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GATA3 Gene Polymorphisms Associated with Allergic Rhinitis in an Iranian Population

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Abstract

Background: The development of allergic rhinitis (AR) is caused by the interaction between genetic predisposition and environmental factors. In this study, the association between *GATA3* single nucleotide polymorphisms and AR in an Iranian population was identified.

Methods: This case-control study was performed on 86 patients with AR and 86 healthy subjects. This study aimed to evaluate a potential association between two *GATA3* SNPs, rs1269486 and rs2229360, and AR. Blood samples were collected and DNA was extracted for the evaluation of these SNPs by RFLP-PCR.

Results: A statistically-significant association was found between rs1269486 and AR ($P<0.001$). The frequencies of the A and GA genotypes were less in patients than in controls. The frequencies of the G allele and the GG genotype were greater in patients than in controls ($P < 0.001$).

Conclusions: SNP rs1269486 of *GATA3* was associated with AR and sensitivity to aeroallergens in our population. Because of the significance of this gene in AR, studying the association between *GATA3* polymorphisms and AR is recommended for other populations.

Keywords: Allergic rhinitis, *GATA3*, Genetic, SNP

Introduction

Rhinitis is the most common allergic disease, affecting about one tenth of the worlds' population, and its incidence is increasing globally (1). Some studies have estimated 9-42% prevalence of allergic rhinitis (AR) (2, 3). Unlike asthma, a potential association between genetic factors and AR has not been intensely studied. Recent investigations have begun to identify genes that might be involved in AR (4, 5). Single nucleotide polymorphism (SNP) studies were performed among Asian populations, especially in Japan and Korea, within the last five years (6). The genes encoding cytokines and receptors in patients with AR were investigated the most (4). In this disease, the role of T helper type 2 (T_h2) cells is greater than that of T helper type 1 (T_h1) cells. Interleukin 4, interleukin 13, and *GATA3* transcription factor are the most important contributing factors in increasing T_h2 function (7). Some studies showed an association between AR and gene polymorphism. Trans-acting T-cell-specific transcription factor ***(GATA3)*** is a transcription factor that regulates the T_h2 response and also suppresses IFN gamma (IFNγ)

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and T_h1 cells. *GATA3* is located on chromosome 10 (10 p15) in T cells and contains six exons (8, 9). There is relatively little data about SNP genotypes of *GATA3* compared with other interleukins in AR patients. In 2007, Huebner and colleagues investigated a possible relationship between SNP genotype of *GATA3* (rs1058240) and AR (10). Allergic rhinitis is highly prevalent in Iran and data on genetic factors of this disease among the Iranian population is lacking. This study aims to investigate the relation of SNPs rs1269486 and rs2229360 of *GATA3* and AR.

Materials and Methods

This study was approved by the Ethical Committee of Mashhad University of Medical Sciences. The case-control study included 86 patients with AR and 86 healthy subjects. AR was confirmed by skin allergy test and Allergic Rhinitis and Impact on Asthma (ARIA) guideline criteria in the participants referred to Ghaem Hospital in Mashhad, Iran. The skin allergy test was negative for all the control group subjects. The control group consisted of patients' relatives, Immunology Department staff, students, and volunteers. All the principles of the Helsinki Convention applied to this study. Patients incurred no additional costs. Inclusion criteria were positive history of AR, positive physical examination, and positive skin-prick test. Patients with possible chronic systemic disorder, atopy, and allergic disease, family history of asthma, allergy, or airway diseases, malignant disease, auto-immune disease, lung disease, psoriasis, or any diffuse dermatitis or respiratory infection in the last 30 days before recruitment were excluded from the study. Patients with diagnoses of AR, who referred to the allergy clinic of Ghaem Hospital, were included to the study. The control group consisted of healthy subjects. A questionnaire and informed consent were completed by each participant in the study. The questionnaire included questions regarding signs and symptoms of AR based on ARIA guidelines. All the participants received a skin-prick test. This test contains extracts of common allergens, including aeroallergens, such as pollen, trees, grasses and weeds. After that, the clinical examination and skin-prick test were carried out for allergic investigations.

After confirmation of AR in patients, 5 ml of venous blood were collected from all subjects in falcon tubes containing EDTA. DNA was extracted using an extraction kit (QIAGEN Germany). Genotyping of SNPs rs1269486 and rs2229360 of *GATA3* was performed using the polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). Genotype of the rs1269436 SNP was from type GG, GA, and AA and the genotype of the rs2229360 SNP was from type CC, CT, and TT. Characterizations of SNPs, primer sequences, PCR products sizes, and restriction enzyme digests are presented in Table 1. The associations of *GATA3* SNPs rs1269486 and rs2229360 with AR were evaluated in this study.

Table 1. SNP, primer sequences, PCR product sizes and restriction enzymes

SNP name	Primer sequences	PCR product (bps)	Restriction enzymes
GATA3 (rs1269486)	F = 5'AGG CTC GGG AAA GAG GTG ACA 3' R = 5'GGC TCC TGC CAA TTC ATT CG 3'	333	*BamH*1
GATA3 (rs2229360)	F = 5'CAC TCC AGC CAC ATG CTG AC3' R = 5'TCA CAG ATG GGG TCC AGA TTC3'	285	*BstU*1

Statistical analysis

The frequencies of alleles and genotypes were calculated using the chi-square test. Data were analyzed using SPSS software (version 16). The proportion of *GATA3* polymorphism among cases and controls was compared using the exact version of McNemar's test for matched data. Odds ratios (comparing the odds of *GATA3* negatives among cases versus controls) and associated 95% confidence intervals were computed through exact logistic regression. Haplotypes were analyzed using Epi Info software Ver.7 as the public domain statistical software for epidemiology developed by the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia (USA).

Results

The average age of the patients was 26.6 years and ranged from 15 to 60 years. The average age of the control group subjects was 27.66 years and also ranged from 15 to 60 years. Thirty-six patients (42%) and 40 control group individuals (47%) were male. No statistically significant difference was found between the two groups with regard to age or gender ($P > 0.05$). Fifty patients (58%) had histories of AR. The skin allergy test was performed on all the participants. All the subjects in the control group tested negative, whereas all the patients tested positive. In the patient group, 44 (51%) were sensitive to tree pollen, 75 (79%) were allergic to grass, 82 (95%) were sensitive to weeds, and 40 (45%) were sensitive to dust mites. Patient characteristics are presented in Table 2.

Table 2. Demographic information of patients and controls

Variable	Case group Number 86 (%)	Control group Number 86 (%)
Sex		
Male	36 (42%)	**40 (47%)**
Female	50 (58%)	**46 (53%)**
Age (year)		
Minimum	5	**15**
Maximum	65	**60**
Average	25	**27**
Family history of asthma	50 (58%)	-
Skin prick test		
Trees	44 (51%)	-
Grass	75 (87%)	-
Weeds	82 (95%)	-
mites	40 (47%)	-

Frequencies of GATA3 alleles and genotypes

The frequencies of the GATA3 rs1269486 and rs2229360 SNP alleles and genotypes are listed in Table 3. A significant difference was found between patients and controls for allele A of rs1269486 ($P \leq 0.001$). Similar results were found for allele G ($P= 0.001$), genotype AG ($P< 0.001$), and genotype AA ($P=0.06$). An association was found between the SNP alleles and genotypes in the patients. No significant difference was found between the groups for allele C of the rs2229360 genotype ($P= 0.48$). Similar results were observed for allele T ($P= 0.48$), genotype CT ($P= 0.7$), and genotype CC ($P= 0.8$). No association was found between SNP rs2229360 and either group.

Table 3. Frequency of Alleles and genotypes of GATA3 gene in patient with allergic rhinitis and control group

SNP	Allele and Genotype	Patients	Controls	*P* Value	Odds Ratio (95% CI)
	A	24(28%)	71(83%)	0.001	0.23(0.13-0.39)
	G	148(86.05%)	101(58.8%)	0.001	4.33(2.5-7.34)
rs1269486	**GA**	22(25.59%)	69(80.24%)	0.001	0.084(0.04-0.17)
	AA	1(1.1%)	1(1.1%)	1	1.00(0.06-10.25)
	GG	63(73.25%)	16(18.6%)	0.001	11.98(5.8-24.6)
	C	160(93%)	164(95%)	0.48	0.66(0.26-1.6)
	T	12(14%)	8(9.3%)	0.48	0.66(0.6-3.86)
rs2229360	**CT**	8(9.3%)	8(9.3%)	0.8	1.00(0.35-2.7)
	TT	2(2.3%)	0.00	-	-
	CC	76(88%)	78(91%)	0.8	0.77(0.29-2.08)

Analysis of GATA3 haplotypes

Different GATA3 haplotypes are presented in Table 4. No associations were found between the H1 (AT) or H3 (TG) haplotypes and either group. There was a significant association between H2 haplotypes (AC) and H4 (GC) haplotypes in the patient group ($P < 0.001$).

Table 4. Frequencies of GATA3 haplotypes in patients and controls

Haplotype	Patients	Controls	*P* Value	Odds Ratio (95% CI)
H1 (AT)	2 (2.3%)	0	-	-
H2 (AC)	23 (27%)	72 (84%)	0.001	0.2 (0.12-0.36)
H3 (TG)	10 (11.6%)	7 (8%)	0.6	1.45 (0.45-3.91)
H4 (GC)	137 (80%)	93 (54%)	0.001	3.3 (2.06-5.35)

Discussion

An association between genetic factors and AR was first reported by Haagerup and colleagues (11). Later, this association was confirmed by Yokounchi et al (12). Most of the work to the date has focused on associations between SNPs and AR (12, 13). The aim of this study was to investigate the *GATA3* rs1269486 and rs2229360 polymorphisms in the pathogenesis of AR. Fig. 1 schematically shows the SNPs in our study in locus rs1269486 in the promoter region and in locus rs 2229360, resulting in the stop codon.

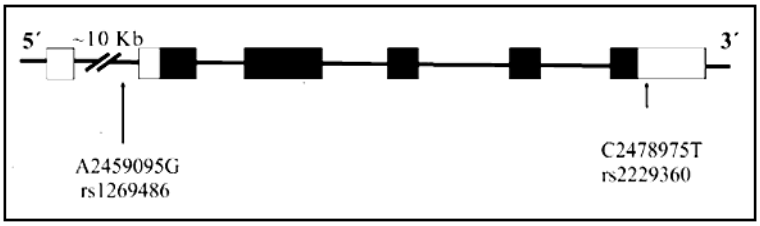

Fig. 1. Location of candidate polymorphisms in *GATA3*. Genotype rs1269486 (A/G) is located in the promoter region and genotype rs2229360 (C/T) is located in the last exon.

The SNPs of these loci were investigated by Zhang and colleagues (14). Their study showed a significant association between *GATA3* genotype, allele, and haplotype polymorphism frequencies in AR patients (15), whereas Pykalainen et. al. found no difference in asthma levels between patient and control groups (8). The percentages of patients and controls with the G allele were 79.1 and 80.7 respectively, whereas percentages for the A allele in rs1269486they in the two groups were 20.9 and 19.3 respectively. Luo and colleagues found a remarkable difference between the GG genotype and G allele frequencies and AR (9). In addition, the GA genotype and A allele frequencies were significantly different between the patient and control groups (13). In our study, the G allele and GG genotype were recognized as risk factors and the A allele and AG genotype as preventive factors for AR. Other studies reported genotype mutations (11) and SNP transcription changes (14) in the RANTS and IL-10 promotor regions, respectively. In these studies, no significant differences were found between patients and controls for C or T alleles of rs2229360 (10). The results of our study were similar to those of previous studies. The frequency of the GC haplotype was significantly greater in our patients than in the controls. However, the frequencies of alleles and haplotypes were not significantly different between the two groups. Conversely, haplotype AC was found in 13% of patients and 42% of healthy subjects ($P < 0.001$).

In our study, the AC genotype appears to have a protective effect in AR, which agrees with the results of the Luo study (9) . Overall, most studies reported cytokine polymorphisms as risk factors for AR in different races. Complex traits such as AR result from interactions between genetic and environmental factors. Although cytokine gene polymorphism variants have been reported in different ethnicities, it seems likely that presently unidentified polymorphisms may influence cytokine function in AR. A better understanding of the pathophysiology of AR and the roles of different cell types in airway inflammation can help us to identify other polymorphisms related to AR. Further studies are needed to investigate an association between cytokine SNPs and AR in Iranian patients of different ethnicities. In addition, identifying associations between cytokine gene polymorphisms and clinical index would aid in the diagnosis and treatments of AR.

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