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Role of interleukin-4 receptor α polymorphism in patients with asthma and its correlation with asthma severity

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Abstract

Asthma is a heterogeneous disease characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness, and cough that vary over time and intensity, together with variable expiratory airflow limitation. A personal history or a family history of allergy is the factor most strongly associated with the development of asthma. Our primary aim was to investigate interleukin-4 receptor α (IL-4Rα) polymorphism to determine whether the presence of the R576 IL-4Rα allele was associated with asthma and whether the presence of the R576 allele influenced the severity of asthma in affected individuals. The data obtained indicated asthmatic patients were characterized by a higher prevalence of positive family history of asthma ($p<0.001$) as compared to controls. It was found that the patients homozygous for mutant alleles had a 1.39-fold increased risk of asthma compared with individuals not homozygous for R576. Also, we found that females had higher odds (1.61-fold) of significant association with asthma ($p=0.09$; odds ratio=1.58). While this report clearly necessitates a more detailed study, it is plausible that IL-4 mutation has a significant role in the development of asthma and, thus, can play an important role in developing targeted therapy.

Key words: bronchial asthma, genetics, IL-4, IL-4 receptor, TH2 cytokine.

Introduction

Asthma is a heterogeneous disease characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and ~~in~~ intensity, together with variable expiratory airflow limitation [1]. It affects 1–18% of the population in different countries [2]. These variations are triggered by factors such as exercise, allergen or irritant exposure. Triggers may be allergic (e.g., house dust mites, cockroach residue, animal dander, mould, and pollens) or non-allergic (e.g., viral infections, exposure to tobacco smoke, cold air, exercise) stimuli [3]. The pathological process begins with the inhalation of an irritant (e.g., cold air) or an allergen (e.g., pollen), which then, due to bronchial hypersensitivity, leads to airway inflammation, infiltration of inflammatory leukocytes into the lung tissue, epithelial damage, tissue remodelling and an increase in mucus production. Infiltrating leukocytes increase the inflammatory process, thus some of these leukocytes release toxic reactive oxygen species (ROS) into the surrounding tissue, resulting in increased oxidative stress and thus leading to a significant increase in airway resistance, which is most pronounced on expiration [4]. Symptoms and airflow limitation may resolve spontaneously or in response to medication, and may sometimes be absent for weeks or months at a time. On the other hand, patients can experience episodic flare-ups (exacerbations) of asthma that may be life-threatening and carry a significant burden to patients and the community. Even though environmental influences play an important role in the development of asthma, there is a strong genetic predisposition. Children who have one parent with asthma have about a 25% chance for the development of asthma, and those whose mother and father both have asthma may have as high as a 50% risk of disease [5]. A personal history or a family history of allergy is the factor most strongly associated with the development of asthma. A no. of genes and loci have been linked to asthma such as IL4, IL4Rα, HLA complex and chromosomal regions on 5q31-33. Genes for the TH2 cytokines IL-4, IL-5, IL- 9 and IL-13 are also present on the locus 5q31-33. IL4 is a key cytokine in the development of allergic inflammation. It plays a critical role in the regulation of IgE and also triggers the process of isotype switching of IgM to IgE [6]. Also, IL4 acts on TH_0 cells and leads to its conversion to TH_2 cells, thus leading to more production of IL4 and other TH2-derived cytokines, ultimately leading to progression of the allergic cascade [7]. IL4 exerts its action by interacting with its receptor IL4Rα which is also the receptor of IL13 [8]. Thus, upon stimulation of IL-4 or IL-13, Janus tyrosine kinase-1 and 3 are activated, followed by activation of the signal transducer and activator of transcription 6 (STAT 6) which has a central role in IgE production. The central role played by the IL-4Rα in regulating IL-4 and IL-13 responses make it a likely candidate gene for atopy and asthma. Several SNPs have been identified in the IL4Rα gene which results

in amino acid substitutions and thus predispose asthma [9]. One of these polymorphisms is Q576R which consists of an A-to-G transition at nucleotide 1902, causing a change from glutamine to arginine at codon 576 (Q576R) in the cytoplasmic domain of the IL-4Rα [10]. Studies have shown that allergic patients harbouring the Q576R mutation have an enhanced CD23 induction in response to IL-4 in their B-lymphocytes and more chances of having the chronic inflammatory response and thus developing asthma [11]. Individuals who possess 1 or 2 copies of this allele had a significantly increased (9.3-fold) relative risk toward the atopic phenotype compared with the wild-type variant with glutamine at position 576 (Q576) [12] however the effect on asthma and its severity has not been evaluated. Due to the scarcity of information about combinations of genes in the Punjab population, we took asthmatics and normal population and studied IL4Rα polymorphism to determine whether the presence of the R576 IL-4Rα allele was associated with asthma and whether the presence of the R576 allele influenced the severity of asthma in affected individuals.

Materials and Methods

Subjects

103 cases and 103 controls were enrolled in the study who presented to the Department of Chest and Tuberculosis, Government Medical College and Rajindra Hospital, Patiala. Asthma was defined by history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that varied over time and intensity together with variable expiratory airflow limitation. Those patients that fit the definition were subjected to spirometry with reversibility along with clinical history and physical examination. Reversibility was defined as increase or decrease in FEV1 of >12% and >200 mL from baseline. Patients were classified as mild, moderate and severe asthma according to GINA 2020. Mild asthma was defined as asthma well controlled with as-needed ICS-formoterol alone, or with low-intensity maintenance controller treatment such as low dose ICS, leukotriene receptor antagonists or chromones. Moderate asthma was defined as asthma well controlled with low dose ICS-LABA. Severe asthma was defined as asthma that required high dose ICS-LABA, to prevent it from becoming 'uncontrolled', or asthma that remained 'uncontrolled' despite this treatment. Controls were the healthy unrelated volunteers. Informed consent was obtained from all participants in these studies.

Q576R polymorphism

DNA was extracted from 5ml of blood of both cases and controls. Briefly, blood was washed three times with 10mM Tris-HCl (pH 8.0), 320mM sucrose, 1% Triton X-100, and 5mM $MgCl_2$

to remove red cells. The resulting pellet then was resuspended in 400mM Tris-HCl (pH 7.0), 150mM NaCl, 60mM EDTA, 1% SDS, and 100 mg/ml proteinase K and incubated at 42°C for 12– 15 h in an orbital shaker. Deproteinization was carried out by the addition of phenol/chloroform. After mixing and centrifugation for 2 min, the aqueous phase was then transferred to a fresh tube, and DNA precipitated by the addition of an equal volume of isopropanol. DNA recovered was washed with ice-cold 70% ethanol, and resuspended in Tris-EDTA buffer. DNA was genotyped for the IL4RA*Q576R polymorphism by means of restriction enzyme digestion. The region surrounding the polymorphism was amplified with the following primers: 5 -GCC CCC ACC AGT GGC TAC C-3 and 5 -GCC TTG TAA CCA GCC TCT CCT-3 . PCR was performed by using 1 µmol/L of each primer. Amplification conditions were optimized; however, the PCR reaction was carried out at 94°C for 5 minutes, 80°C for the addition of the Taq DNA polymerase, and then 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 second. The PCR product after amplification were of 123 bp in length and then were digested with 2U of MspI restriction enzyme. The digest product was then run on a 2% agarose gel and the banding pattern was studied: 107- and 16-bp fragments from the *Q576 allele and 89-, 18-, and 16-bp fragments from the *576R allele.

Statistics

The allele and genotype frequencies in patients with asthma were compared to a control non-asthmatic population. All the groups were tested for Hardy-Weinberg equilibrium using $\chi 2$ analyses. The alleles frequencies of the IL-4R polymorphisms between controls and patients were assessed using Pearson's two-tailed chi-square test or Fisher's exact test. A p value <0.05 was considered to be statistically significant. Using logistic regression, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the relationship of alleles.

Results

To determine the role of the R576 allele in asthma, 103 cases of asthma were genotyped and the results are summarized in Table 1. No significant group difference in height, weight, BMI, marital status or habitation between cases and controls was found. However, healthy examinees were 14% older as compared to the examined asthma patients ($p = 0.010$). At the same time, asthmatic patients were characterized by a higher prevalence of positive family history of asthma ($p < 0.001$) as compared to controls.

The allele frequency of Q576(wild allele) was found to be 62.13% and 65.05%among cases and controls respectively. The allele frequency of R576 (mutant allele)was found to be 7.77% amongst cases and 5.82% amongst controls. Using wild allele (QQ) as the reference genotype it was observed that the mutant variant (RR) showed some association towards susceptibility

for asthma (p= 0.55). It was found that the patients homozygous for mutant alleles had a 1.39-fold increased risk toward asthma compared with individuals not homozygous for R576 (Table 2).

Our data exhibited that the homozygosity for the R576 allele increased the susceptibility towards asthma, thus we also explored its relation with asthma susceptibility. Patients were classified as mild, moderate or severe asthma according to GINA 2020. IL4 576 allele polymorphism was assessed in these groups. When homozygosity for the R576 allele was compared homozygous it was found that individuals possessing 2 alleles of R576 had a 2-fold susceptibility towards asthma (OR= 1.98; 95%CI=0.64-6.10; p=0.23) (Table 3).

R576 homozygous allele was found to be more associated with patients having a family history of asthma and patients with this homozygous R576 allele had 2-fold increased susceptibility for asthma as compared to patients carrying the wild type genotype (OR=2.03,95%CI=0.52-7.86) (Table 4).

We next analysed whether the interaction of SNP with gender could alter the risk of asthma. Q576 allele was seen in 56.7%(n= 34/60) and 60%(n=36/ 60) amongst female asthmatics and controls respectively. Out of 40 males we studied Q576 allele was found in 75%(n=30/40) and 77.5%(n=31/40) amongst male asthmatics and controls respectively. When studied for homozygosity, homozygous R576 allele was found in 10%(n=6/60) and 6.7%(n=4/60) amongst female asthmatics and controls and amongst males homozygous R576 allele was found in 5%(n=2/20) amongst both male asthmatics and controls. Thus we found that females had higher odds(1.61 fold) of significant association with asthma (p value 0.09 OR= 1.58) (Table 5). However as the sample size was smaller , further studies need to be done to find the definite association.

Discussion

It is well known that asthma has a familial nature and is strongly associated with atopy several genes are responsible for this. Three types of genes have been identified playing a role in the pathogenesis of asthma which are susceptibility genes, disease-modifying genes and drug modifying genes. We studied the susceptibility gene 576R of IL4Rα in our study. As we know IL-4 receptor alpha is a vital element by which extracellular IL-4 and IL-13 cytokines signalling is transduced which affects the immune response. This receptor stimulates the B lymphocytes and then leads to the production of IgE and IgG in response to allergens. The crucial role of IL-4Rα in the pathogenesis of allergic disorders has driven multiple studies aiming toward the development of therapeutic compounds to modulate their function.

We studied the SNP Q576R and found that mutant allele (RR) showed some association towards susceptibility for asthma. The patients with both mutant alleles were 1.39 times more

susceptible to the development of asthma than patients with wild genotype (QQ). On the contrary, the cases that were carrying a single copy of the variant allele *i.e.* heterozygous variant (QR) showed no association towards asthma susceptibility. However, previous reports have shown a strong association of asthma susceptibility with mutant allele and variation in our result may be due to wide ethnic variation. Not many studies have been done in the Indian population to assess the association. Zhang et al studied frequencies of IL-4RA gene in asthmatics and controls in Indian and Chinese population and found no significant difference in the Indian subjects (P>0.05) but the frequency of R576 haplotype was lower in the Chinese asthmatics than the Chinese controls (P = 0.046; odds ratio = 0.511). Thus, the variation in the association can be attributed to different genetic backgrounds and ethnicity.

In addition to increasing the susceptibility to the disease, we also found out that the patients with both the mutant alleles (RR) were twice more likely to have severe asthma than the patients with both the wild alleles (QQ) but was found to be statistically non-significant. However, a study done by Garcia et al. [13] in 2005 found no association of 576R polymorphism with asthma phenotype or with asthma severity but when they analysed 33C>T and 576Q>R polymorphism together they found that patients who carried both the T allele of -33C>T*IL4* and the A allele of 576Q>R*IL4RA* had an increased risk of severe asthma. Also, a study done by Rosa et al in 1999 [12] found that homozygosity for the R576 allele was associated with a severe asthma phenotype (p value=0.15). The varied results in different studies may be attributed to asthma being a complex disease and IL-4R α polymorphisms alone do not always influence the susceptibility or severity of the disease. The severity of the disease also depends on several other gene interactions and also on other factors such as climate condition, occupation and compliance to the treatment and these factors also need to be studied in further studies.

As talked earlier asthma is a disease characterized by familial nature and children of asthmatic parents are at a higher risk of developing asthma. We studied the association of 576R towards predisposing of asthma and found out that patients with a family history of asthma, carrying both the mutant variants (RR) had a 2-fold increased susceptibility for asthma when compared to patients carrying the wild type genotype (OR=2.03,95%CI=0.52-7.86) but was found to be statistically insignificant. This may be due to the small sample size as a very a smaller number of patients with a family history of asthma were enrolled in the study.

In our study, we found that the Q576R polymorphism mutant allele (RR) was found more in female asthmatics than female controls (p value=0.09; Crude OR 1.58) but was statistically non-significant. Also, in males, the mutant allele was not found to be significantly associated amongst asthmatics and controls. But a study done by Muhsen et al. [14] in 2014 found that females had higher odds of significant association with asthma when genders were nested

within A/A genotype or A/G-G/G genotype. We failed to find any significant association between gene polymorphism and gender. Even though the polymorphism was found more in female asthmatics than controls it was statistically insignificant. Ethnic variation may be the reason for this as is the case with IL4 polymorphism and susceptibility to asthma explained above.

Conclusions

We found a significant association of gene mutation increasing the susceptibility of asthma. This study can be used as a reference for future studies directly targeting the treatment according to the mutation as personalised medicine is the future. Targeting IL4 receptor can act as a safe and effective treatment especially in those where corticosteroids cannot be given. Also it can be tried in non-allergic forms of asthma. On the basis of our current knowledge of the differentiation of IL-5-producing TH2-like lymphocytes, this process is also dependant on IL-4 and should be susceptible to suppression by IL-4R therapy. Thus this targeted therapy can act as the new wonder therapy of asthma.

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Table 1. Distribution of individuals (asthmatics and healthy subjects).

Marital status	Married	5(71%)	16 (84%)	64 (83%)	89 (86%)
	Unmarried	2 (29%)	3 (16%)	13 (17%)	14 (14%)
Habitation	Rural	1 (14%)	8 (42%)	28 (36%)	40 (39%)
	Urban	6 (86%)	11 (58%)	49 (64%)	63 (61%)
Smoking	Yes	1 (14%)	3 (16%)	10 (13%)	3 (3%)
	No	6 (86%)	16 (84%)	67 (87%)	100 (97%)
Family history of asthma	Yes	1(14%)	5 (26%)	30 (39%)	1 (1%)
	No	6 (86%)	14 (74%)	47 (61%)	102 (99%)
Symptom duration	Years	6.2±10.5	7.5±8.8	12.5±12.2	
Asthma since childhood	Yes	1 (14%)	5 (26%)	29 (38%)	
	No	6 (86%)	14 (74%)	48 (62%)	
Inhaler usage	Yes	3 (43%)	16 (84%)	60 (78%)	
	No	4 (57%)	3 (16%)	17 (22%)	
Inhaler usage duration	Years	0.03±0.05	2.95±5.6	5.18 ± 8.45	

Table 2. Frequency of R576 and Q576 IL-4Rα alleles in asthmatics and controls.

Genotype (Q576R)	Cases (103), N(%)	Controls (103), N(%)	Crude OR	p-value
QQ	64 (62.13)	67 (65.05)	1.00 (Reference)	
QR	31 (30.10)	30 (29.13)	1.08 (0.58-1.98)	0.78
RR	8 (7.77)	6 (5.82)	**1.39** (0.45-4.24)	0.55
QR+RR	39	36	1.13 (0.64-2.00)	0.66

Table 3. Effect of R576 and Q576 IL-4Rα allelic variants on asthma severity.

Genotype (Q576R)	Cases (103)	Controls (103)	Crude OR	p-value
Severe				
QQ	45	67	1.00 (Reference)	
QR	24	30	1.19 (0.61-2.29)	0.60
RR	8	6	**1.98 (0.64-6.10)**	0.23
QR+RR	32	36	1.32 (0.72-2.43)	0.36
Moderate				
QQ	13	67	1.00 (Reference)	
QR	6	30	1.03 (0.36-2.97)	0.95
Mild				
QQ	6	67	1.00 (Reference)	
QR	1	30		

Table 4. Association of IL4 polymorphism with family history of asthma.

Genotype (Q576R)	Cases (103)	Controls (103)	Crude OR	p-value
No				
QQ	42	67	1.00 (Reference)	
QR	21	30	**1.41 (0.57-2.20)**	0.89
RR	4	6	1.08 (0.29-4.01)	0.89
QR+RR	25	36	1.13 (0.59-2.10)	0.69
Yes				
QQ	22	67	1.00 (Reference)	
QR	10	30	1.01 (0.42-2.40)	0.97
RR	4	6	2.03 (0.52-7.86)	0.30
QR+RR	14	36	1.18 (0.45-2.59)	0.67

Table 5. Association of IL4 polymorphism towards risk of asthma on basis of gender.

Genotype (Q576R)	Cases	Controls	Crude OR	p-value
Males				
QQ	30	31	1.00 (Reference)	
QR	11	10	1.13 (0.42-3.06)	0.80
RR	2	2	1.03 (0.13-7.81)	0.97
QR+RR	13	12	1.11 (0.44-2.80)	0.81
Females				
QQ	34	36	1.00 (Reference)	
QR	20	20	1.14 (0.55-2.37)	0.5
RR	6	4	1.58 (0.41-6.12)	0.09
QR+RR	26	24	1.05 (0.48-2.30)	0.88