



Research paper

The *IL6R* gene polymorphisms are associated with sIL-6R, IgE and lung function in Chinese patients with asthma

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ABSTRACT

Background: sIL-6R is involved in a variety of inflammatory diseases. The present study analyzed the potential associations between two *IL6R* gene polymorphisms (rs2228145 and rs12083537) and asthma in a Han Chinese population. **Methods:** A cohort of 394 patients and 395 healthy controls were genotyped to detect the two polymorphisms using SNaPshot. In 66 asthma patients and 49 controls, peripheral eosinophil and serum immunoglobulin E (IgE) levels were determined using a routine blood test, and sIL-6R levels were measured by ELISA. **Results:** No statistically significant differences were detected between the patients and controls in the distribution of the two independent *IL6R* polymorphisms ($p > 0.05$). However, rs2228145 C and rs12083537 G were significantly associated with decreased lung function in patients with asthma; the rs2228145 A–C variant was also associated with increased sIL-6R and IgE levels. In addition, sIL-6R levels were positively associated with IgE and inversely associated with lung function in patients with asthma. **Conclusions:** Our results provide evidence that rs2228145 C and rs12083537 G are associated with poor lung function in patients with asthma. Furthermore, the rs2228145 A–C variant is associated with levels of sIL-6R and IgE.

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1. Introduction

Asthma is a chronic inflammatory respiratory disease affecting more than 300 million people worldwide characterized by airway obstruction, hyperproduction of immunoglobulin E (IgE), mucus hypersecretion and hyperresponsiveness (Murphy & O'Byrne, 2010; Holgate, 2012). The pathological processes of asthma may be influenced by a combination of both genetic and environmental factors, but the detailed mechanism has not been fully explained.

Interleukin 6 (IL-6), like other inflammatory cytokines, is involved in allergic asthma (Doganci et al., 2005a; Neveu et al., 2010; Rincon & Irvin, 2012; Ferreira et al., 2011). IL-6 can directly act on target cells via specific membrane-bound IL-6 receptor (mIL-6R), which is known as classical IL-6 signaling. An alternative IL-6 signaling pathway is mediated by the soluble IL-6 receptor (sIL-6R), which is termed trans-signaling (Jones

et al., 1999). sIL-6R can be produced either by proteolysis of the membrane receptor or by alternative mRNA splicing (Horiuchi et al., 1994; Lust et al., 1992; Mullberg et al., 1993; Rose-John & Heinrich, 1994). The persistently elevated IL-6 may down-regulate mIL-6R and make cells unresponsive to IL-6. Such a refractory state can be overcome by the elevation of sIL-6R (Taga et al., 1989). Thus, sIL-6R plays an important role in the responses mediated by IL-6. The activity of sIL-6R is implicated in a variety of inflammatory diseases, such as inflammatory bowel disease (Brulhart et al., 2010; Rose-John et al., 2009), rheumatoid arthritis (Febbraio et al., 2010) and asthma (Robinson et al., 2015; Ullah et al., 2015; Cho et al., 2015), and there are anti-IL-6R therapies, which target sIL-6R as well as the membrane-bound mIL-6R (Mima & Nishimoto, 2009).

An important genetic determinant of sIL-6R levels is the rs2228145 single-nucleotide polymorphism (SNP) (formerly rs8192284), which is located in exon 9 of the *IL6R* gene on chromosome 1 (Galicia et al., 2004). Rs2228145 is specifically localized to amino acid position 358, which is the main cleavage site of *IL6R* (Mullberg et al., 1994). The functional polymorphism of rs2228145 A–C results in the 358 Aspartic acid substituted by Alanine (Asp358Ala) (Hamid et al., 2004). Notably, rs2228145 C allele carriers (Ala358 allele) show an increase in sIL-6R levels (Galicia et al., 2004). Rs2228145 is also associated with a lower level of lung function (Hawkins et al., 2012) and an increased risk of asthma (Ferreira et al., 2011). Another SNP site, rs12083537 A/G, located in intron 1, 2.9 kb away from exon 1 of *IL6R*. The rs12083537 G SNP is also associated with higher sIL-6R levels, and may be associated

Abbreviations: sIL-6R, soluble IL-6 receptor; IgE, immunoglobulin E; IL-6, interleukin 6; mIL-6R, membrane-bound IL-6 receptor; SNP, single nucleotide polymorphism; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; HWI, Hardy-Weinberg equilibrium; FEV₁, the forced expiratory volume in 1 second; ppFEV₁, percent predicted FEV₁; ppFVC, percent predicted forced vital capacity; FEF 25–27%, the average forced expiratory flow during the mid (25–75%) portion of the FVC; MAF, minor allele frequency; Hapmap-HCB, haplotype map of Han Chinese from Beijing; OR, odds ratio; 95%CI, 95% confidence; Th1, T helper type 1; Th2, T helper type 2; CD4, cluster of differentiation 4; CD25, cluster of differentiation 25; IL-4, interleukin 4; IL-5, interleukin 5; IL-13, interleukin 13; IL-10, interleukin 10; Tregs, regulatory T cells; BMI, body mass index.

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with an increased risk of asthma (Revez et al., 2013). However, there has been no data published on an association of the two *IL6R* alleles to asthma risk or lung function in the Chinese population.

In the present case-control study of a Han Chinese population, we measured the associations of the two *IL6R* polymorphisms (rs2228145 and rs12083537) to asthma risk, sIL-6R, serum IgE levels and lung function. Furthermore, the correlations between sIL-6R and serum IgE, lung functions were determined.

2. Material and methods

2.1. Participants

In total, 394 patients and 395 controls from a Han population in China were included in this case-control study. Asthma subjects were patients seeking treatment for asthma symptoms at the Affiliated Hospital of Guangdong Medical College (Zhanjiang, China) between January 2013 and December 2014. None of the patients received treatment for asthma during the 4 weeks prior to their participation in this study. A clinical diagnosis of probable asthma was established according to the criteria of the Global Initiative for Asthma guidelines (Bateman et al., 2008). The age and sex matched control subjects were recruited through advertisements at the Health Examination Center of the Affiliated Hospital of Guangdong Medical College. The control groups were confirmed to be healthy according to their medical history and general examinations. All subjects who had a history of oral corticosteroid use, the occurrence of a respiratory tract infection within 4 weeks of enrolment in the study and any chronic cardiopulmonary disease other than asthma (including Chronic Obstructive Pulmonary Disease) were excluded.

This study was approved by the Ethics Committee of Affiliated Hospital of Guangdong Medical College. All experiments on human subjects were conducted based on the Declaration of Helsinki 2013 (Muthuswamy, 2014), and written informed consent was obtained from each of the participants.

2.2. Pulmonary function tests

Spirometry was measured using standard spirometric techniques (POS Instrumentation, Inc., Louisville USA) according to the American Thoracic Society (ATS) guidelines as we previously described (Wang et al., 2015).

2.3. Measurement of sIL-6R and IgE levels and peripheral eosinophils

Venous blood was sampled after clotting for 1 to 2 h, the serum was obtained. The sIL-6R levels were determined by an enzyme-linked immunosorbent assay (ELISA) using a commercial Human IL-6 Receptor ELISA Kit (USCN Life Science Inc. Wuhan, China) specific for human IL-6R, according to the manufacturer's instructions. The limit of detection in this assay was 0.83 ng/mL. Blood eosinophil counting was associated with allergic asthma and characterizes disease severity in patients with severe asthma (Garcia et al., 2013; Giovannelli et al., 2015). The cell counts/percentages of peripheral blood eosinophils and the serum IgE levels and were each determined from a routine blood test in patients and control subjects.

2.4. DNA extraction and genotyping

Genomic DNA was extracted from peripheral whole blood samples from all of the patients and controls using the TIANamp blood DNA extraction kit (Tiangen Biotech, Beijing, China), following to the manufacturer's instructions. A total of 789 individuals were genotyped for the two SNPs (rs12083537 A/G and rs2228145 A/C) using the SNaP-shot technique (Applied Biosystems, Foster City, CA, USA) according to

the manufacturer's protocol, as previously described (Ma et al., 2014). The primers used in this study are summarized in Table 1.

2.5. Statistical analysis

General characteristics and laboratory findings between cases and controls were compared by Chi-square tests (categorical variables) or t-test (continuous variables). Allele frequencies, genotype distribution of cases and controls were determined using the online software SNPStarts (<http://bioinfo.iconcologia.net/snpstats/start.htm>) (Sole et al., 2006), OR and p values were calculated by multivariate unconditional logistic regression and adjusted for age, gender and smoking status. Allele frequency and deviation of the genotype were estimated using the Hardy-Weinberg equilibrium (HWE). The deviation from Hardy-Weinberg equilibrium was estimated by calculating of Chi-square test. The sIL-6R levels between cases and controls were compared with a t-test. The correlations between sIL-6R and IgE or FEV₁ in either cases or controls were estimated by Spearman's rank correlation analysis. The sIL-6R level and asthmatic characteristics differences between genotypes were tested by Kruskal-Wallis test (three groups) or t-test (two groups).

Data were analyzed using SPSS 19.0 (IBM, NY, USA) and GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, CA, USA). The data are shown either as the means $\pm$ SD or as n (%), unless stated otherwise. The criterion for statistical significance was defined as $p \leq 0.05$ for all the tests.

3. Results

3.1. Characteristics of patients with asthma and controls

The mean year of age for the patients with asthma and healthy subjects was 45.37 and 45.23, respectively. There were no significant differences in both age and gender composition between patients with asthma and healthy subjects. In terms of characteristics associated with asthma, there were significant differences between patients and healthy subjects. Compared to healthy subjects, the patients with asthma exhibited increased serum IgE levels (542.00 ± 792.00 IU/ml vs. 48.00 ± 56.00 , $p < 0.0001$), increased percentages of peripheral eosinophils and peripheral eosinophil numbers ($4.90 \pm 3.70\%$ vs. $2.30 \pm 1.30\%$ and $0.40 \pm 0.35 \times 10^9/L$ vs. $0.16 \pm 0.10 \times 10^9/L$, respectively, both $p < 0.0001$), lower predicted percentage forced expiratory volume in 1 s (FEV₁) ($74.95 \pm 24.70\%$ vs. $91.24 \pm 15.38\%$, $p < 0.0001$), lower FEV₁/forced vital capacity values (FEV₁/FVC) ($72.72 \pm 14.97\%$ vs. 88.61 ± 8.80 , $p < 0.0001$) and lower average forced expiratory flow during the mid (25–75%) portion of the FVC (FEF 25–75%) ($53.87 \pm 58.66\%$ vs. $85.15 \pm 24.26\%$, $p < 0.0001$). There was no significant difference in smoking status between patients and healthy subjects ($p = 0.08$) (Supporting Table 1).

3.2. The genotype and allele frequency distributions in patients and controls

The distributions of the genotype frequencies of the two *IL6R* SNPs (rs2228145 and rs12083537) complied with the Hardy-Weinberg equilibrium in both patients and controls (all $p > 0.05$, data not shown).

Table 1
Primers used in this study.

rs12083537	PCR primers	Forward 5'-TGGGGTAGGGGGTGGTTAGAGA-3' Reverse 5'-GGTGGGACAGGATTACAGAGA-3'
	Extension primer	5'-TTTTTTTTTCCCACTAGAGGGGAGTAITTTTACCC-3'
rs2228145	PCR primers	Forward 5'-ATGGCCTGTTGGTTGGCAGA-3' Reverse 5'-TGCAGAGGAGCGTTCCGAAG-3'
	Extension primer	5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTGTGGGCACTGGTACTGAAGAAGAA-3'

Minor allele frequency (MAF) detected in this study were rs2228145 C = 0.338 and rs12083537 G = 0.079, respectively, which is close to the reported MAF of the haplotype map of Han Chinese from Beijing (Hapmap-HCB) rs2228145 C = 0.36 and rs12083537 G = 0.114. The genotype distributions and allele frequencies of the rs2228145 and rs12083537 polymorphisms in all patients and the controls are presented in Table 2. The results show that the rs2228145 A/C and the rs12083537 A/G polymorphisms have no significant influence on the risk of asthma.

Because of the gender differences in asthma onset and respiratory symptoms (Hansen et al., 2015; Takeda et al., 2013), we stratified the allele frequencies and genotypes by gender. However, no statistically significant differences in genotype or allele frequency distribution were found for rs2228145 and rs12083537 between patients and controls in either males or females (supporting Tables 1 and 2).

3.3. sIL-6R levels were increased in patients with asthma and associated with serum IgE and lung function

sIL-6R levels were measured in 66 patients with asthma and 49 control subjects. In patients with asthma, increased sIL-6R levels were associated with higher serum IgE ($r = 0.3435$, $p = 0.0047$) and a lower percent predicted FEV₁ ($r = -0.2528$, $p = 0.0406$) (Fig. 1A, and B, respectively). However, we failed to find associations between sIL-6R levels and FVC, FEV₁/FVC or FEF 25–75% (Supporting Fig. A, B, C). In healthy control subjects, no associations were found between sIL-6R levels and serum IgE, FEV₁, FVC, FEV₁/FVC or FEF 25–75% (Fig. 1C, D and Supporting Fig. D, E, F respectively).

3.4. The rs2228145 A/C and rs12083537 A/G variation associated with sIL-6R levels in patients with asthma

Generally, the plasma levels of sIL-6R were significantly higher in patients with asthma (129.3 ± 46.54) than in the controls (95.63 ± 29.88) ($p < 0.05$) (Fig. 2A). Another experiment was performed to determine whether the IL6R rs2228145 and rs12083537 polymorphisms had effects on sIL-6R level in 49 controls and 66 patients with asthma. The patients carrying the rs2228145 CC genotype presented with higher sIL-6R levels compared to the AA group (159.9 ± 42.52 ng/ml vs. 111.1 ± 34.19 ng/ml; $p = 0.0014$) (Fig. 2B; Table 3). In regard to rs12083537, in the 66 samples, we only obtained serum from one patient with the GG genotype (107.61 ng/ml). Though the patients carrying the AG genotype showed a slightly higher sIL-6R levels compared to the AA group, the difference failed to achieve statistical significance ($p = 0.9185$) (Fig. 2C; Table 3). However, no significant differences showed between rs2228145 A/C or rs12083537 A/G variations and sIL-6R levels in control subjects (Fig. 2D, E; supporting Table 3).

3.5. The rs2228145 and rs12083537 polymorphisms associated with IgE and lung function in patients with asthma

Because of its association with lower lung function and higher serum IgE, the presence of increased peripheral eosinophils is an important phenotype in asthma (Am. Thorac. Soc., 2000). We first evaluated the rs2228145 and rs12083537 SNPs for an association with lung function, serum IgE and peripheral eosinophils, respectively, in the patients with asthma. After adjusting for sex, age and smoking status at enrollment, the C allele of rs2228145 showed an association with a lower percent of predicted FEV₁ and higher serum IgE. Subjects who carry the CC genotype for rs2228145 (Ala358) had the lowest mean percent of predicted FEV₁ (66.16%) and the highest serum IgE levels (792.4 ng/ml) (both $p \leq 0.05$). Although subjects carrying the CC genotype (Ala358) also showed the lowest mean percent FEV₁/FVC (71%) and FEF 25–75% (45.12%), this difference failed to achieve statistical significance. In regard to rs12083537, the GG genotype carriers had the lowest mean percent predicted FEV₁ (42.10%) and FEF 25–75% (17.78) (both $p < 0.05$) (Table 3). There were no significant differences between genotypes in rs2228145 and rs12083537 for the distributions of predicted FVC, the peripheral eosinophil counts and the peripheral eosinophil ratio.

4. Discussion

4.1. sIL-6R increased in patients with asthma

IL-6 signaling is associated with asthma control and poor lung function and to the greatest frequency with asthma symptoms (Simpson et al., 2006; Hastie et al., 2010; Al-Ramli et al., 2009). IL-6 acts on target cells by binding to IL-6R that is in a complex with the gp130, resulting in the activation of the associated gene expression. In the present study, we demonstrated that the levels of sIL-6R increased in patients with asthma (129.3 ± 46.54 ng/ml vs. 95.63 ± 29.88 ng/ml), near to the levels reported in Japanese patients with asthma (132 ± 31 vs. 111 ± 16 ng/ml) (Yokoyama et al., 1997). These results suggest that the circulating levels of sIL-6R may be a characteristic of airway inflammation.

4.2. Variation in rs2228145 was correlated with sIL-6R and IgE and lung function in patients with asthma

In the present study, we found that the sIL-6R levels in patients carrying the rs2228145 CC allele were higher than in those carrying AA allele. It has been reported that the rs2228145 C allele (Ala358) was strongly associated with increased sIL-6R levels in severe asthma patients (Galicia et al., 2004), which is consistent with our results. The sIL-6R is produced by proteolysis of the mIL-6R or as a result of alternative mRNA splicing (Peters et al., 1998). Both mechanisms can be affected by rs2228145. The rs2228145 A to C variant leads to both enhanced proteolytic cleavage of mIL-6R (Garbers et al., 2014) and upregulated

Table 2
Genotype and allele frequency distributions of rs2228145 and rs12083537 in patients with asthma and controls.

Model	rs2228145					rs12083537				
	Genotype	Asthma	Control	OR (95%CI)	p value	Genotype	Asthma	Control	OR (95%CI)	p value
Codominant	A/A	166 (42.1%)	171 (43.3%)	1.00	0.31	A/A	332 (84.3%)	337 (85.5%)	1.00	0.71
	C/A	181 (45.9%)	189 (47.9%)	1.05 (0.77–1.44)		G/A	58 (14.7%)	55 (14%)	0.90 (0.59–1.37)	
	C/C	47 (11.9%)	35 (8.9%)	0.71 (0.42–1.18)		G/G	4 (1%)	2 (0.5%)	0.56 (0.10–3.18)	
Allele	A allele	513 (65%)	531 (67%)	0.91 (0.73–1.14)	0.42	A allele	722 (92%)	729 (93%)	0.87 (0.59–1.27)	0.47
	C allele	275 (35%)	259 (33%)			G allele	66 (8%)	59 (7%)		

OR: odds ratio; 95%CI: 95% confidence interval;

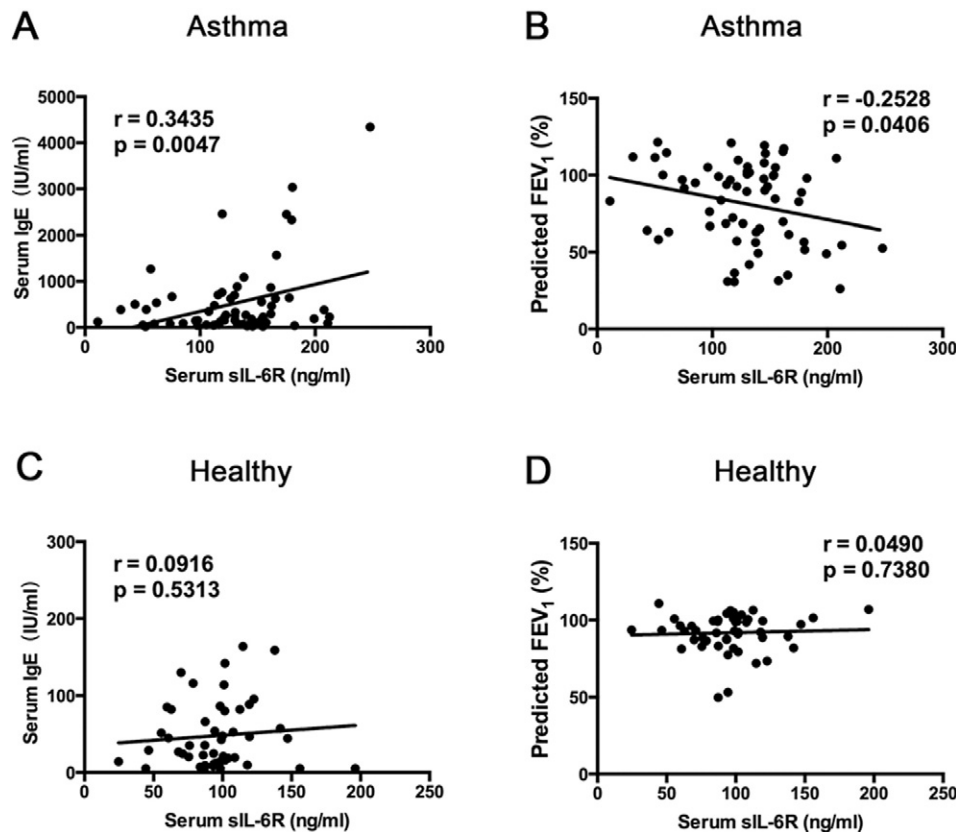


Fig. 1. Correlations between sIL-6R and Serum IgE or FEV₁. The Spearman's rank correlation analysis showed that the sIL-6R levels correlated positively with the levels of serum IgE ($r = 0.3435$, $p = 0.0047$) (A) and inversely with the levels of FEV₁ ($r = -0.2528$, $p = 0.0406$) (B) in patients with asthma. However, there was no significant correlation found between sIL-6R levels and serum IgE (C) or FEV₁ (D) in healthy subjects.

transcription of sIL-6R (Ferreira et al., 2013), which results in high levels of sIL-6R (Melzer et al., 2008) and low mL-6R expression (Ferreira et al., 2013).

Our results confirmed that patients with asthma were associated with an increased IgE concentration in serum. Moreover, in patients with asthma we observed that sIL-6R levels were positively correlated with serum IgE levels. Subjects who carry the CC allele for rs2228145 had higher serum IgE levels than patients with the AA and AC alleles. IgE is a mediator of airway inflammation response and is considered an important biomarker for asthma (Bachert & Zhang, 2012; Beeh et al., 2000). IL-4 promotes the production of IgE (Berton et al., 1989), and had been considered to be a target for blocking IgE production in allergic asthma (Barnes, 2001). IL-6 promotes endogenous IL-4 production in CD4 T cells (Diehl et al., 2002; Neveu et al., 2009). Thus, elevated IL-6 or IL-6R might result in the increased IgE production. Our results confirmed that patients with asthma were associated with an increased IgE concentration in serum. Moreover, in patients with asthma we observed that sIL-6R levels were positively correlated with serum IgE levels. Subjects who carry the CC allele for rs2228145 had higher serum IgE levels than patients with the AA and AC alleles. This intriguing phenotype in the present study may be explained by the effect of rs2228145 C in promoting sIL-6R level, therefore enhancing IgE producing through IL-4.

Furthermore, we observed that asthma patients with the C allele of rs2228145, which is associated with lower lung function compared to the alternate A allele in asthma patients with European ancestry (Hawkins et al., 2012), are consistently associated with a decrease in the predicted FEV₁ compared to those carrying the reference polymorphism site. Although the mechanisms of the sIL-6R association to lung function remain largely unknown, we speculate that sIL-6R probably functions via IL-6 trans-signaling. An important caveat to consider is the therapeutic treatment of allergic asthma patients with already-

high IgE levels and low predicted FEV₁ may have differential effects on rs2228145 A and rs2228145 C carriers.

4.3. The rs12083537 G is associated with decreased lung function

The rs12083537 polymorphism is an *IL6R* SNP related to body mass index (BMI) (Edwards et al., 2012) and altered levels of circulating C-reactive protein (Qi et al., 2009). IL-6R can regulate the synthesis and release of C-reactive protein in response to IL-6, suggesting that functional effects of rs12083537 may have a role in influencing IL-6R expression. A study of two large cohorts reported that the rs12083537 allele A was associated with a slightly increased sIL-6R level and a higher risk of asthma (Revez et al., 2013). However, in the present study, we detected a slight increase in sIL-6R levels for rs12083537 AG carriers compared to those levels in AA carriers, although this difference failed to achieve statistical significance. The rs12083537 variant is located in intron 1 of *IL6R*, which is a region with high H3K4Me1 lysine methylation (Consortium, 2012), and a potential regulatory region for IL-6R transcription. The detailed molecular mechanism underlying the association between sIL-6R levels and rs12083537 still remain to be elucidated. *IL6R* regulatory variants have been reported to influence protein levels similarly in healthy individuals (Galicía et al., 2004; International Genetics of Ankylosing Spondylitis, C., 2013) and patients with ankylosing spondylitis, coronary heart disease and airway inflammation (Ferreira et al., 2013; International Genetics of Ankylosing Spondylitis, C., 2013; Interleukin-6 Receptor Mendelian Randomisation Analysis, C., 2012). Studies using allergen stimulation or gene expression in immune cells types could possibly help to characterize the function of the rs12083537 variant.

An anti-IL-6R monoclonal antibody (tocilizumab) has been approved as an effective biological drug for rheumatoid arthritis and systemic juvenile arthritis therapy (Tanaka et al., 2011), and has been suggested

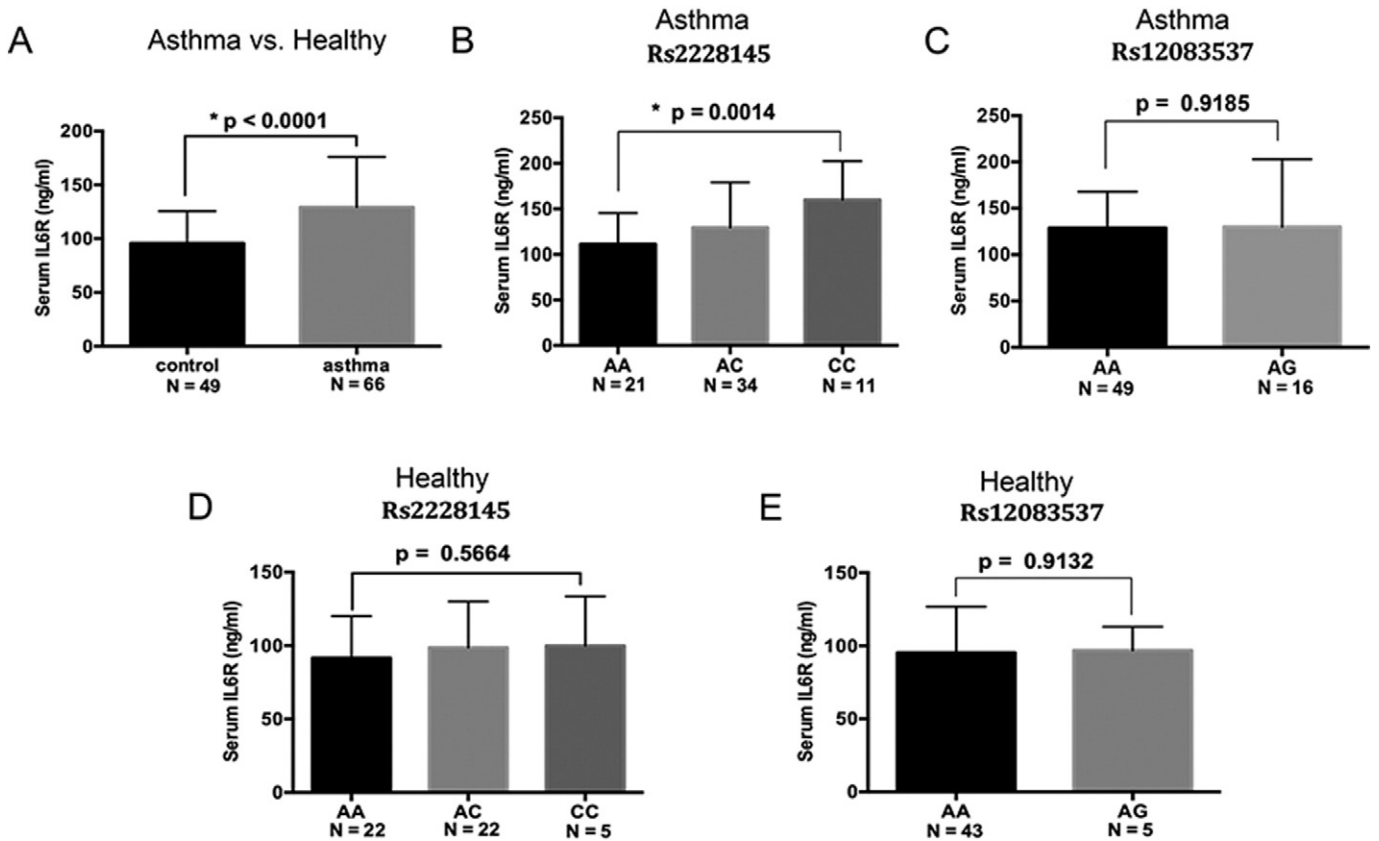


Fig. 2. sIL-6R levels with three and two different genotypes of rs2228145 and rs12083537, respectively, in serum. The sIL-6R levels from serum of asthmatic patients and healthy subjects were measured by ELISA. (A): sIL-6R levels of asthmatic patients (129.3 ± 46.54) were significantly higher than that of healthy controls (95.63 ± 29.88); (B): sIL-6R distribution of rs2228145 AA and CC carriers in asthmatic patients; (C): sIL-6R distribution of rs12083537 AA and AG carriers in asthmatic patients; (D): sIL-6R distribution of rs2228145 AA and CC carriers in healthy controls. (E) sIL-6R distribution of rs12083537 AA and AG carriers in healthy controls.

as a treatment for asthma (Ferreira et al., 2011; Doganci et al., 2005b); therefore, pharmacogenetic studies of the clinical response to tocilizumab should also consider the influences of the genetic determinant of sIL-6R levels, namely rs2228145 and rs12083537. Prospective studies will be

necessary to test the hypothesis that tocilizumab might be effective in asthma therapy, particularly for carriers of the C allele of rs2228145 or the G allele of rs12083537. The *IL6R* variants rs2228145 C and rs12083537 G might be useful as DNA markers to identify asthma patients

Table 3
Distributions of asthmatic characteristics between *IL6R* genotypes in patients with asthma

	rs2228145				rs12083537			
	Genotype	No.	Mean $\pm$ SD	p-Value ^a	Genotype	No.	Mean $\pm$ SD	p-Value ^a
sIL-6R (ng/ml)	AA	21	111.1 $\pm$ 34.19	0.0014*	AA	49	128.7 $\pm$ 39.38	0.9185
	CA	34	129.6 $\pm$ 49.44		GA	16	129.8 $\pm$ 73.16	
	CC	11	159.9 $\pm$ 42.52		GG	1	107.61	
Serum IgE (ng/ml)	AA	166	472.8 $\pm$ 572.3	0.05*	AA	332	540.9 $\pm$ 600.5	0.95
	CA	181	540.6 $\pm$ 859.7		GA	58	542.6 $\pm$ 629.7	
	CC	47	792.4 $\pm$ 1102		GG	4	634.9 $\pm$ 232.2	
Peripheral eosinophil (10^9 /ml)	AA	166	0.3893 $\pm$ 0.3851	0.35	AA	331	0.3953 $\pm$ 0.3013	0.98
	CA	180	0.4302 $\pm$ 0.3339		GA	58	0.4500 $\pm$ 0.5494	
	CC	47	0.3447 $\pm$ 0.2367		GG	4	0.3250 $\pm$ 0.0500	
Peripheral eosinophil ratio(%)	AA	166	4.565 $\pm$ 3.244	0.16	AA	332	4.918 $\pm$ 3.772	0.25
	CA	181	5.304 $\pm$ 4.12		GA	58	4.707 $\pm$ 3.312	
	CC	47	4.320 $\pm$ 3.227		GG	4	3.793 $\pm$ 1.188	
Predicted FEV ₁ (%)	AA	163	76.01 $\pm$ 24.36	0.04*	AA	325	74.92 $\pm$ 24.71	0.02*
	CA	178	76.21 $\pm$ 24.11		GA	57	77.46 $\pm$ 23.79	
	CC	45	66.16 $\pm$ 26.95		GG	4	42.10 $\pm$ 15.11	
Predicted FVC (%)	AA	179	90.45 $\pm$ 62.43	0.08	AA	325	88.59 $\pm$ 47.86	0.58
	CA	194	85.59 $\pm$ 19.11		GA	57	88.02 $\pm$ 18.62	
	CC	61	76.01 $\pm$ 24.17		GG	4	65.10 $\pm$ 16.98	
FEV ₁ /FVC (%)	AA	163	72.16 $\pm$ 14.20	0.46	AA	325	72.71 $\pm$ 14.96	0.09
	CA	178	73.67 $\pm$ 14.56		GA	57	73.91 $\pm$ 14.91	
	CC	45	71.00 $\pm$ 18.91		GG	4	57.05 $\pm$ 9.163	
FEF 25–75%	AA	151	55.94 $\pm$ 82.47	0.57	AA	306	53.97 $\pm$ 62.31	0.02*
	CA	170	54.20 $\pm$ 32.73		GA	53	56.00 $\pm$ 32.76	
	CC	42	45.12 $\pm$ 31.53		GG	4	17.78 $\pm$ 9.577	

* p < 0.05

with lower lung function who might be more effective in anti-IL-6R therapy.

4.4. No significant differences were detected between asthmatics and controls in the distribution of the two *IL6R* polymorphisms

In the present study, we failed to find the association of rs2228145 A variant and asthma risk (OR = 0.91, $p = 0.42$). However, the effect of this SNP is perfectly in line with a previous observation of Ferreira et al (Ferreira et al., 2011). The asthma GWAS reported an OR of 1.09 for the allele that increases sIL-6R (rs4129267 T, a SNP in high LD with rs2228145). The increasing allele in the current manuscript is allele C. If the OR is reported for allele A, and the OR for that allele is 0.91, meaning that it decreases the risk of asthma in this population, that is in line with direction of effect of the previous study by Ferreira et al. In fact, the effect size also appears to be the same. The lack of finding a statistically significant association in the current study may be due to a lack of power.

In a recent study by Revez, J.A., et al., the functional *IL6R* polymorphisms rs12083537 G were found to increase asthma risk (OR = 1.039, $p = 0.0419$) (Revez et al., 2013). However, in the present study, the variants showed a null association with asthma risk (OR = 0.87, $p = 0.47$). Possible causes for the discrepancy between our results and those of Revez's study may include the differences in the composition of the study populations, or the smaller sample size used in our study. Moreover, similar to other phenotypes such as schizophrenia (International Schizophrenia et al., 2009) and height (Yang et al., 2010), asthma risk can be influenced by multiple genes with potentially hundreds or thousands of variants that individually explain only a very small fraction of asthma cases. Consistent with this hypothesis, through quantitative genetic scores, Manuel A R Ferreira (Ferreira et al., 2011) observed that each common SNP individually influences asthma risk only weakly (Moffatt et al., 2010). These results suggest that many SNPs are either in linkage disequilibrium with true causal SNPs that influence disease risk weakly, but are common in the population. Alternatively, many SNPs could be in linkage disequilibrium with causal SNPs that strongly increase disease risk but are rare in populations. Rs12083537 may be variants that are in linkage disequilibrium with other true causal variants.

Several limitations of the present study need to be acknowledged. Firstly, there was no correction for descent by the use of principal components. This may have resulted in spurious effects of genetic variants on phenotypes. Secondly, as mentioned above, in this study, a type II error may have resulted from the limited power and suppressed a potential relationship. Thirdly, when interpreting results from this study, it is important to recognize that clinically diagnosed cases are probably exposed to different environmental risk factors. Similarly, we could not exclude the possibility that the controls were undiagnosed asthmatics, given the limited information on their atopic status. Thus, the power to explore associations with asthma may have been reduced. Fourthly, in computing the associations of the *IL6R* genotypes and alleles with asthmatic characteristics, we chose an alpha level of 0.05 for determining statistical significance, which is not strict in the light of the number of tests and the possibility of false positive results should be taken into account. Conclusions in the present study need repeated by a larger sample size and more rigorous statistical tests.

5. Conclusion

In the present study involving 394 Southern Han Chinese patients with asthma, we demonstrated that two independent *IL6R* variants, rs2228145 A/C and rs12083537 A/G were related to the influence of sIL-6R on the lung function of patients with asthma. The rs2228145 C variant was also associated with higher sIL-6R and IgE levels. Increased sIL-6R levels were associated with higher serum IgE levels and lower lung function. However, in a case-control study involving 394 patients and 395 healthy controls, we did not find positive associations of the

two variants with asthma risk. Our results provide evidence that rs2228145 C and rs12083537 G associated with low lung function in patients with asthma and that the rs2228145 A–C variant may affect the expression of sIL-6R and IgE.

Author contributions

Yajun Wang and Huiting Hu: equal contributions to the study's conception and design, the collection and assembly of the data, the data analysis and interpretation, and the writing of the manuscript.

Yajun Wang: Ph.D. Affiliated Hospital of Guangdong Medical College; the revising of the manuscript.

Huiting Hu: Master Candidate, Guangdong Medical College; the revising of the manuscript.

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Yan Zhen: Ph.D. Affiliated Hospital of Guangdong Medical College; the revising of the manuscript.

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Wen Li: Master of Science, Affiliated Hospital of Guangdong Medical College; data analysis and interpretation.

Min Liang: Master of Science, Affiliated Hospital of Guangdong Medical College; collection and assembly of the data, and the data analysis.

Bin Wu: Master of Science, Affiliated Hospital of Guangdong Medical College; data analysis and interpretation.

Guoda Ma: Ph.D., Affiliated Hospital of Guangdong Medical College; study conception and design, financial support, administrative support, and final approval of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.gene.2016.03.026>.

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Supporting Table 1. General characteristics and laboratory findings of patients with asthma and controls.

Characteristics	Groups				p value
	Asthma		Controls		
	Value (mean ± SD)	Measured number (n)	Value (mean ± SD)	Measured number (n)	
Total		394		395	
Age (years)	45.37 ± 15.77		45.23± 12.66		0.8907
Gender					0.972
Male (n, %)		173		173	
Female (n, %)		221		222	
Serum IgE (IU/ml)	542.00 ± 792.00	394	48.00 ± 56.00	129	<0.0001
Serum sIL-6R (ng/ml)	95.6 ± 29.88	66	129.30 ± 46.54	49	<0.0001
Eosinophils (%)	4.90 ± 3.70	394	2.30 ± 1.30	129	<0.0001
Eosinophils (×10 ⁹ /L)	0.40 ± 0.35	393	0.16 ± 0.10	129	<0.0001
Spirometry data					
FEV ₁ (% of predicted)	74.95 ± 24.70	386	91.24 ± 15.38	129	<0.001
FVC (% of predicted)	88.26 ± 44.56	386	88.96 ± 15.03	129	0.8611
FEV ₁ /FVC (%)	72.72 ± 14.97	386	88.61± 8.80	129	<0.001
FEF25-75 (%)	53.87 ± 58.66	386	85.15± 24.26	129	<0.001
Smoking status, n					0.08
(%)					
Current		54		38	
Former		28		20	
Never		312		336	

Supporting Table 2. Genotype and allele frequency distributions of rs2228145 in male and female controls.

Model	Male					Female				
	Genotype	Asthma	Control	OR (95%CI)	p value	Genotype	Asthma	Control	OR (95%CI)	p value
Codominant	A/A	77	82	1.00	0.31	A/A	89	89	1.00	0.56
	C/A	77	81	1.22 (0.76-1.96)		C/A	104	108	0.91 (0.59-1.39)	
	C/C	19	10	0.64 (0.27-1.54)		C/C	28	25	0.70 (0.36-1.34)	
Dominant	A/A	77	82	1.00	0.65	A/A	89	89	1.00	0.46
	C/A-C/C	96	91	1.11 (0.71-1.75)		C/A-C/C	132	133	0.86 (0.57-1.29)	
Recessive	A/A-C/A	154	163	1.00	0.2	A/A-C/A	193	197	1.00	0.33
	C/C	19	10	0.58 (0.25-1.34)		C/C	28	25	0.74 (0.40-1.36)	
Overdominant	A/A-C/C	96	92	1.00	0.24	A/A-C/C	117	114	1.00	0.94
	C/A	77	81	1.31 (0.83-2.06)		C/A	104	108	0.98 (0.66-1.47)	
Allele	A allele	231	245	0.96 (0.68-1.38)	0.84	A allele	282	286	0.86 (0.63-1.16)	0.31
	C allele	115	101			C allele	160	158		

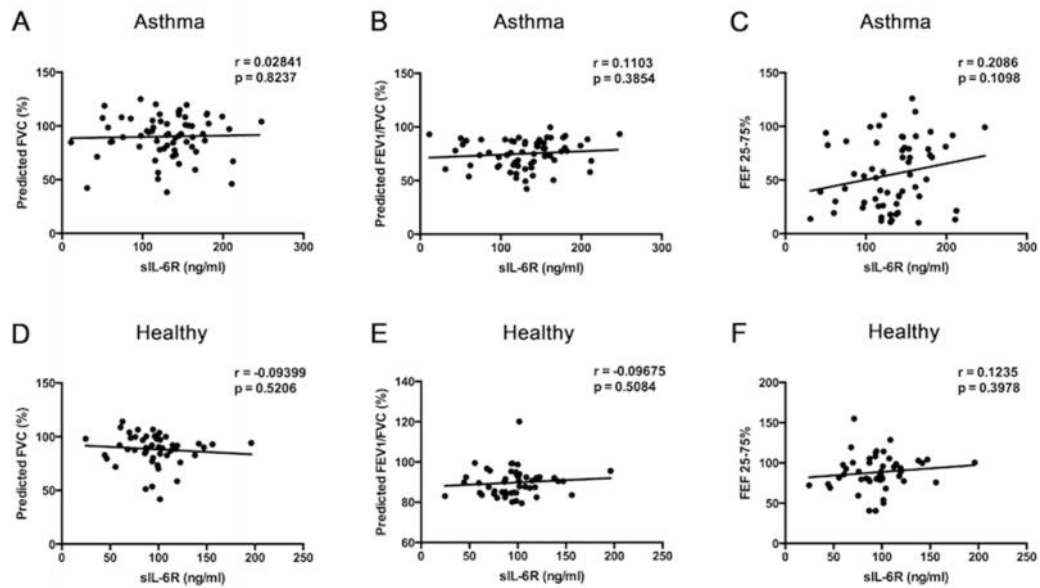
Supporting Table 3. Genotype and allele frequency distributions of rs12083537 in male and female controls.

Model	Male					Female				
	Genotype	Asthma	Control	OR (95%CI)	p value	Genotype	Asthma	Control	OR (95%CI)	p value
Codominant	A/A	145	147	1.00	0.95	A/A	187	190	1.00	0.55
	G/A	27	25	0.94 (0.50-1.74)		G/A	31	30	0.86 (0.49-1.53)	
	G/G	1	1	1.47 (0.07-29.32)		G/G	3	1	0.34 (0.03-3.32)	
Dominant	A/A	145	147	1.00	0.87	A/A	187	190	1.00	0.47
	G/A-G/G	28	26	0.95 (0.52-1.75)		G/A-G/G	34	31	0.82 (0.47-1.42)	
Recessive	A/A-G/A	172	172	1.00	0.8	A/A-G/A	218	220	1.00	0.33
	G/G	1	1	1.48 (0.07-29.59)		G/G	3	1	0.35 (0.04-3.39)	
Overdominant	A/A-G/G	146	148	1.00	0.83	A/A-G/G	190	191	1.00	0.64
	G/A	27	25	0.93 (0.50-1.74)		G/A	31	30	0.87 (0.49-1.55)	
Allele	A allele	317	319	0.97 (0.54-1.73)	0.92	A allele	405	410	0.79 (0.48-1.32)	0.37
	G allele	29	27			G allele	37	32		

Supporting Table 4. Distributions of asthmatic characteristics between IL6R genotypes in controls.

rs2228145					rs12083537			
	Genotype	No.	Mean $\pm$ SD	P value ^a	Genotype	No.	Mean $\pm$ SD	P value ^a
Serum IL6R (ng/ml)	AA	22	91.53 $\pm$ 28.48	0.6934	AA	43	95.37 $\pm$ 31.54	0.9132
	CA	22	98.73 $\pm$ 31.32		GA	5	96.95 $\pm$ 16.10	
	CC	5	99.99 $\pm$ 33.72		GG	1	99.89	
Serum IgE (ng/ml)	AA	61	54.99 $\pm$ 72.04	0.3473	AA	115	49.73 $\pm$ 58.10	0.4410
	CA	57	40.06 $\pm$ 32.66		GA	12	28.81 $\pm$ 21.05	
	CC	11	46.72 $\pm$ 141.94		GG	2	28.40 $\pm$ 27.30	
Peripheral eosinophil (10⁹/ml)	AA	61	0.1591 $\pm$ 0.1034	0.2843	AA	115	0.1607 $\pm$ 0.0982	0.4402
	CA	57	0.1698 $\pm$ 0.0999		GA	12	0.1475 $\pm$ 0.0897	
	CC	11	0.1182 $\pm$ 0.0542		GG	2	0.2450 $\pm$ 0.2333	
Peripheral eosinophil ratio (%)	AA	61	2.270 $\pm$ 1.333	0.5287	AA	115	2.267 $\pm$ 1.374	0.7596
	CA	57	2.313 $\pm$ 1.440		GA	12	2.050 $\pm$ 1.092	
	CC	11	1.816 $\pm$ 0.7221		GG	2	2.750 $\pm$ 1.626	
Predicted FEV₁ (%)	AA	61	94.19 $\pm$ 12.10	0.1138	AA	115	92.06 $\pm$ 13.95	0.2196
	CA	57	88.36 $\pm$ 17.99		GA	12	86.52 $\pm$ 23.29	
	CC	11	89.77 $\pm$ 15.39		GG	2	77.45 $\pm$ 39.10	
Predicted FVC (%)	AA	61	92.65 $\pm$ 12.85	0.0193	AA	115	90.60 $\pm$ 13.41	0.0628
	CA	57	84.93 $\pm$ 15.94		GA	12	83.61 $\pm$ 19.28	
	CC	11	89.40 $\pm$ 17.39		GG	2	71.55 $\pm$ 28.64	
FEV₁/FVC (%)	AA	61	87.20 $\pm$ 6.533	0.2226	AA	115	88.46 $\pm$ 7.875	0.8661
	CA	57	89.99 $\pm$ 10.17		GA	12	89.69 $\pm$ 15.81	
	CC	11	89.19 $\pm$ 11.47		GG	2	90.35 $\pm$ 11.24	
FEF 25~75%	AA	61	86.47 $\pm$ 21.48	0.5636	AA	115	86.06 $\pm$ 22.77	0.5324
	CA	57	85.25 $\pm$ 28.22		GA	12	78.98 $\pm$ 34.63	
	CC	11	77.03 $\pm$ 17.02		GG	2	73.05 $\pm$ 45.89	

Supporting Figure.



Supporting Figure. Correlations between sIL-6R and predicted FVC, FEV₁/FVC or FEF_{25~75}%. The Spearman's rank correlation analysis showed the correlations between sIL-6R levels and FVC, FEV₁/FVC or FEF_{25~75}% in patients with asthma (A, B, C) or healthy subjects (D, E, F).