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IL-13 Gene Polymorphisms and Their Association with Atopic Asthma and Rhinitis in Pakistani Patients

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ABSTRACT

Interleukin-13 (IL-13) is known to be a key regulator in immunoglobulin E (IgE) synthesis, mucus hypersecretion and airway hyperresponsiveness. Single nucleotide polymorphisms in IL-13 are associated with allergic phenotypes in several ethnically diverse populations.

This study was performed in 214 atopic patients (asthma n=108, allergic rhinitis n=106) and sex-matched healthy controls (n=120). Genotyping of IL-13 gene polymorphisms was performed using polymerase chain reaction-based restriction fragment length polymorphism method.

A statistically significant association of the A-1512C polymorphism in IL13 gene was observed with atopy ($p<0.001$; $\chi^2=19.0$). Upon stratification of the data into asthma and AR association was revealed with both asthma ($p=0.01$; $\chi^2=8.80$) and AR ($p<0.001$; $\chi^2=24.3$) in Pakistani patients. Higher odds ratio (OR 95% CI) was observed for AR 3.42 (2.04-5.76) relative to asthma 2.40 (1.41-4.09) for the C allele compared to controls.

In conclusion the study provides the evidence that A-1512C polymorphisms in IL-13 is a risk factor for asthma and AR.

Keywords: Allergic rhinitis(AR); Atopic asthma; Interleukin-13 (IL-13); Polymorphism

INTRODUCTION

IL-13 is a critical cytokine located on chromosome 5q, a region frequently linked to asthma, allergic rhinitis (AR), airway responsiveness, and other related phenotypes. Higher expression of IL-13 at both mRNA and protein level was observed after exposure to allergen, in the sputum and bronchoalveolar lavage (BAL) fluid of the allergic asthma patients and other related traits such as high eosinophil counts and airway hyper-responsiveness.[1-4] In AR, IL-13 was observed to be expressed in the nasal mucosa of patients with perennial atopic rhinitis after allergen provocation. [5, 6]

To support the key role of IL-13 mediator in asthma, several groups have studied IL-13 in mouse

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models. IL-13 null mice were unable to produce goblet cells responsible for mucus over-production in asthma and could not recover the basic IgE levels even upon stimulation by IL-4.[7,8] IL-13 is a major contributor in the development of a late nasal response therefore inhibition of IL-13 may be considered an important therapeutic application in preventing the persistent nasal blockage in AR.[9] To further support function of IL-13 in allergic responses recently several single nucleotide polymorphisms (SNPs) have been reported for their association with the allergic diseases such as asthma and AR. [10, 11] Graves *et al* have identified seven polymorphisms in various parts of the IL-13 gene. Among these polymorphisms two were in the promoter region C-1055T and A-1512C. [12] C-1055T polymorphism was reported with altered regulation of IL-13 and strong involvement of TT genotype in the Dutch population. In a recent meta-analysis of C-1055T SNP in asthma, a strong association was observed in Caucasians. [13] In addition to these two SNPs, one was found in the third intron of the IL-13 gene and three were found in the 3′ untranslated region (G+2525A, C+2580A and T+2749C).[12]

Another important SNP, Gln144Arg also known as +2044 G/A (Arg130Gln) studied in different populations, is present in the coding region of exon 4 and was found to be associated with asthma in British and Japanese populations[10] along with increased total serum IgE (TsIgE) levels in the American and German cohorts. [12] Individuals with the GA +2044 genotype had a 40% increased risk of asthma compared to the Homozygous GG and Asians were considered at high risk compared to Caucasians. Vladich et al investigated the functionality of the IL-13 R130Q variants in peripheral blood cells of normal individuals and showed that the IL-13 130Q variant was more active than the wild type IL-13 130R variant, and was less neutralized by soluble IL-13 receptor-alpha. [14] Similarly, in a meta-analysis of IL-13 SNPs in AR, a strong association of +2044 A allele was observed.[15]

In most of the previous studies the constant genetic associations of IL-13 polymorphisms have been observed with asthma and related traits across diverse ethnic populations. They help to strong contention that IL-13 acts as a major candidate for its association with asthma and atopy. Therefore, the aim of the present case control study was to evaluate the role of IL-13 SNPs in the Pakistani atopic asthma and AR patients.

MATERIALS AND METHODS

Subjects

The case control study included 120 controls and 214 atopic patients (108 atopic asthma patients and 106 AR patients). Selection criteria for the patients and controls were described previously. [16] To be qualified as a control for the study, healthy individual had no history of allergy, and negative skin prick test to different aeroallergens. TsIgE levels were also lower than 100 IU/ml in controls. Patients were diagnosed for atopic asthma according to the following criteria: Skin prick test positive reactions to at least one of the aeroallergens (pollens) being tested as allergens. Allergic asthma patients had a history of attacks of short breathlessness along with wheezing due to chest tightness. Allergic rhinitis patients had symptoms of sneezing, runny nose, nasal obstruction, itchy nose and rhinorrhea. All the patients were recruited from the Allergy Centre of National Institute of Health Islamabad Pakistan.

Genomic DNA and genotyping

This study conforms to the tenets of the Helsinki declaration and has been approved by the Departmental Review and Ethics Committee. All subjects were briefed about the study in their local language and written informed consent was obtained before obtaining their blood samples. Genomic DNA was obtained using a standard phenol chloroform method.[17]

Genotyping of SNPs

Performed for the detection of three polymorphisms in IL-13 gene by using the PCR-RFLP procedure.

Genomic DNA was genotyped for three non-synonymous SNPs located in IL-13: rs20541 (c.431G>A; p.Gln144Arg) in exon 4, rs1881457 (c.-1470A>C) and rs847 (c.*695T>C) in 5’ and 3’ UTR, respectively. The primers given in table 1 were used to amplify products, which spanned polymorphic sites in IL-13 gene. The polymerase chain reaction (PCR) mixture contained 2.5U *Taq* polymerase (Fermentas, Burlington, Ontario), 1x PCR buffer (10mM Tris-HCl, pH 9.0, 50mM KCl, 0.1% Triton X-100, 0.01% gelatin) (Fermentas, Burlington, Ontario), 1.5mM $MgCl_2$, 10pmol each primer, and 50ng DNA. PCR amplification was performed with an initial denaturation at 95 ^{0}C for 5 min, followed by 35 cycles of denaturation at 95^{0}C for 45 s, primer annealing given

Table 1. List of primers used for genotyping IL-13 SNPs

Primers	Primers Sequence	°C†	PCR Product
G+2044A_ F	CTTCCGTGAGGACTGAATGAGACGGTC	60	235
G+2044A_R	GCAAATAATGAGCTTTCGAAGTTTCAGTGGA		
A-1512C _F	CAACCGCCGCGCCAGCGCCTTCTC	65	245
A-1512C_ R	CCGCTACTTGGCCGTGTGACCGC		
C+2749T _F	GGACAGGGACCCACTTCACAC	44	390
C+2749T _R	GCTAACATATTTAATATTTATGTAC		

°C†: annealing temperature

in table 1 for 45 s, extension at 72^0C for 45 s, followed by a final extension for 7 min at 72^0C. PCR products were separated on a 2% agarose gel.

For genotyping of three SNPs rs20541, rs1881457 and rs847, 16 µl aliquot of PCR product was subjected to restriction enzyme digestion at 37 °C overnight with 10 U of *NlaIV*, *BstUI*, and *BsrGI* restriction enzymes, respectively according to the manufacturer's instructions (Fermentas, Burlington, Ontario). The resulting digested products were resolved on 3% agarose gels (Table 2).

Statistical Analysis

The associations between the genotype and allele frequencies in patients compared to controls were analyzed by computing the Pearson chi-square (χ^2) and odds ratio (OR 95% CI) using statistical software StatCalc EpiInfo package v.6 (Atlanta, GA).

RESULTS

The distribution of the IL-13 genotype and allele frequencies of SNPs rs20541, rs1881457 and rs847 were analyzed in asthma and AR patients sensitized to allergens and control individuals.

Table 2. Genomic Sequence polymorphisms Analysis of IL-13 Gene by RFLP

cDNA Coordinates of SNPs	rs #	AA change	Position	R. E	RFLP Fragments
Genomic Sequence polymorphisms Analysis of IL-13 Gene					
c.431G>A	rs20541	p.Gln144Arg	Exon 4	*NlaIV*	G=209,26 A=178,31,26
c. -1470A>C	rs1881457	NA	5'UTR	*BstUI*	A= 214,20,9,2 C=192,22,20,9,2
c.*695T>C	rs847	NA	3'UTR	*BsrGI*	T= 223, 142, 25, C= 223,167

Table 3. Genotypic frequencies of IL-13 polymorphisms in controls and atopic patients

Genotypes	Controls (120)	Patients (214)	*p*-value (χ^2)
Interleukin 13 G+2044A			
GG	47 (39.2)	81 (37.9)	0.24 (2.83)
GA	44 (36.7)	64 (29.9)	
AA	29 (24.1)	69 (32.2)	
Interleukin 13 A-1512C			
AA	97 (80.8)	123 (57.5)	<0.001 (19.06)
AC	18 (15)	64 (29.9)	
CC	5 (4.2)	27 (12.6)	
Interleukin 13 T+2749C			
TT	5 (4.2)	18 (8.4)	0.30 (2.37)
TC	16 (13.3)	31 (14.5)	
CC	99 (82.5)	165 (77.1)	

Table 4. Genotypic frequencies of IL-13 polymorphisms in controls, asthma and AR

Genotypes	Controls (120)	Asthma (108)	*p*-value (χ^2)	AR (106)	*p*-value (χ^2)
Interleukin 13 G+2044A					
GG	47 (39.2)	46 (42.6)	0.52 (1.29)	35 (33.0)	0.11 (4.27)
GA	44 (36.7)	32 (29.6)		32 (30.2)	
AA	29 (24.1)	30 (27.8)		39 (36.8)	
Interleukin 13 A -1512C					
AA	97 (80.8)	70 (64.8)	0.01 (8.80)	53 (50)	<0.001 (24.03)
AC	18 (15)	24 (22.2)		40 (37.7)	
CC	5 (4.2)	14 (13)		13 (12.3)	
Interleukin 13 T+2749C					
TT	5 (4.2)	10 (9.25)	0.21 (3.08)	8 (7.6)	0.19 (3.26)
TC	16 (13.3)	10 (9.25)		21 (19.8)	
CC	99 (82.5)	88 (81.5)		77 (72.6)	

The A-1512C SNP showed a marked difference in genotype frequencies of non-atopic controls and atopic patients [*p*<0.001 (19.06)] while no significant difference was found for G+2044A and T+2749C SNPs when the patients were compared to controls (Table 3).

Upon the stratification of the data according to diseases type, A-1512C SNP was significantly associated with both atopic conditions asthma and AR. In controls, 80.8% individuals had the homozygous wild type genotype compared to patients of asthma (64.8%) and AR (50%). The homozygous CC variant was observed in 4.2% controls relative to 13% in asthma [*p*=0.01 (5.76), OR 0.29 (0.09-0.91)] and 12.3% in AR [*p*=0.02 (5.03), OR 0.31 (0.09-0.98)]. No significant association of the T+2749C SNP was observed either in asthma [*p*=0.21 (3.08)] or in AR [*p*= 0.19 (4.27)] (Table 4).

No significant difference in allelic frequencies was observed for G+2044A polymorphism in asthma [*p*=0.98 (0.00), OR 1.00 (0.68-1.48)] while in AR a significant difference in allele frequencies relative to controls was observed [*p*=0.04 (3.98), OR 1.46 (0.99-2.15)] (Table 5).

DISCUSSION

In the current study we evaluated the role of three SNPs rs20541 (G+2044A), rs1881457 (A-1512C) and rs847 (T+2749C) in the IL-13 gene in allergic asthma and AR patients sensitized to pollens. We did not found association with the G+2044A and T+2749 polymorphisms with both asthma and AR. Although association of A allele in case of G+2044A SNP was observed in a meta-analysis of six populations with AR.[15] Instead of this SNP in Pakistani asthma and AR patients, strong association was observed with the A-1512C polymorphism which might be due to diverse genetic heterogeneity among different populations.

Table 5. Allele frequencies of IL-13 polymorphisms

Alleles	Controls	Asthma	p-value (χ^2)	OR (95% CI)	AR	p-value (χ^2)	OR (95% CI)
Interleukin 13 G+2044A							
G	138 (57.5)	124 (57.4)	0.98 (0.00)	1.00 (0.68-1.48)	102 (48.1)	0.04 (3.98)	1.46 (0.99-2.15)
A	102 (42.5)	92 (42.6)			110 (51.9)		
Interleukin 13 A-1512C							
A	212 (88.3)	164 (75.9)	<0.001	2.40 (1.41-4.09)	146 (68.9)	<0.001	3.42 (2.04-5.76)
C	28 (11.7)	52 (24.1)	(12.10)		66 (31.1)	(25.89)	
Interleukin 13 T+2749C							
T	26 (10.8)	30 (13.9)	0.32 (0.99)	0.75 (0.41-1.37)	37 (17.5)	0.04 (4.11)	0.57 (0.32-1.02)
C	214 (89.2)	186 (86.1)			175 (82.5)		

For the first time, in the British population, evidence was provided for involvement of A-1512C SNP in IL-13 gene with the total IgE levels but functional effect of this polymorphism was not studied. [18]

Our study supported the fact that this SNP alone was enough to change the IgE levels as we found significant association of A-1512C with both asthma and AR.

From the results of the present study, we do believe that the polymorphisms of IL-13 gene are playing an important role in atopic diseases and it might be a good target for the therapeutic intervention. In the previous studies, it was also observed that high levels of IL-13 were expressed in atopic and non-atopic asthma [19, 20], atopic dermatitis [21], and allergic rhinitis. [22] IL-13 is produced by Th2 cells and is important in the patho-physiological allergic reactions and its effects were the hallmark features of the asthma such as increased secretion of the mucus, high levels of IgE, AHR, eosinophil recruitment.[23-26] Therefore, IL-13 has an important role in inflammation and is responsible for driving the epithelial cells into a hypersecretory phase which is a cause of increased allergic reactions in the airways. [27] IL-13 also has an important role in the contraction of the smooth airway muscles and in the development of the allergic airway hyper-reactivity.

In conclusion we found a strong association of A-1512C polymorphism with both asthma and AR in Pakistani patients. Further studies conducted on the functional level are required to contribute to the existing knowledge on these respiratory diseases.

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