Original Article

Relationship between Foxp3–3279 (rs376158) Polymorphism and Dust Mite Allergic Conjunctivitis

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Abstract

Purpose: To investigate the genotyping of Foxp3-3279 (A/C, rs376158) genes in patients with dust mite-induced allergic conjunctivitis from Guangdong province and to explore the association between these genes and the susceptibility to dust mite allergic conjunctivitis.

Methods: In total, 80 patients with dust mite allergic conjunctivitis and 103 healthy Han Chinese were enrolled in the study and received genotyping of Foxp3-3279 (A/C, rs376158) by PCR-SSP technique.

Results: Genotype frequency of Foxp3-3279 AA, CA, and CC in patients with dust mite allergic conjunctivitis were 1.25%, 25.00% and 73.75%, respectively. Gene frequency of C and A in patients with dust mite allergic conjunctivitis were 86.25% and 13.75% with no significant difference from healthy counterparts (both $P>0.05$).

Conclusion: Foxp3-3279 polymorphisms did not significantly differ between patients with dust mite allergic conjunctivitis and healthy controls, implying that this genetic locus is probably not an independent risk factor of the underlying pathogenesis of dust mite allergic conjunctivitis. (***Eye Science 2014; 29:151–154***)

Keywords: dust mites; dust mites allergic conjunctivitis; foxp3-3279 (rs376158); polymorphisms

Introduction

Allergic conjunctivitis is considered an IgE-mediated type I allergic disease. Previous studies reported that 88.41% of allergic conjunctivitis cases in Guang dong province, China were due to dust mite allergen[1]. This study is designed to investigate the distribution of Foxp3-3279(–rs376158) polymorphisms in patients diagnosed with dust mite allergic conjunctivitis and to provide a preliminary discussion of the molecular mechanism and hereditary factors of dust mite allergic conjunctivitis in a Guangdong population to provide effective prevention and treatment of dust mite allergic conjunctivitis.

Materials and methods

Study materials

Study subjects: The enrolled subjects were unrelated local residents with a similar diet who resided in Guangdong province. The experimental group consisted of 80 patients diagnosed with dust mite allergic conjunctivitis in our hospital between 2007 and 2011; 35 were males, 45 were females, and all were aged 7–61 years. The course of disease ranged from 1 to over 30 years. The diagnostic criteria were drawn largely from "Chinese ophthalmology"[2]. A skin test solution of the allergen was provided by our hospital laboratory. Based on medical history, clinical examination and necessary laboratory tests were conducted for final diagnosis. The control group consisted of 103 healthy volunteers, mainly medical staff from our hospital; 48 were males, 55 were females, and all were aged 20–50 years. The possibility of allergic diseases was excluded. No statistical significance was observed between the two groups regarding gender and age. No participant had a history of malignant tumors or autoimmune diseases. None had used glucocorticoids or other immunodepressants within the recent 4 weeks. Prior to the study, all patients discontinued use of antihistamine and glucocorticoid drugs. Those without immunodeficiency diseases, connective tissue diseases, or dermatographism were

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included. Those complicated with asthma episodes, an acute cutaneous anaphylaxis delayed examination and skin wounds were excluded from this study.

Materials: A DNA extraction kit (Gentra, U.S.) was used. The Foxp3 sequence in GenBank was used to design the primers using Primer Premier 5.0 and the specificity was validated by Blast software[3]. The primer sequences of Foxp3 were: Forward 1: 5′-CTGGCTCTCTCCCCAACTGA-3′, Forward 2: 5′-CTGGCTCTCTCCCCAACTGC-3′, Reverse: 5′-ACAGAGCCCATCATCAGACTCTCTA-3′, synthesized by Sangon Biotech, Shanghai, China. TaqDNA polymerase and dNTP were supplied by Beijing Dingguo Biotech, China.

Methods

Genome DNA was extracted with phenol/chloroform.

PCR-SSP of Foxp3-3279 (A/C, rs376158): A 30 μL reaction system was prepared in a 0.5 mL centrifugal tube as follows: 3.0 μL template DNA, 11.0 μL primer 1, 1.0 μ L primer 2, 3.0 μL DNTP, 3.0 μL 10× PCR buffer, 1.0 μL Taq DNA polymerase, and made up to 30.0 μL with water.

PCR conditions: Pre-denaturation at 94℃ for 3 min, denaturation at 94℃ for 1 min, annealing at 60℃ for 1 min, extension at 72℃ for 1 min for 31 cycles and extension at 72℃ for 5 min. Following PCR amplification, the presence of specific amplified products was confirmed by 1.5% agarose gel electrophoresis.

Allergen detection: Prior to skin tests, adrenalin, promethazine, emergency medicine, oxygen, sputum suction apparatus, and a tracheotomy package were fully prepared. The operating procedures and evaluation of the skin test results were performed according to the standard[4].

Statistical analysis

SPSS 17.0 statistical software was used for data analysis. Allele frequency, genotype frequency, correlation between polymorphism and diseases, relative risk rank, and ratio comparison among groups were statistically analyzed by *chi*-square tests.

Results

The length of the desired amplified Foxp3 was 334bp after PCR amplification.

Reproducibility experiment: A total of 10 samples were used for each test. The PCR amplification for each sample was repeated 5 times and the same results were obtained.

Foxp 3-3279 (A/C, rs376158) allele: The length of amplified Foxp3-3279 products was 333 bp. In both groups, 10% of the test samples were subjected to repeated PCR amplification and the same results were obtained.

Foxp3-3279 allele: For the CC genotype, amplification was observed only when the F2 primer was used; for the AA genotype, amplification was noted only when the F1 primer was used; for the AC genotype, amplification was observed when either the F1 or F2 primer was used (Figure 1). Figure 3 shows the sequence of Foxp3-3279 DNA Further sequencing revealed that the Foxp3-3279 base had a dimorphism of C or A, characterized as three genotypes of CC and AA homozygotes, and an AC heterozygote.

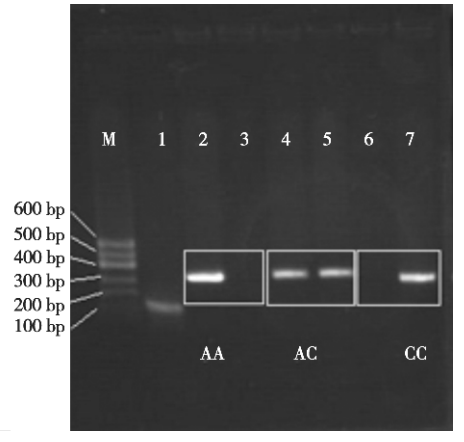

Figure 1 Agarose gel electrophoresis of the Foxp3–3279 allele

Lane 1: β-Actin; lanes 2 and 4: positive PCR amplified products of F1 primer; lane 6: negative PCR amplified products of F1 primer; lanes 5 and 7: positive PCR amplified products of F2 primer; lane 3: negative PCR amplified products of F2 primer; lane M: DNA marker, as illustrated in Figures 1 and 2. The three genotypes of Foxp3-3279 in healthy Chinese subjects were found to conform to Hardy-Weinberg equilibrium ($P>0.05$), suggesting population genetic equilibrium. The data originated from the same Mendelian population, confirming the

reliability of sampling and genotyping results. Sequence of each genotype: the three genotypes of CC, AA, and AC were consistent with PCR results (Figure 3).

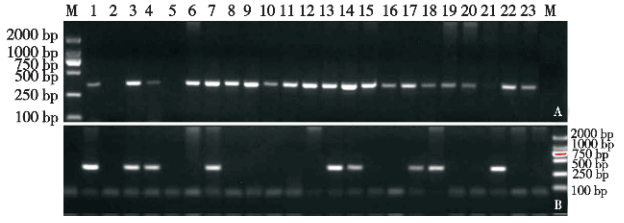

Figure 2 PCR-ARMS of Foxp3-3279 genotype

A:M:DNA marker; 1-2,4-12,14-18,22: positive C allele; 3,13,19-21,23: negative C allele. B:M:DNA marker; 4,7-8,12-14,16-18,20-22: positive A allele; 1-3,5-6,9-11,15,19,23: negative A allele.

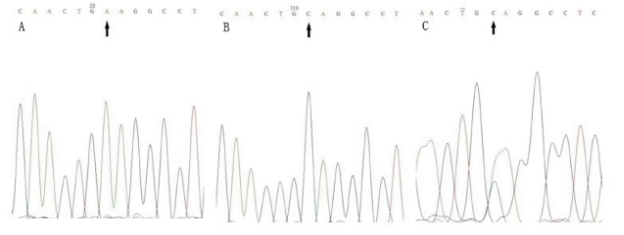

Figure 3 Sequence of each Foxp3-3279 genotype

A: sequence of Foxp3-3279AA genotype; B: sequence of Foxp3-3279AC genotype; C: sequence of Foxp3-3279CC genotype.

Foxp3-3279 genotype and allele frequency: no statistical significance was observed in genotype and allele frequency between two groups, as shown in Table 1.

Discussion

The Foxp3 gene is located on the short arm of the X chromosome (Xp 11.23) and belongs to the family of forkhead/winged-helix transcription factors. Foxp3 mutation mainly consists of a point mutation - an mRNA splicing deletion zipper alteration-which may cause X-linked autoimmunity-allergic dysregulation syndrome (XLLAD). XLLAD patients consistently present with serious eczema, high levels of IgE, eosinophilia, and severe food allergy. Detection of Foxp3 mRNA expression on CD4+CD25+ Treg cells could directly reflect the status of CD4+ CD25+ Treg cells, which play a pivotal role in treatment of multiple autoimmune diseases and allergic illnesses.

Allergic disease refers to the specific condition induced by IgE when the host fights against dust mite, pollen, and animal proteins, *etc.*, and includes such diseases as asthma, allergic rhinitis, allergic conjunctivitis, and atopic dermatitis[5]. Human and animal experiments demonstrated that mucous membrane immune tolerance of intake allergen was correlated with the development of CD4+ CD25+ T cells[6]. Down-regulation of Foxp3 gene expression was also found in the nasal secretion of patients with allergic rhinitis[7]. Allergic conjunctivitis is usually complicated by a variety of allergic diseases. The complicated rhinitis, asthma, eczema, and allergic conjunctivitis share common allergen-induced pathological and physiological reactions in the conjunctiva, nasal mucosa, respiratory tract mucosa, and skin. SNP alteration of Foxp3 is hypothesized to reduce the quantity of CD4+ CD25+ Treg or influence its function, thereby causing immune tolerance dysfunction. Based upon this hypothesis, our study was designed to explore the association between SNP alterations of the Foxp3 gene and the susceptibility to allergic conjunctivitis.

Genotype frequency of Foxp3-3279 AA, CA, and CC in patients with dust mite allergic conjunctivitis were 1.25, 25.00, and 73.75%, respectively. Gene frequency of C and A in patients with dust mite allergic conjunctivitis were 86.25% and 13.75%, with no significant difference from healthy counterparts (both $P>0.05$). Foxp3-3279 polymorphisms did not

Table 1 Foxp3-3279 genotype and allele frequency in the control and study groups

Grouping	Number of cases	Genotype			Allele	
		C/C	C/A	A/A	C	A
Study group	80	59(73.75%)	20(25.00%)	1(1.25%)	138(86.25%)	22(13.75%)
Control group	103	75(72.82%)	25(24.27%)	3(2.91%)	175(85.95%)	31(15.05%)
χ^2		0.02	0.01		0.12	0.12
P		>0.05	>0.05	=0.31	>0.05	>0.05

significantly differ between patients with dust mite allergic conjunctivitis and healthy controls, implying that this genetic locus is probably not an independent risk factor for the underlying pathogenesis of dust mite allergic conjunctivitis.

The relationship between Foxp3 polymorphism and allergic conjunctivitis provides novel evidence for analyzing the molecular genetic mechanism and hereditary factors underlying allergic conjunctivitis. The genes involved with allergic conjunctivitis are complex and diverse and the incidence and progress of allergic conjunctivitis is subject to multiple genes. In certain steps and pathway of diseases, one single genetic variation may exert moderate influence upon the prevalence of diseases, whereas different genetic variations may magnify the influence upon the incidence of illnesses. Consequently, providing an explanation of the holistic process of allergic conjunctivitis is extremely difficult using one single gene or pathway. In addition, the influence of environmental factors upon the incidence of illnesses should not be neglected. Previous studies have demonstrated that for susceptible individuals, whether SNP alteration acts as a protective or risk factor mainly depends upon the degree of exposure to the environment[8]. In the present study, both cases and healthy controls were included, but a larger sample-size study is urgently needed. Additionally, the potential involvement of multiple genes in the same pathway among difference races should be investigated to reach a final conclusion.

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