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Role of interleukin-6 polymorphisms in the development of allergic rhinitis

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ABSTRACT. The aim of this study was to investigate the role played by the *IL-6* rs1800795 (-174G/C) and rs1800796 (-572G>C) polymorphisms in the susceptibility to allergic rhinitis in a Chinese population. A total of 265 patients with allergic rhinitis and 265 controls from our hospital were enrolled in this study. The *IL-6* rs1800795 and rs1800796 polymorphisms were genotyped by polymerase chain reaction coupled with restriction fragment length polymorphism. The results of the χ^2 statistical analysis revealed significant differences in the allele frequencies of *IL-6* rs1800795 between patients with allergic rhinitis and controls (χ^2 = 4.52, P = 0.03). Multivariate logistic regression analyses revealed that individuals with the C allele of IL-6 rs1800795 were susceptible to increased risk of allergic rhinitis, compared to those expressing the G allele (adjusted OR = 1.31; 95%CI = 1.01-1.68). In conclusion, the results of our study indicated that the *IL-6* rs1800795 polymorphism was associated with an increased risk of allergic rhinitis.

Key words: Interleukin-6; Polymorphism; Allergic rhinitis

INTRODUCTION

Allergic rhinitis is a common chronic disorder and atopic disease that usually co-exists with asthma (Bousquet et al., 2001). The mechanistic and pathological characteristics of allergic rhinitis are similar to those of allergic asthma. The clinical symptoms of allergic rhinitis include sneezing, nasal congestion, and rhinorrhea; additionally, this disease affects the peripheral blood, bone marrow, and the lungs (Dávila et al., 2009). The etiology of allergic rhinitis is not well understood; allergic rhinitis is caused by many environmental factors. However, not all of the individuals exposed to similar environmental factors develop allergic rhinitis, which suggests that environmental factors play an important, but not conclusive, role in the development of allergic rhinitis.

Several factors have been reported to play an important role in the pathogenesis of allergic rhinitis (Pawankar et al., 2015; Wan et al., 2015). Disequilibrium in the Th1/Th2 immune response could cause selective eosinophil accumulation in the nasal mucosa and production of allergen-specific immunoglobulin. Interactions between allergen-specific immunoglobulins and inhaled allergens in the upper airway could also play an important role in promoting the inflammatory process of allergic rhinitis (Dávila et al., 2009; Huang et al., 2013). Interleukin-6 (IL-6) is a well-known pro-inflammatory and immunoregulatory cytokine; the *IL-6* gene plays a role in the maintenance of the inflammatory response. Two functional proteins are found in the *IL-6* gene: the secreted C-terminal peptide with cytokine function and N-terminal product with cell cycle control function. Several previous studies have reported that genetic variants of the *IL-6* gene are correlated with various autoimmune diseases (Gu et al., 2008; Gao et al., 2008; Xue et al., 2009). However, few studies have reported the association between the two common SNPs in IL-6 and the development of allergic rhinitis. Therefore, the aim of this study was to investigate the correlations between polymorphisms in *IL-6* at the rs1800795 (-174G/C) and rs1800796 (-572G>C) loci and susceptibility to allergic rhinitis in a Chinese population.

MATERIAL AND METHODS

Patients with allergic rhinitis were recruited from the Yi Du Central Hospital between January 2011 and October 2014. Allergic rhinitis was diagnosed based on the guidelines of the American Thoracic Society (1987). All cases (N = 282) were newly diagnosed; among the identified patients, 265 agreed to participate in our study, with a participation rate of 93.97%. An equal number (N = 265) of healthy subjects without a history of allergic rhinitis were selected from the Yi Du Central Hospital as controls. Each control was age- and gender-matched to the patients. Data regarding the demographic and clinical characteristics of the patients with allergic rhinitis and of the control subjects were collected from their medical records, and their sensitivity to allergens was tested using a commercially available skin prick test.

Signed informed consent forms were obtained from all participants prior to the study. The study protocol was approved by the Clinical Research Ethics Committee of the Yi Du Central Hospital.

DNA extraction and genotyping

Blood samples (5 mL) was obtained from each subject for DNA extraction. DNA was extracted from the buffy-coat fractions using the TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). The *IL-6* rs1800795 and rs1800796 polymorphisms were genotyped by polymerase chain

reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). Primers for PCR amplification and single base extension (SBE) assays were designed using the Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA). The forward and reverse primers for *IL-6* rs1800795 were 5'-GCCTCAATCAGCAGGTAAGC-3' and 5'-CCGGCTGATTCCAAAGGTTA-3' and the ones for rs1800796 were 5'-TGGCAAATTGGAGTCAGTGT-3' and 5'-GGGAAGCCTCCCATTA TGTTG-3'. The PCR was performed using the following cycling program: one cycle of DNA denaturation at 94°C for 5 min; followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min; and a final extension step at 72°C for 5 min. Enzyme digestion followed the identification process. The PCR products were digested with the restriction endonuclease and analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide; the gels were visualized under UV light.

Statistical analysis

Frequencies were used to describe the distribution of categorical variables, and means ± standard deviation was used for continuous variables. Differences between patients with allergic rhinitis and control subjects with respect to frequency distributions of the selected demographic variables and risk factors for allergic rhinitis were evaluated using the chi-squared test or the Student *t*-test. The distributions of genotypes in cases and controls were tested for deviation from the Hardy-Weinberg equilibrium. Logistic regression was performed to assess the association between *IL-6* rs1800795 and rs1800796 polymorphisms and the risk of allergic rhinitis. The results are reported as odds ratios (ORs) and 95% confidence intervals (CIs). $P < 0.05$ indicated a statistical significant difference. Statistical analyses were conducted on the SPSS v.16.0 software platform (SPSS, Inc., Chicago, IL, USA).

RESULTS

The distributions of demographic and clinical characteristics of patients with allergic rhinitis and controls are summarized in Table 1. The mean ages of patients with allergic rhinitis and control subjects were 23.70 ± 7.65 and 24.65 ± 8.10 years, respectively. The study subjects and controls comprised 154 females and 111 males each. We observed no significant differences between patients with allergic rhinitis and controls in terms of age and gender ($P > 0.05$). Among the 265 patients with allergic rhinitis, 202 (76.23%) showed perennial onset and 63 (23.77%) presented seasonal onset; 124 patients (46.79%) were affected as a result of house dust mites, while 59 (22.26%) and 82 (30.94%) patients showed reactions to pollen and mixed allergens, respectively.

We observed significant differences in the allele frequencies of *IL-6* rs1800795 between patients with allergic rhinitis and controls using the χ^2 test ($\chi^2 = 4.52$, $P = 0.03$); however, there were no significant differences in the genetic distributions of rs1800796 ($\chi^2 = 2.51$, $P = 0.11$) (Table 2). The genotypic distributions of *IL-6* rs1800795 and rs1800796 in the controls conformed to the HWE, with P values (for HWE) of 0.43 and 0.42, respectively.

Multivariate logistic regression analyses revealed that individuals with the C-allele of *IL-6* rs1800795 were associated with increased risk of allergic rhinitis compared to the G allele; the adjusted OR was 1.31 (1.01-1.68) (Table 3). However, no significant associations were found between the *IL-6* rs1800796 polymorphism and the development of allergic rhinitis.

Table 1. Characteristics of patients with allergic rhinitis and controls.

Variables	Patients	%	Controls	%	χ^2 test	P value
Age (years)						
<25	144	54.34	139	52.45		
>25	121	45.66	126	47.55	0.19	0.66
Gender						
Females	154	58.11	154	58.11		
Males	111	41.89	111	41.89	0.00	1.00
Time of onset						
Perennial	202	76.23				
Seasonal	63	23.77				
Allergen category						
House dust mite	124	46.79				
Pollens	59	22.26				
Mixed allergens	82	30.94				

Table 2. Distributions of *IL-6* rs1800795 and rs1800796 polymorphisms.

IL-6 gene	Patients	%	Controls	%	χ^2 test	P value	P value for HWE
rs1800795							
GG	88	33.21	107	40.38			
GC	122	46.04	118	44.53			
CC	55	20.75	40	15.09	4.29	0.12	0.43
G allele	298	112.45	332	125.28			
C allele	232	87.55	198	74.72	4.52	0.03	
rs1800796							
GG	99	37.36	107	40.38			
GC	114	43.02	118	44.53			
GG	52	19.62	40	15.09	2.88	0.24	0.42
G allele	312	117.74	332	125.28			
C allele	218	82.26	198	74.72	2.51	0.11	

HWE = Hardy Weinberg equilibrium.

Table 3. Association between *IL-6* rs1800795 and rs1800796 polymorphisms and risk of allergic rhinitis.

IL-6	Patients	%	Controls	%	OR (95%CI)	P value
rs1800795						
GG	88	33.21	107	40.38	1.0 (Ref.)	-
GC	122	46.04	118	44.53	1.26 (0.85-1.87)	0.24
CC	55	20.75	40	15.09	1.67 (0.99-2.83)	0.04
G allele	298	112.45	332	125.28	1.0 (Ref.)	-
C allele	232	87.55	198	74.72	1.31 (1.01-1.68)	0.03
rs1800796						
GG	99	37.36	107	40.38	1.0 (Ref.)	-
GC	114	43.02	118	44.53	1.04 (0.70-1.55)	0.82
GG	52	19.62	40	15.09	1.41 (0.83-2.38)	0.18
G allele	312	117.74	332	125.28	1.0 (Ref.)	-
C allele	218	82.26	198	74.72	1.17 (0.91-1.51)	0.21

OR = odds ratio; CI = confidence interval.

We further analyzed the association between the *IL-6* rs1800795 polymorphism and the development of allergic rhinitis based on the gender, age, time of onset, and allergen category. Logistic regression analyses revealed that the *IL-6* rs1800795 polymorphism was not correlated with the gender, age, time of onset, and allergen category in increasing the risk of allergic rhinitis.

DISCUSSION

In this hospital-based case-control study, we investigated the role of two important polymorphisms in the *IL-6* gene, rs1800795 and rs1800796, in the risk of allergic rhinitis, as well as their interaction with environmental factors in the development of allergic rhinitis. In this study, the *IL-6* rs1800795 polymorphism was found to be associated with an increased risk of allergic rhinitis.

The results of our study showed that the *IL-6* rs1800795 polymorphism was correlated with increased risk of allergic rhinitis; in fact, this significant association existed even after adjusting for confounding variables. The *IL-6* gene is located on chromosome 7p21; the *IL-6* rs1800795 and rs1800796 regions are at the 5'-flanking region of the *IL-6* promoter (Terry et al., 2000). Previous experimental studies have reported that the G allele of *IL-6* rs1800795 is responsible for the production of higher levels of IL-6, compared to the CC genotype; additionally, the G allele of *IL-6* rs1800796 was correlated with higher serum IL-6 levels than those obtained with the CC genotype (Fishman et al., 1998; Jerrard-Dunne et al., 2004). Moreover, studies have also reported correlations between *IL-6* rs1800795 and rs1800796 gene polymorphisms and increased risk of chronic arthritis, lipid abnormalities, insulin resistance, and diabetic nephropathy (Fernández-Real et al., 2000; Pawlik et al., 2005; Zamora-Ginez et al., 2013; Karadeniz et al., 2014).

Some previous studies have reported an association between polymorphisms in the *IL-6* gene and the development of allergic rhinitis (Nieters et al., 2004; Nasiri et al., 2014). Nieters et al. (2004) reported an association between the genetic variants of the *IL-6* gene and the development of hay fever in a German population, while Nasiri et al. (2014) discovered, in a case-control study conducted in an Iranian population, that the rs1800795 polymorphism in *IL-6* was a predisposing factor for allergic rhinitis. However, no studies have reported any associations between the *IL-6* gene polymorphisms and the development of allergic rhinitis in a Chinese population. In our study, the *IL-6* rs1800795 polymorphism was found to be associated with an increased risk of allergic rhinitis. Further studies must be performed to confirm these results.

Our study has several limitations. First, the patient and control subjects were selected from only one hospital, which could cause a selection bias. However, this bias was corrected by matching the controls with the patients. Second, the role of polymorphisms other than those in the *IL-6* gene in the development of allergic rhinitis was not studied. Finally, the limited sample size may lead to a lack of power, which may explain the failure to find an association between the rs1800795 polymorphism and allergic rhinitis.

In conclusion, the results of our study indicate that the *IL-6* rs1800795 polymorphism is associated with an increased risk of allergic rhinitis. Future studies using larger sample sizes and employing similar or different analytical strategies may help elucidate the impact of these polymorphisms on the development of allergic rhinitis.

Conflicts of interest

The authors declare no conflict of interest.

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