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Association between *TNFAIP3* Gene Polymorphisms and Risk of Allergic Rhinitis in a Chinese Han Population

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ABSTRACT

Tumor necrosis factor alpha-inducible protein 3 (TNFAIP3) gene polymorphisms have been reported to be associated with the susceptibility to several immune-related diseases. Here we investigated the effect of *TNFAIP3* gene polymorphisms on the risk of allergic rhinitis (AR) in a Chinese Han population.

The case-control study included 540 AR patients and 524 healthy controls. Genotyping for *TNFAIP3* polymorphisms (rs5029928, rs9494885, rs10499194, rs610604, and rs7753873) were performed using restriction fragment length polymorphism analysis and DNA sequencing. Allele and genotype frequencies were compared between patients and controls. The rs9494885 TC genotype (corrected p (p=0.0032); odds ratio (OR)=2.06, 95% confidence intervals (CI): 1.40-3.04) and C allele (p=0.0056; OR=1.94, 95% CI: 1.35-2.76) were more frequent in AR patients compared with controls.

The frequencies of the rs9494885 TT genotype (p=0.0029; OR=0.49, 95% CI: 0.33-0.72) and T allele (p= 0.0056; OR=0.52, 95% CI: 0.36-0.74) were lower in AR patients than that in controls. A higher frequency of the rs7753873 AC genotype (p=0.0023; OR=1.96, 95 %CI: 1.38-2.77) and C allele (p=0.0012; OR=1.74, 95% CI: 1.26-2.40) and a lower frequency of the rs7753873 AA genotype (p=0.0040; OR=0.53, 95% CI: 0.38-0.75) and A allele (p=0.0012; OR=0.58, 95% CI: 0.42-0.80) were observed in AR patients.

TNFAIP3 gene polymorphisms (rs9494885 and rs7753873) are associated with the susceptibility to AR in the Chinese Han population.

Keywords: Allergic rhinitis; Gene polymorphisms; Risk; Single nucleotide polymorphisms; TNFAIP3

INTRODUCTION

Allergic rhinitis (AR) is a common inflammatory disease of the nasal mucosa, affecting 10-30% of the population worldwide.[1] The prevalence of AR has increased markedly over the last 2 decades in China,

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especially western regions such as Chongqing city.[2] The overall incidence of AR in Chongqing in 2008 was estimated to be 32.3%.[2] Major clinical manifestations of AR include nasal congestion, rhinorrhoea, nasal itching, and sneezing. The etiology of AR is multifactorial including genetic and environmental factors.[3] Single nucleotide polymorphisms (SNPs) in numerous genes such as *IL-23R*, *MRPL4*, and *TNF-α* have been related to the susceptibility to AR.[4,5]

Tumor necrosis factor alpha-induced protein 3 (TNFAIP3) encodes ubiquitin modifying enzyme (A20) that acts as a negative regulator of TNF-induced NF-κB activity.[6] TNFAIP3 plays a key role in modulating inflammatory responses in different biological settings.[7,8] For instance, it has been reported that TNFAIP3 deficiency in myeloid cells causes erosive polyarthritis resembling rheumatoid arthritis.[7] Several lines of evidence indicate that activation of NF-κB signaling contributes to the pathogenesis of AR.[9] Given the regulatory effect of TNFAIP3 on NF-κB activity, we suggest a causal link between TNFAIP3 function and AR development.

SNPs in *TNFAIP3* gene have been identified to be related to the susceptibility to a variety of autoimmune disorders, such as rheumatoid arthritis,[10] systemic sclerosis,[11] systemic lupus erythematosus,[12] psoriasis, [13] and type 1 diabetes.[14] These findings suggest TNFAIP3 as a common risk gene for a number of immune-related diseases. However, there are few reports on the associations between *TNFAIP3* gene polymorphisms and the risk of AR. In this study, we sought to determine the impact of SNPs in TNFAIP3 on the susceptibility to AR in a Chinese Han population.

MATERIALS AND METHODS

Ethics Statement

This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (approval number: CMU-EAR-2012-25). Written informed consents were obtained from all adult participants and from parents or legal guardians of children.

Patients

From May 2012 to June 2013, 540 AR patients aged 6 to 69 years were enrolled in this study. AR was diagnosed according to the Allergic Rhinitis and its Impact on Asthma (ARIA) criteria.[15] The patients presented with 2 or more common symptoms of AR (e.g. nasal congestion, rhinorrhoea, nasal itching, and sneezing) for 4 or more days in each week during the last year before this study. All of them were treated with antihistaminics and topical steroids at the First Affiliated Hospital of Chongqing Medical University (Chongqing, China). A skin prick test (SPT; Allergopharma, Hamburg, Germany) was performed to identify specific allergens in each patient. A total of 18 inhaled allergens were examined, including house dust, grass, tree, mold, food, and weed panel allergens. A positive SPT result was defined as that where a wheal is larger than or equal to one half of the diameter of the histamine control, and at least 3 mm larger than the diameter of that shown by the negative control. A total of 524 hereditarily-unrelated healthy individuals were enrolled as controls. They did not show clinical features or family history of allergies and had not experienced an upper respiratory tract infection within the 4 weeks prior to the study. All subjects of this study were of Chinese Han ethnic origin and from the Chongqing city of China.

SNP Selection and Genotyping

We studied 5 SNPs (i.e., rs5029928, rs9494885, rs10499194, rs610604 and rs7753873) in human *TNFAIP3* gene on chromosome 6q23, which have been documented to be associated with immune diseases.[10] The SNPs rs10499194, rs7753873, and rs9494885 are located in the intergenic region of TNFAIP3, while the SNPs rs610604 and rs5029928 are located in an intron of TNFAIP3.

The 5 SNPs were genotyped by restriction fragment length polymorphism (RFLP) analysis. Briefly, peripheral blood samples were collected from participants and stored at -70°C until use. Genomic DNA was isolated from blood leukocytes using the Qiagen DNA Blood Mini kit (Qiagen, Valencia, CA, USA). Amplification of target DNA was performed by PCR using the primers listed in Table 1. PCR conditions were as follows: initial denaturation at 95°C for 5 min, 37 cycles of denaturation at 95°C for 30 s, annealing at 58-62°C for 30 s, and extension at 72°C for 30 s, and final extension at 72°C for 5 min. The PCR products were digested with specific restriction enzymes (Table 2) at 37°C for at least 4 h. Digestion products were visualized on a 4% agarose gel

Table 1. PCR primers and product sizes

SNP	PCR primers	Product size (bp)
rs5029928	GGGAGAAGAGTTTGAGTAAC	596
	CTC CATTGCCTTAGCTGC	
rs610604	TCCCC TGCTCGCTGTTTT	626
	GCAGACACACTCAAAGGCGC	
rs7753873	AAGTCCCAGATTTGCTCTCCCAG	304
	TTTACACGACAGGCCTCACCAG	
rs9494885	TACCAGCCACATAGCAAGCA	234
	TTTCTCCCACATATGCCCTG	
rs10499194	CCACCTTGAATTTCTTAGCTCTG	444
	TTTGGAG TGCAGTGGCGC	

Table 2. Restriction enzymes and length of restriction fragments

SNP	Restriction enzyme	Length of restriction fragments
rs5029928	*Xap*I (*Apo*I)	CC: 596 bp
		CT: 596 bp, 396 bp, 200 bp
		TT: 396 bp, 200 bp
rs610604	*Sac*I	AA: 626 bp
		AC: 626 bp, 371 bp, 255 bp
		CC: 371 bp, 255 bp
rs7753873	Tsp509I	AA: 169 bp, 135 bp
		AC: 304 bp, 169 bp, 135 bp
		CC: 304 bp
rs9494885	*Hinf*I	TT: 234 bp
		CT: 234 bp, 175 bp, 59 bp
		CC: 175 bp, 59 bp
rs10499194	*Mse*I/ Tru1I	CC: 444 bp
		CT: 444 bp, 342 bp, 102 bp

and stained with Goodview (SBS Genetech, Beijing, China).

Genotypes were confirmed using direct sequencing by the Invitrogen Biotechnology Company (Guangzhou, China) in 20% of randomly selected PCR products.

Statistical Analysis

Call rates for the SNPs studied were compared between AR patients and controls by the χ^2 test. The χ^2 test was also applied to compare demographic characteristics and the allele and genotype frequencies between the patients and controls. Estimation of genotype frequencies was performed by direct counting. The online software platform SHEsis (http://analysis2.bio-x.cn/myanalysis.php) was used to analyze the haplotype and probabilities. We used non-risk alleles as the reference, and tested all the other haplotypes. Logistic regression analysis was used to analyze the genotype allele, controlling for age, gender, and occupation as the co-variables. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to determine the association of SNPs with the risk of AR. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 13.0 for Windows (SPSS Inc., Chicago, Ill. USA). *p* values were corrected (*Pc*) for the number of alleles tested using the Bonferroni correction method. A p_c value of <0.05 was considered statistically significant.

RESULTS

Subject Characteristics

Demographic and clinical characteristics of the participants are presented in Table 3. The patient group consisted of 364 males and 176 females, with a mean age of 32 years (SD=7).

The control subjects included 318 males and 206 females and had a mean age of 33 years (SD=8). There was no significant difference between the 2 groups with respect to mean age and gender distribution. Over 50% of the patients were allergic to house dust mite, 21.9% to pollens, and 20.4% to mixed allergens.

Table 3. Demographic and clinical characteristics of participants

Characteristic	AR patients (n=540)	Healthy controls (n=524)
Age, mean (SD), years	32 (7)	33 (8)
Male/female	364/176	318/206
Allergen category, n (%)		
House dust mite	312 (57.8)	
Pollens	118 (21.9)	
Mixed allergens	110 (20.4)	

Table 4. Frequencies of alleles and genotypes of *TNFAIP3* polymorphisms in AR patients (n=540) and controls (n=524)

SNP	Allele	AR, n (%)	Control, n (%)	*p*	p_c	OR (95%CI)
rs5029928	C	1024(94.8)	994(94.8)	1.000	NS	0.99(0.68-1.46)
	T	56(5.2)	54(5.2)	1.000	NS	1.01(0.69-1.48)
	CC	485(89.8)	472(90.1)	0.919	NS	0.97(0.65-1.45)
	TC	54(10.0)	50(9.5)	0.873	NS	1.05 (0.70-1.58)
	TT	1(0.2)	2(0.4)	0.619	NS	0.48(0.04-5.36)
rs9494885	C	90(8.3)	47(4.5)	0.000377	0.0056	1.94(1.35-2.76)
	T	990(91.7)	1001(95.5)	0.000377	0.0056	0.52(0.36-0.74)
	CC	3(0.6)	2(0.4)	1.000	NS	1.46(0.24-8.76)
	TC	84(15.6)	43(8.2)	0.000216	0.0032	2.06(1.40-3.04)
	TT	453(83.9)	479(91.4)	0.000192	0.0029	0.49(0.33-0.72)
rs10499194	C	1057(97.9)	1016(96.9)	0.219	NS	1.45(0.84-2.49)
	T	23(2.1)	32(6.1)	0.219	NS	0.69(0.40-1.19)
	CC	517(95.7)	492(93.9)	0.212	NS	1.46(0.85-2.53)
	TC	23(4.3)	32(6.1)	0.212	NS	0.68(0.40-1.19)
	TT	0(0.0)	0(0.0)			
rs610604	A	991(0.918)	938(0.895)	0.087	NS	1.31(0.97-1.75)
	C	89(0.082)	110(0.105)	0.087	NS	0.77(0.57-1.03)
	AA	457(0.846)	420(0.885)	0.064	NS	1.36(0.99-1.87)
	AC	77(0.143)	98(0.187)	0.057	NS	0.73(0.52-1.01)
	CC	6(0.011)	6(0.011)	1.000	NS	0.97(0.31-3.03)
rs7753873	A	972(0.900)	985(0.940)	0.000777	0.012	0.58 (0.42-0.80)
	C	108(0.100)	63(0.060)	0.000777	0.012	1.74(1.26-2.40)
	AA	434(0.084)	464(0.885)	0.000266	0.0040	0.53(0.38-0.75)
	AC	104(0.193)	57(0.109)	0.000156	0.0023	1.96(1.38-2.77)
	CC	2(0.004)	3(0.006)	0.682	NS	0.65(0.11-3.88)

OR=odds ratio; 95% CI=95% confidence interval; p_c=corrected *p* value; NS=not significant.

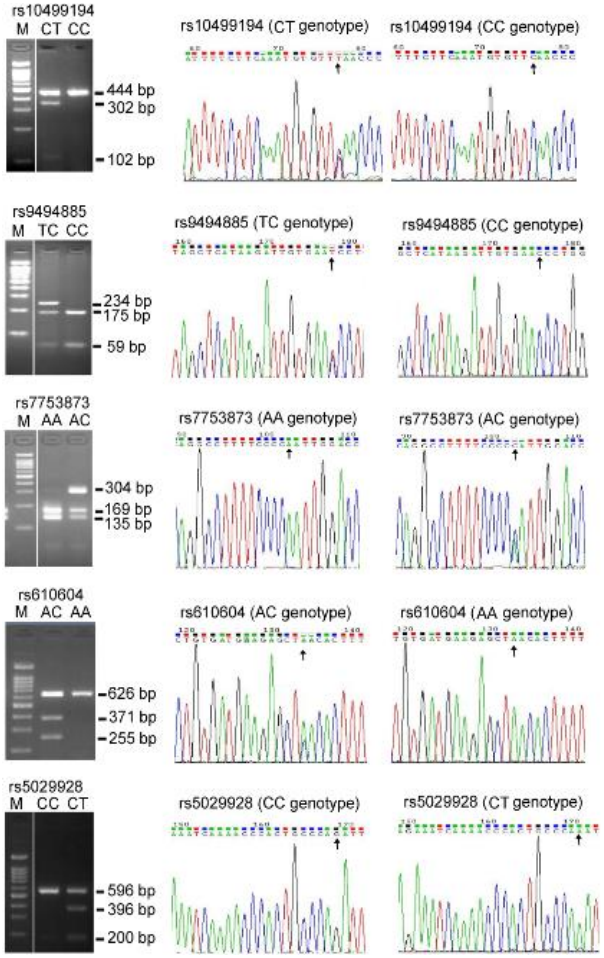

Figure 1. RFLP and DNA sequencing analysis of genotypes at the SNPs (rs5029928, rs9494885, rs10499194, rs610604, and rs7753873) of *TNFAIP3* gene. Left panels: DNA fragment was amplified by PCR and digested with specific restriction enzymes. RFLP products were resolved in 4% agarose gels and stained with Goodview. M: 100 bp DNA marker; CC, TC, AA, AC, GC: genotypes of the SNPs tested. Right panels: Representative sequencing chromatogram results for the SNPs tested.

Associations between the TNFAIP3 Polymorphisms and AR

The genotype distributions of the 5 examined SNPs in the *TNFAIP3* gene were in Hardy-Weinberg equilibrium in both the AR and control groups. The call rate for each SNP was 100%. There were no statistically significant differences in the proportions of missing genotype data between cases and controls (p>0.05). The results of genotypic and allelic frequency analysis are shown in Table 4.

There were significant differences between AR patients and controls concerning the frequencies of SNPs rs9494885 and rs7753873. AR patients showed a significantly higher prevalence of the TC genotype (p_c=0.0032; OR=2.06, 95% CI=1.40-3.04) and C allele (p_c=0.0056; OR=1.94, 95% CI=1.35-2.76) of rs9494885, compared with the controls. In contrast, the frequencies of the TT genotype (p_c=0.0029; OR=0.49, 95% CI=0.33-0.72) and T allele (p_c=0.0056; OR=0.52, 95% CI=0.36-0.74) of rs9494885 were significantly lower in AR patients than that in controls. Compared with the controls, AR patients had a significantly increase in the frequencies of the rs7753873 AC genotype (p_c=0.0023; OR=1.96, 95% CI=1.38-2.77) and C allele (p_c=0.0012; OR=1.74, 95% CI=1.26-2.40) and decrease in the frequencies of the rs7753873 AA genotype (p_c=0.0040; OR=0.53, 95% CI=0.38-0.75) and A allele (p_c=0.0012; OR=0.58, 95% CI=0.42-0.80). The other 3 SNPs examined did not display significant associations with AR. Additionally, linkage disequilibrium structure analysis revealed that the 5 SNPs studied did not exhibit strong linkage disequilibrium with each other.

DISCUSSION

A variety of SNPs in *TNFAIP3* gene region have been identified to be associated with immune diseases. For instance, the 2 SNPs (rs10499194 and rs2230926) in the *TNFAIP3* gene region affect the risk of rheumatoid arthritis in the northern Chinese Han population.[10] Liu et al reported that the rs5029924 SNP in the *TNFAIP3* promoter is associated with the risk of systemic inflammatory response syndrome in acute pancreatitis patients.[16] Zhou et al established the link between the TNFAIP3 rs5029939 SNP and susceptibility to systemic lupus erythematosus in Chinese patients.[17] In this study, we showed that 2 novel SNPs (rs9494885 and rs7753873) in *TNFAIP3* gene have a significant impact on the risk of AR in the Chinese Han population. Our results demonstrated that the rs9494885 TC genotype and C allele were more frequent in AR patients than in healthy controls, suggesting their contribution to the pathogenesis of AR.

In contrast, the rs9494885 TT genotype and T allele had significantly lower frequencies in AR patients than in controls, suggesting their protection against AR. In line with our findings, Li et al identified the rs9494885 SNP of *TNFAIP3* gene as a strong risk factor for Behcet's disease in the Chinese Han population.[18] Our data also demonstrated that the rs7753873 SNP of *TNFAIP3* gene was significantly associated with the susceptibility to AR. The rs7753873 AC genotype and C allele were found to be predisposing factors to AR, while the rs7753873 AA genotype and A allele may confer protection against this disease. However, the rs7753873 and rs9494885 SNPs of *TNFAIP3* are not associated with Fuchs' syndrome in the Chinese Han population.[19] The rs9494885 SNP but not s7753873 SNP of *TNFAIP3* gene is significantly associated with the risk of Vogt-Koyanagi-Harada disease.[20] These findings suggest that TNFAIP3 plays different roles in multiple disease contexts.

The NF-κB pathway has an important role in immunity and inappropriate NF-κB activity has been linked with many autoimmune and inflammatory diseases.[21] TNFAIP3 functions as a negative feedback modulator of NF-κB signaling,[22] thus playing a pivotal role in the pathogenesis of various immune diseases. Indeed, there are significant associations between the SNPs in *TNFAIP3* gene and the susceptibility to autoimmune disorders, including rheumatoid arthritis,[10] systemic sclerosis,[11] systemic lupus erythematosus,[12] psoriasis,[13] and type 1 diabetes. [14] Our data confirmed the impact of *TNFAIP3* gene polymorphisms on the susceptibility to AR, suggesting its involvement in the pathogenesis of this disease. However, the biological function of this gene in AR remains to be further elucidated.

The 5 SNPs selected in this study have been commonly explored in autoimmune diseases such as rheumatoid arthritis,[10] systemic lupus erythematosus,[12] and psoriasis. [13] To ensure the quality of our results, we selected AR patients strictly according to the criteria of ARIA and enrolled unrelated healthy individuals from the same geographic region as that of AR patients. The patients and controls were comparable with regard to age, gender, and ethnicity. To verify the results of genotyping by PCR-RFLP, DNA sequencing was performed on 20% of randomly selected PCR products. The results of the 2 genotyping methods were in complete agreement.

However, some limitations of this study should be noted. First, no information on the association of *TNFAIP3* gene polymorphisms with the AR classification is available. Although the present data reveal no relationship between the SNPs (rs5029928, rs10499194, and rs610604) and the risk of AR, we cannot exclude the possibility that the SNPs may have an impact on the susceptibility to some specific allergens. Second, there is a lack of mechanistic evidence for the role of *TNFAIP3* in the pathogenesis of AR. Finally, the effect of *TNFAIP3* gene polymorphisms on the susceptibility to AR in ethnic populations needs to be explored.

We demonstrated that the 2 SNPs (rs9494885 and rs7753873) of *TNFAIP3* gene are significantly correlated to the susceptibility to AR in the Chinese Han population. Further studies are required to understand the biological function of TNFAIP3 in the pathogenesis of AR.

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