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The Role of Interleukin-4 and 13 Gene Polymorphisms in Allergic Rhinitis: A Case Control Study

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Abstract

Background: Allergic Rhinitis (AR) is an IgE-mediated inflammatory disorder with high morbidity rates. The eitiology of this disease is understood to occur from a complex interaction between genetic and environmental factors. T helper type 2 cells have been shown to have a crucial role in atopic disease due to their production of the cytokines, intelukin *(IL)-13* and *IL-4*, involved in inflammation. Research has shown single nucleotide polymorphisms (SNP) of the *IL-13* and *IL-4* genes to be associated increased levels of IgE and with allergic diseases such as, allergic rhinitis, asthma, and atopic dermatitis. Specifically, the rs2243250 SNP of *IL-4* and the rs20541 SNP of *IL-13* have been shown to be associated with AR.
Methods: A case-control study was designed to investigate the relationship between the two SNPs rs2243250 and rs20541 with the incidence of AR. The SNPs were examined in patients with AR and healthy controls (86 patients and 86 controls). Blood samples were collected and DNA was extracted to evaluate the SNPs by RFLP-PCR.
Results: Recessive analysis model of the *IL-13* gene (GG vs. AA+AG) revealed that the GG genotype was more common in AR patients (P=0.36) (OR=0.8 [81% CI 0.38-1.6]). For the *IL-4* gene (TC vs. TT+CC), the TC genotype was more common in AR patients (P = 0.0022)(OR=0.71 [60% CI 1.41-5.02]). Furthermore, in the *IL-4* gene, the 590 T>C polymorphism had a significant association with AR. However, no association was found between AR and the *IL-13* rs20541 polymorphism.
Conclusions: Our findings suggest that the *IL-13* polymorphism (rs20541, Exo 4, G>A, Arg130Gln) and *IL-4* polymorphism (rs2243250= C-590T, promoter, T>C) are co-associated with AR and sensitivity to aeroallergens. However, this study used a cohort of AR patients and healthy controls from the northeast of Iran. Given the influence of ethnicity and environment on genetics, further investigation is needed to elucidate the role of SNPs in *IL-4* and *IL-13* in AR among different populations.

Keywords: Allergic rhinitis, Interleukin 4, Interleukin 13, Single nucleotide polymorphism.

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Introduction

Allergic rhinitis (AR) is a highly prevalent inflammatory disorder impacting the upper respiratory tract (1, 2). The development of AR occurs from a complex interaction between environmental and genetic factors. The presence of AR is characterized by elevated levels of plasma immunoglobulin (Ig)E and an overexpression of T helper type 2 (Th2) cytokines (3).

Although the etiology remains to be clearly elucidated, the pathogenesis of AR has been found to involve different inflammatory cells including, mast cells, $CD4^+$ T cells, B cells, macrophages, and eosinophils infiltrating the nasal cavity following exposure to a stimulating allergen (4). In AR, dysregulation of the Th1/Th2 immune response leads to the production and binding of allergen-specific immunoglobulin IgE to mast cells (5, 6). When the upper airway is exposed to allergens, the allergens interact with the IgE antibodies which leads to the release of a number of cytokines, chemokines and inflammatory mediators by mast cells. This leads to the accumulation of T cells, basophils, mast cells, and eosinophils within the nasal cavity (5-7). The release of cytokines, specifically, interleukin *(IL)-4* and *IL-13*, are known to hold a key role in the pathogenesis of AR (4, 8).

Genetic factors have been found to have a strong association with the development and severity of AR, and response to treatment (3, 9, 10). Chromosome 5q31.1 has been largely investigated as this region of the genome contains several candidate genes for allergic disease, including the gene for the Th2 cytokines, *IL-4* and *IL-13* (11). Within allergic rhinitis, the Th2 response predominantly entails T cells infiltrating the nasal cavity and releasing *IL-4* and *IL-13*. The release of *IL-4* and *IL-13* significantly contributes to the IgE-mediated allergic inflammation (5). *IL-4* is a B cell growth factor known to have a large role in the allergic response. Here, *IL-4* and *IL-13* is involved in promoting isotype class switching from IgM to IgE, which encourages T cell differentiation towards a Th2 cell phenotype, and enhances the antigen presenting capacity of B cells (12-14). In addition, *IL-13* shares many biological activities with *IL-4* and holds a critical role in the allergic response as it is also involved in isotype class switching to IgE. However, the release of *IL-13* by Th2 lymphocytes has a role in modulating the inflammatory allergic response and promotes tissue remodeling in an allergic diseases similar to AR (15) .

Single nucleotide polymorphisms (SNPs) in both the *IL-4* and *IL-13* genes have been reported to be associated with allergic responses (15-21). With respect to *IL-4*, the C-590T (rs2243250) SNP located in the promoter region has been previously shown to correlate with an increased risk of AR. This SNP is understood to up-regulate *IL-4* gene expression and subsequently increase the levels of plasma IgE, exacerbating the symptoms of AR (17-20). Furthermore, the *IL-13* gene has several SNPs which are often associated with allergic disorders (21-24). Seven polymorphisms have been reported within the *IL-13* gene however the SNP, Arg130Gln (rs20541), located in exon 4 of the *IL-13* gene, has been identified to have the strongest association with high levels of plasma IgE (21,24) and with the development of AR (19, 21, 25).

In this study, we assumed that the functional polymorphisms in *IL-4* and *IL-13*, rs2243250 and rs20541, respectively contribute to the susceptibility of AR. To test this, we performed a genotype analysis for the C-590T SNP in *IL-4* and the Arg130 Gln SNP in *IL-13* in a cohort of patients from the north east region of Iran.

Materials and methods

Study design and subjects

This study was approved by the Ethical Committee of Mashhad University of Medical Sciences. The case-control study included 86 patients with allergic rhinitis and 86 healthy subjects. AR was confirmed by the skin prick test, and diagnosed according to the Allergic Rhinitis and Impact on Asthma (ARIA) guideline criteria for all participants (2). A questionnaire was provided to each participant, including signs and symptoms of allergic rhinitis based on the ARIA guidelines. Both groups were asked to read and sign informed consent forms. Following this, clinical examination and skin-prick tests were conducted to determine the presence of allergies. Specifically, the skin-prick test, contained extracts of common allergens including aeroallergens (such as pollen, trees, grasses and weeds) and dust mites, was performed to ensure the diagnosis of allergy in all the participants. The skin prick tests were negative in the control group. The control group consisted of relatives of patients, staff in the Immunology department, students, and volunteers. Healthy subjects were placed in the control group. Individuals with possible chronic systemic disorder, atopy and allergic disease, a family history of asthma, allergy and airway diseases were excluded from the control group. The principles of the Helsinki Convention apply to this study and no additional cost was required from the patients. The inclusion criteria were as follows for the AR patients: a positive history of allergic rhinitis, a positive physical examination, and positive skin-prick test. Patients with a diagnosis of allergic rhinitis, who referred to the allergy clinic of Ghaem Hospital, were included in the study. Patients with malignant disease, auto-immune disease, lung disease, psoriasis or any diffuse dermatitis or respiratory infection 30 days prior to recruitment for the study were excluded from the patient group.

DNA extraction

After confirming the presence or absence of allergic retinitis in the participants, 5ml of venous blood was collected from both the patient and control groups in falcon tubes containing EDTA. DNA was extracted using an extraction kit (QIAGEN Germany Cat No. /ID: 69504).

Genotyping

Genotyping of the two SNPs, rs2243250 of the *IL-4* gene and Andrs20541 of the *IL-13* gene, was performed using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). Characteristics of the SNPs, primer sequences, PCR products size and restriction enzymes are provided in Table 1.

Table 1. The characteristics of SNPs, primer sequences, PCR product size and restriction enzymes

Name of SNP	Primer sequence	PCR product (bp)	Restriction Enzyme
IL-4 (rs2243250)	(F)= 5’-CTTCCGTGAGGACTGAATGAGAC-3’ ®=5’GCAAATAATGATGCTTTCGAAGTTTCAG3’	195	AvaII
IL-13 (rs20541)	(F)= 5’-TAAACTTGGGAGAACATGGT-3’ (R)= 5’-TGGGGAAAGATAGAGTAATA-3’	236	NLAIV

PCR was carried out in a thermal cycler in a total volume of 20 µl. This mixture contained 1 µl of each primer, 1 µl of the extracted DNA, 2 µl $MgCl_2$, 0.4 µl of each deoxynucleotide triphosphate, 0.25 µl of Taq DNA polymerase, 2 µl of 10X buffer and 12.35 µl of nuclease-free water. The genotype of the rs2243250 polymorphism was from type CC, CT, and TT, while the genotype of the rs20541 polymorphism was from type AA, CC and AC. The DNA was denatured at 93 °C for 3 min, and temperature cycling was set at 93 °C for 30s, 57 °C (P1) or 62 °C (P2) for 30s, and 72 °C for 2 min, followed by a final extension at 72 °C for 10 min. The sizes of the generated PCR products were 100 bp. The PCR products were cleaved by AvaII for rs2243250 and NLAIV for rs20541 as follows: 25 µl of the PCR product was added to 0.5 µl of the enzyme, and then 2.5 µl of buffer and 5 µl of PCR product was added to 17 µl of sterile distilled water, then incubated at 37 °C for 1 hour prior to running *Polyacrylamide gel* electrophoresis. The digestion produced 210 bp and 26 bp fragments for rs20541, and produced 175 bp and 20 bp for rs2243250.

Statistical analysis

The genotype and allele frequencies were calculated through chi-square test and data analysis was carried out using SPSS software (version 16). The proportion of *IL-4* and *IL-13* polymorphisms among the patients and controls was compared using the exact version of McNemar’s test for matched data. Odds ratios (comparing the odds of IL-4 and IL-13 negatives among cases versus controls) and associated 95% confidence intervals were computed through an exact logistic regression.

Results

The average age of patients in this study was 26.6 years (ranging from 15 to 60 years old), the average age in the control group was 27.66 (ranging from 15 to 60 years old). In the patient group, 36 (42%) were males, while 40 (47%) individuals in the control group were males. There was no significant difference between the two groups regarding age or gender ($P > 0.05$). All patients in this study had a history of AR. The presence or absence of allergy was determined by the skin-prick test in all participants. For all individuals in the control group, the test result was negative and the test was determined positive for all subjects in the patient group. There were 44 patients who were sensitive to tree pollen (51%) and 75 patients (79%) were also allergic to grass. Additionally, 82 (95%) participants were allergic to weeds, and 40 were allergic to dust (45%). Patient characteristics are presented in Table 2.

Table 2. Demographic information for the subjects in the patient and control group

Variable	Case group Number 86(%)	Control group Number 86 (%)
Sex		
Male	36 (42%)	40 (43%)
Female	50 (58%)	46 (54%)
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 (79%)	-
Weeds	82 (82%)	-
mites	40 (40%)	-

Frequency of alleles and genotypes of IL-13 and genes

Enzymatic cleavage of the 195 bp PCR product of the *IL-4* promoter region resulted in two fragments of 175bp and 20 bp. The restriction site is eliminated by C allele. For exon 4 of *IL-13* gene, the 236 bp PCR product was cleaved into two fragments of 210 bp and 26 bp. The restriction site is eliminated by G allele.

The distribution of *IL-4* and *IL-13* genotypes, and allele frequencies of the *IL-4* SNP rs2243250 and *IL-13* SNP rs20541, were analyzed in the patient and control groups (Table 3). Frequency of the rs2243250 (C) allele in AR patients was found to be increased by 39.16% when compared to controls. Furthermore, the distribution of the CC genotype was significantly higher (23.6%) in AR patients compared to controls.

Table 3. Allele and genotypes frequencies of the IL-4 and IL-13 genes in patient with allergic rhinitis and controls.

SNP	Allele Genotype	Case	Control	PV	Odds ratio (95%CI)
rs2243250 T>C	C	47 (28%)	(69%)	0.0001	6.3 (3.8-9.7)
	T	125 (72%)	52 (31%)	0.001	0.1 (0.1-0.2)
	CC	8 (10%)	34 (40%)	0.0001	6.3 (2.7-11.8)
	TC	31 (36%)	52 (60%)	0.0022	0.71 (1.41-5.02)
	TT	47 (54%)	0 (0)	0.0001	0
rs20541 G>A	G	150 (86%)	(80%)	0.23	0.76 (0.42-1.39)
	A	22 (14%)	27 (20%)	0.24	1.31 (0.71-2.2)
	GG	70 (81%)	66 (76%)	0.36	0.8 (0.38-1.66)
	AG	16 (19%)	20 (24%)	0.28	1.32 (0.63-2.7)

The C allele in rs2243250 was associated with an increased the risk of AR by 6.3-fold (95%CI: 3.8-9.7). The CC genotype of rs2243250 in *IL-4* was associated with a 6.3-fold (95% CI: 2.7-11.8) increased risk of AR occurrence. However, the SNP rs20541 in exon 4 of the *IL-13* gene was not found to be associated with the risk of AR.

Discussion

AR is a multifaceted disorder that originates from a complex interaction of genetic and environmental factors that can lead to the dysregulation of the immune system (3, 26). The present study was aimed at evaluating the association of SNPs in the *IL-4* promoter (rs2243250; C-590T) and exon 4 of the *IL-13* gene (rs20541; Arg130Gln) with the incidence of AR in a cohort of patients from northeastern Iran.

It has been well-documented that IL-4 has a critical role in the pathogenesis of AR, particularly in the late phase of the disease (5). Mast cells and T helper cells are the main source of IL-4 promoting the differentiation of Th2 cells and IgE production (5, 27). Our results showed an association between the C-590T SNP in the *IL-4* promoter gene and AR risk. However, the Arg130Gln SNP in *IL-13* showed no significant association with the risk of AR. We also found that the CC genotype and C allele of the C-590T polymorphism had a positive association with the incidence of AR. These finding are in accordance with work from previous studies evaluating the relationship between *IL-4* SNPs and the risk of various allergic-related diseases (18). A large meta-analysis evaluating several published human studies revealed that the T allele and TT genotype of the C-590T polymorphism in the *IL-4* gene promoter have a positive association with AR risk (18).

The promoter region of the *IL-4* gene contains a 590C/T SNP that is understood to interact with nuclear transcription factors and regulate *IL-4* expression. Specifically, the T allele has been shown to enhance the binding of nuclear transcription factors to the promoter region, ultimately upregulating the *IL-4* expression (19, 28, 29). Furthermore, it was found that there is a relationship between *IL-4* -590C/T polymorphisms and IgE levels. The substitution of C with T in the -590 position of the *IL-4* promoter has been observed to increase IgE levels in patients with asthma (30, 31). In China, AR patients with the TT polymorphism have been found to have significantly higher levels of IgE and an increased risk of AR than those with the CT/CC genotypes (19). Contrary to previous findings, Movahedi et al. reported that in AR patients from an Iranian population in Tehran, the CC genotype in the -590C/T SNP within *IL-4* gene was associated with increased risk of AR. Patients with the TT genotype were found to have a negative association with AR (20). The inconsistencies may be explained by the variations in allele frequencies among different ethnic groups. Therefore, the TT genotype in the population in the north east of Iran may be more frequent than in those from Tehran. The contradictory results from our study and the Tehran study are supported by previous research that has shown similar conflicting findings. A separate study observed a protective effect for the CC genotype when compared to the TC and TT genotypes of patients with allergic-related disorders, including AR, asthma, and atopic dermatitis (32). Similarly, additional studies of patients with rhino conjunctivitis and hay fever showed no significant association between the -590C/T SNP and allergy (33, 34). Although we did not observe any association between the Arg130Gln SNP in *IL-13* and a risk of AR, it has been frequently reported that this SNP is associated with plasma levels of IgE and AR risk (19, 22, 31, 35-38). Lack of an association in our study may be due to the differences in ethnic backgrounds and the effect of the environment on the genetic background of our examined population. This is supported by research from a meta-analysis showing that the contradictory findings regarding the association of specific SNPs with allergic rhinitis is likely a result of the genetic variation among different ethnicities and the effect of different environmental factors (38). The various results reported from different studies regarding genetic polymorphisms of cytokines in allergic rhinitis indicate different roles for particular genetic polymorphisms among various ethnicities. Diseases with complex traits such as allergic rhinitis are likely to be explained by complex genetic interactions, as well as the genetic and environmental factors. Therefore, different racial groups could express different polymorphisms within candidate genes.

Due to the differences among various studies examining the relationship between the genetic polymorphisms of cytokines, levels of cytokines production, and gene expression, additional research is necessary to further identify and evaluate the SNPs in genes involved in allergic rhinitis and the relationship with the serum level of inflammatory cytokines and IgE. Furthermore, it is suggested that this genetic relationship be evaluated in other Iranian races. Another suggestion would be to incorporate a greater number of clinical indices to provide a more accurate assessment of the relationship between the genetic polymorphism of cytokines, and the development and severity of allergic rhinitis. These efforts will help to create improved diagnostic and medical treatments for allergic rhinitis.

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The authors declare no conflicts of interest for this report.

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