with some studies suggesting that numerous genes are associated with asthma, and different gene variants are involved among different ethnic groups.[3]

Genome-wide association studies (GWAS), a genetic approach to identifying complex traits, have discovered some variants on the *IL-1 receptor-like 1 (IL1RL1)* and *thymic stromal lymphopoietin (TSLP)* genes are associated with asthma. The *IL1RL1* gene, also known as *ST2*, is a potential candidate gene for asthma and atopy. A GWAS for eosinophil counts related to sequence variants discovered a variant (rs1420101), and the first locus associated with asthma on the *IL1RL1* gene was found in 9392 Icelanders and was successfully replicated in 12 118 Europeans and 5212 East Asians ($P = 5.3 \times 10^{-14}$).[4] As a member of the Toll-IL-1 receptor superfamily, IL1RL1 is a receptor on mast and Th2 cells and also exists in serum in a soluble form. The complex formed by soluble IL1RL1 and IL-33 regulates Th2 cells through Toll-like receptor pathways to play an important role in the development and progression of asthma.[5] The *TSLP* gene encodes a cytokine released from infected airway epithelial cells, which is bound up with inflammation response by activating T cells. The overexpression of TSLP protein can be found in the epithelium and lamina propria of the airway of asthmatic patients, especially in severe ones.[6] By far, substantial research has indicated that IL1RL1 and TSLP are critical to the immune pathogenesis of asthma.

Single nucleotide polymorphisms (SNPs) located in the *IL1RL1* and *TSLP* genes have recently been authenticated to be evidently associated with asthma among different races, such as Europeans and Africans.[7] A variant (rs1420101) in *IL1RL1* is related to asthma among nine European and one East Asian sample set ($P = 5.5 \times 10^{12}$),[4] but shows weak association with the disease among Australians ($P = 0.46$).[8] Another SNP (rs1837253) located in *TSLP* is a locus of protection from asthma with a *P* value of 0.0058.[9] But two variants (rs2289276 and rs2289278) in the *TSLP* gene have been demonstrated to be related to asthma among the Chinese Han population.[10] The genetic background of various peoples differs because of their ethnic customs and habits. Therefore, verifying an SNP in different ethnic groups and identifying various loci in a candidate gene are necessary. In view of the new trend in asthma GWAS, some relative genetic researches on asthma among the Chinese population have concentrated on Han people but only a few has paid attention to the Zhuang population in Guangxi. This ethnic group lives in southern China and comprises about 15 million of the population. Their lifestyle is extremely unique because of endogamy. They have the least contact with the outside world because of their uniqueness and geographical location. Two variants (rs3771180 in *IL1RL1* and rs1837253 in *TSLP*) associated with asthma among North Americans[3] have never been replicated in Zhuang people. Therefore, this research aims to identify the relationship between the two variants and asthma and the two variants' related phenotypes among the Zhuang population from Guangxi.

2 | MATERIALS AND METHODS

2.1 | Subjects

A total of 123 asthmatic patients and 100 healthy controls were recruited in this study. All patients were diagnosed based on the Global Initiative for Asthma (2008). The controls had no history of allergic diseases, including asthma, allergic rhinitis, and atopic eczema. Both groups were recruited from the Department of Respiration in the First Affiliated Hospital of Guangxi Medical University, Guangxi, China, from February 2010 to April 2013. All participants were not of kin, and three or more than three generations are Zhuang people. The Ethics Committee of the First Affiliated Hospital, Guangxi Medical University, approved the study, and informed consent was obtained from all participants.

2.2 | DNA extraction and genotyping

Peripheral blood was collected in EDTA-treated evacuated tubes. Genomic DNA was extracted using a Blood DNA extract kit (Tiangen) and then stored at −80°C for genotype analysis.

Primers were designed using Primer3 Online, with technical support from the Shanghai Biowing Applied Biotechnology Company. The primer sequences are shown in Table 1. The destination DNA sequences were amplified via the multiplex PCR method on a 20 μL reaction comprising 1 μL of genomic DNA, 2 μL of buffer, 0.6 μL of Mg^{2+} (3.0 mmol/L), 2 μL of dNTP (2.0 mmol/L), 0.2 μL of 1 U Taq enzyme, 12.2 μL of ddH_2O, and 2 μL of primer mixture. The amplification was performed using a GeneAmp PCR system 9600 with a pre-denaturation at 95°C for 2 minutes and then 40 cycles of denaturation at 94°C for 30 seconds, annealing at 53°C for 90 seconds, and extensions at 65°C for 30 seconds and for 10 minutes. For multiple LDR reaction, 4 μL of multiplex PCR product, comprising 1 μL of buffer, 1 μL of each probe mix (2 pmol/μL; probe sequences are listed in Table 2), 0.05 μL Taq DNA ligase (2 U), and 4 μL ddH_2O, was used. The fluorescent products of LDR were distinguished via an ABI sequencer 3730.

TABLE 1 Primer DNA sequences of two SNPs

Primer name	Upper	Lower	PCR length
rs3771180	TGGCCAAATCTATGACTTGTT	TCCTCTCAAGGGATTACTCAATG	116
rs1837253	AGGGCTACCCCTTGACTCAC	CCAACCAGGATTTGCAAGAA	136

TABLE 2 Probe sequences of LDR

Probe	Sequence (5′-3′)	LDR length
rs3771180_modify	P-CACCAGCATTTTTGAACAAGTCATATTTTTTTTTTTTTTTT	
	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs3771180_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	155
	TTTTTTTTACATCAAGAATTCTTAGTACATGATT	
rs3771180_C	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	157
	TTTTTTTTTTTACATCAAGAATTCTTAGTACATGATG	
rs1837253_modify	P-TTGTTTATGTATAAAAGGATCTTTTTTTTTTTTTTTTTTTT	
	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs1837253_C	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	175
	TTTTTTTTTTTTTTTTTTTTTGTAATTTGCTTCATAGTTTAGACACG	
rs1837253_T	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	177
	TTTTTTTTTTTTTTTTTTTTTGTAATTTGCTTCATAGTTTAGACACA	

TABLE 3 Characteristics of control subjects and cases

Group	Cases (n = 123)	Control subjects (n = 100)	*P*
Gender			
Male	50 (40.7%)	52 (52.0%)	0.091
Female	73 (59.3%)	48 (48.0%)	
Age (y)	39.06 ± 12.33	28.34 ± 3.31	0.000
Weight (kg)	54.98 ± 10.31	55.48 ± 10.00	0.736

2.3 | Statistical analysis

The gene frequency distribution in the control group was tested via the Hardy-Weinberg equilibrium, and the comparison of genotype frequencies between the cases and controls was confirmed via the chi-square test. The pertinence among the genetic models (variant allele, homozygote and heterozygote genotypes, and dominant and recessive models) of the two SNPs and asthma was unveiled via logistic regression analysis. The odds ratios (OR) with corresponding 95% confidence intervals (CI) were calculated. Statistical significance was set at $P < 0.05$ (two-tailed), and statistical analyses were conducted using SPSS software version 22.0 with adjustments in sex, age, and weight (Table 5). Allelic association analysis in a replication study was performed via the SHEsis (http://www.bio-x.cn/analysis/).

3 | RESULTS

3.1 | Characteristics of subjects

The genders and weights of the case and control subjects were well matched ($P > 0.05$), but the patients in this study were nearly 10 years older than the control subjects ($P < 0.05$; Table 3).

3.2 | Genotype and allele frequencies of SNPs

The genotype data from rs3771180 and rs1837253 of the control group were consistent with the Hardy-Weinberg equilibrium (*P* values were 0.677 and 0.618, respectively). The genotypic distributions of the loci, as shown in Table 4, demonstrated that a statistical difference exists between the case and control groups in terms of rs1837253 but not for rs3771180. The contributions of genetic polymorphisms to asthma were demonstrated by comparing the genetic models of two loci (Table 5). Figures indicated that rs1837253 was correlated with asthma. All genetic models of rs1837253 showed evident association with asthma except for the recessive model (OR = 2.71; 95% CI = 0.88-8.32,

TABLE 4 The genotypic distributions of the SNPs

SNPs ID	Genotype	Cases (n = 123)	Control (n = 100)	χ^2	*P*
rs3771180				1.491	0.222
	CC	106 (86.89)	92 (92.00)		
	AC	16 (13.11)	8 (8.00)		
	AA	0 (0.00)	0 (0.00)		
rs1837253				13.469	0.001
	TT	30 (24.60)	48 (48.00)		
	CT	69 (56.60)	41 (41.00)		
	CC	23 (18.90)	11 (11.00)		

TABLE 5 The relativity between loci and asthma in different genetic models

SNP	Genetic model	OR*	95% CI*	P*
rs3771180				
	A/C	1.820	0.505-6.552	0.360
	AA/CC	—	—	—
	AC/CC	1.873	0.506-6.934	0.348
	AA+AC/CC	1.873	0.506-6.934	0.348
	AA/AC+CC	—	—	—
rs1837253				
	C/T	2.147	1.268-3.633	0.004
	CC/TT	4.831	1.417-16.468	0.012
	CT/TT	2.686	1.202-6.001	0.016
	CC+CT/TT	3.007	1.387-6.517	0.005
	CC/CT+TT	2.712	0.884-8.316	0.081

*Adjustments for sex, age, and weight.

$P = 0.081$). Results are as follows: allele comparison (OR = 2.15; 95% CI = 1.27-3.63; $P = 0.004$); homozygote genotype comparison (OR = 4.83; 95% CI = 1.47-16.47; $P = 0.012$); heterozygote genotype comparison (OR = 2.69; 95% CI = 1.20-6.00; $P = 0.016$); and dominant model comparison (OR = 3.01; 95% CI = 1.39-6.52; $P = 0.005$). Each genetic model of rs3771180 had no correlation with asthma ($P > 0.05$).

4 | DISCUSSION

To the best of our knowledge, this study is the first to focus on the genetic associations between SNPs in *TSLP* and *IL1RL1* and asthma among the Zhuang population in Guangxi. We found that rs1837253 was significantly associated with asthma patients, whereas rs3771180 showed no difference between patients and controls not only in genotype and allele frequencies but also in the different genetic models.

Substantial research has shown significant genetic associations between the two loci and asthma in distinctive ethnicities. A SNP (rs3771180), located at the *IL1RL1* proximal promoter, was found to be associated with atopic asthma among French Canadians and ordinary asthma among European Americans, African Americans, and Latin Americans[3,11] and related to asthma and hay fever in Swedish twins.[12] No research has been conducted on this locus among Asian populations. In contrast to findings from previous studies, our research demonstrated no association between rs3771180 and asthma among Zhuang people. For rs1837253, presenting the upstream of the transcription start site of the TSLP gene showed a significant association with adult asthma among the Japanese population ($P = 0.023$).[13] Jian-Qing H found that the A allele of rs1837253 was associated with protection from asthma and airway hyper-responsiveness among Canadian and Australian populations, whereas Bunyavanich discovered that the T allele of rs1837253 was inversely associated with allergic rhinitis in boys with asthma in Costa Rica.[14] Interestingly, a study in Costa Rica showed that the T allele of rs1837253 was associated with a reduced risk of asthma in men, but the T allele of another *TSLP* SNP rs2289276 was associated with a reduced risk of asthma in women.[15] As in the previous experiments, we obtained the same results; rs1837253 was associated with asthma susceptibility and may increase the risk of asthma among Zhuang people. Analyzing the risk and protective mutant alleles was difficult because of the lack of SNP haplotype profiles.

As is usually the case in studies of this sort, results are inconsistent with other studies possibly because of small samples, technology hiccups, and the distinction of ethnic groups. In general, the result of a study is better when the sample size is increased. However, we can only contact some of the Zhuang people. Moreover, whether these patients can represent the Zhuang population and whether the sample size is large enough are unclear. Furthermore, experimental error and data dealing error are inevitable incidents. The differences between Zhuang people and other nationalities reflect the contrast of genes among ethnic groups in various environments, with diverse genetic backgrounds and social and cultural habits. In summary, a larger sample is necessary for a better GWAS.

Although several GWAS allow us to recognize the polymorphisms of most genes, we still do not know the specific functions or biologic effects of these loci on disease pathogenesis. Asthma-associated SNPs can reportedly achieve functional alterations in some pathways through several mechanisms, such as changing the level of mRNA expression or altering protein functions via amino acid substitutions. Shimizu et al[16] validated functional SNP rs6543116 in the *ST2* distal promoter region regulating ST2 expression, which activated the Th2 response. *IL1RL1* gene SNP rs11693697 played its biologic role primarily by regulating *IL1RL1* mRNA levels.[17] In Brazilians, the A allele of *IL1RL1* rs1041973 was positively associated with IL-5 production and Ig E levels; expression quantitative trait loci analysis also suggested that polymorphism regulated the expression of the *IL1RL1* gene via amino acid substitution.[18] *TSLP* SNP rs1837253 showed correlation with TSLP protein secretion in human nasal epithelium but not with *TSLP* mRNA levels.[19,20] In recent years, a new technology called Hi-C has been widely applied by researchers worldwide to reveal various epigenomic mechanisms of various diseases. This technology can potentially be used for asthma pathogenesis.

In conclusion, *TSLP* rs1837253 was successfully duplicated among Zhuang people in our study, whereas replication of *IL1RL1* rs3771180 was unsuccessful. This study had limited samples, and experimental errors were still observed; thus, further studies and larger samples are necessary for future replication.

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