

Association of Interleukin-10 Gene Promoter Polymorphism in Allergic Patients

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Aim: Allergic diseases are increasing alarmingly worldwide affecting >30% of the population, including India. Allergy is the result of interaction of the epitopes on the protein with the immunoglobulin E (IgE). T helper cell-2 cytokines promote allergen-specific IgE antibody and induce eosinophil-dominated inflammatory tissue responses. Interleukin-10 (*IL-10*), an antiinflammatory cytokine, plays a major role in the development of the allergy. The cytokine gene polymorphism of $-592C \rightarrow A$ (rs1800872) and $-1082G \rightarrow A$ (rs1800896) of *IL-10* may influence the expression of the protein. Hence, the current study was aimed to evaluate the persistent association between these variants in the susceptibility of the disease. **Methods:** The allelic and genotype frequencies corresponding to *IL-10* ($-592C \rightarrow A$; $-1082G \rightarrow A$) were determined in 94 allergic patients and 100 controls. Genomic typing was performed with polymerase chain reaction with sequence-specific primers. **Result:** The genotype AA at -592 position ($p < 0.000$; odds ratio [OR] 9.92; 95% confidence interval [CI] = 5.06–19.42) and GG at *IL-10*-1082 position ($p < 0.04$; OR = 2.47; 95% CI = 1.003–4.96) was associated significantly in patients compared with controls. A considerable frequency of A-A haplotype in the patients and C-A, C-G haplotypes in controls was observed. A highly noteworthy difference was found in diplotype frequencies of A/A-A/A and A/A-G/A in patients and A/C-G/G and A/C-G/A in the controls. **Conclusion:** Our results indicate that haplotype and diplotype frequencies of the *IL-10* locus may confer susceptibility to allergic patients.

Introduction

ALLERGY IS WIDESPREAD among all population with rising numbers all over the world. Recent studies suggest that 20%–30% of the Indian population are suffering from allergic rhinitis and that 15% develop asthma (Anand *et al.*, 2003). Allergy is characterized by T helper cell-2 (Th2)-type hyperresponse involving various inflammatory cells such as mast cells, eosinophils, T lymphocytes, B lymphocytes, fibroblast, and so on. These cells have the potential to actively participate in the inflammatory process by synthesizing cytokines, which regulate the inflammatory mechanisms (Chung and Branes, 1999; Magnan *et al.*, 2000; Barnes, 2001).

Allergic phenotypes are biologically characterized by an imbalanced T helper cell response skewed toward a Th2 immune response associated with elevated serum immunoglobulin E (IgE) levels (Imboden *et al.*, 2006). High serum total IgE, positive allergen-specific IgE, or skin-prick tests to common allergen are routinely practiced markers for the detection of allergy. But none of these surrogates has high sensitivity

and specificity (Pekkanen and Pearce, 1999; Pearce *et al.*, 2000; Remes and Pekkanen, 2002).

Alternatively, the prototype of T helper type 1 (Th1)/Th2 plays a key role in the pathogenesis of allergy. Earlier studies have reported that high levels of Th2 cytokines such as interleukin-10 (*IL-10*) regulate the immune response (Chung and Branes, 1999) and facilitate the inhibition of interferon- γ and granulocyte macrophage colony-stimulating factor, thus increasing risk of asthma, atopy, and bronchial hyperreactivity (John *et al.*, 1998).

Many of the cytokine polymorphisms specifically expressing high levels of cytokines have been reported to influence susceptibility to various diseases (Bidwell *et al.*, 1999). Presence of a variant allele such as “C” at -592 and “G” at -1082 of *IL-10* gene promoter region has been reported to be involved in the regulation of gene expression (Turner *et al.*, 1997).

Very few studies have been carried out to evaluate the role of these variants in development of allergy. Hence, the current study was carried out to evaluate the association of *IL-10*–

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TABLE 1. BRIEF DESCRIPTION OF POLYMERASE CHAIN REACTION OF THE GENES UNDER STUDY

Cytokine	Primer sequence	Rs number	PCR conditions
IL-10-1082G/A	Forward primer: gtaagcttctgtggctggagtc A reverse primer: 5'-aacactactaaggctctttgggta-3' G reverse primer: 5'-aacactactaaggctctttgggtg-3'	rs1800896	(30 cycles) Denaturation: 94°C Annealing: 60°C Extension: 72°C
IL-10-592C/A	A reverse primer: 5'-atcctgtgacccgcctgta-3' C reverse primer: 5'-atcctgtgacccgcctgtc-3' forward primer: 5'-tgagaaataattgggtcccc-3'	rs1800872	(30 cycles) Denaturation: 94°C Annealing: 60°C Extension: 72°C

PCR, polymerase chain reaction.

592A→C and -1082G→A variants in the susceptibility of allergy in a South Indian population (Hyderabad).

Materials and Methods

Ninety-four allergic patients (asthma, rhinitis, and utricaria) who attended the Allergy Clinic at Mahavir Hospital and Research Center with confirmed diagnosis as per the Allergic Rhinitis and Its Impact on Asthma guidelines and 100 healthy individuals as a control group were included in the study. An equal number of men and women with a mean age of 32.4 years were taken into the study. The subjects below 12 years and above 65 years and pregnant women were excluded. The study was approved by the Institutional Ethics Committee and informed consent was obtained from all the subjects. About 2–4 mL of blood was drawn in an EDTA tube from each subject.

DNA analysis

Genomic DNA was isolated from peripheral blood by using a Qiagen kit (Cat# 51206) according to manufacturer's protocol. The IL-10-1082G→A and -592A→C promoter polymorphisms were evaluated by the method of Shin *et al.* (2005a). The details have been mentioned in Table 1.

Statistical analysis

The genotype association between allergic patients and controls was examined by using odds ratio (OR) with 95%

confidence interval (CI). A *p*-value of <0.05 was considered statistically significant.

Results

Distribution of IL-10-592C→A and IL-10-1082A→G genotypes and allelic frequencies

In the case of IL-10-592C→A polymorphism, the "AA" genotype and the "A" allele were associated significantly with the disease ($p < 0.000$, OR=9.922, 95% CI=5.06–19.42 and $p < 0.00$, OR=3.51, 95% CI=2.293–5.380, respectively). The frequency of the "A" allele was significantly higher in patients (73%) than the controls (44%), while the frequency of C allele is 27% in the patients and 56% in controls (Table 2).

In IL-10-1082G→A polymorphism, genotype frequency revealed that the majority of them were heterozygotes (52% and 64%, respectively) and the GG genotype was significantly associated with the disease ($p < 0.04$; OR=2.47; 95% CI=1.003–4.96). The allelic frequency in patient group was 49% of A and 51% of G, whereas the control group showed 40% of A and 60% of G allele (Table 3).

Frequencies of haplotypes of IL-10-592C→A and -1082A→G genotypes

The analysis of haplotype frequencies at the above mentioned two positions, A-A haplotypes was more frequent in the patients, while in controls the C-A and C-G haplotypes

TABLE 2. DISTRIBUTION OF GENOTYPE AND ALLELIC FREQUENCIES IN PATIENT AND CONTROL GROUPS OF IL-10-592C/A

Variable genotype	Asthma patients	Controls	p-Value	OR value (95% CI)
IL-10 (-592)	n=94	n=100		
A/A	63	17	0.000 ^a	9.922 (5.06–19.42)
C/C	19	29	0.156	0.620 (0.321–1.198)
C/A	12	54	0.000 ^a	0.125 (0.061–0.255)
IL-10 (-592)	n=188	n=200		
A	138 (0.73)	88 (0.44)	0.000 ^a	3.51 (2.293–5.380)
C	50 (0.27)	112 (0.56)	0.000 ^a	0.285 (0.186–0.436)

^a $p < 0.05$.

OR, odds ratio; CI, confidence interval.

TABLE 3. DISTRIBUTION OF GENOTYPE AND ALLELIC FREQUENCIES IN PATIENT AND CONTROL GROUPS OF *IL-10*-1082A/G

Variable genotype	Allergic patients	Controls		
<i>IL-10</i> (-1082)	<i>n</i> = 79	<i>n</i> = 100	<i>p</i> -Value	OR value (95% CI)
A/A	13	28	0.07	0.506 (0.245–1.050)
G/G	14	8	0.04 ^a	2.47 (1.003–6.101)
G/A	52	64	0.875	1.083 (0.585–2.005)
<i>IL-10</i> (-1082)	<i>n</i> = 158	<i>n</i> = 200		
A	78 (0.49)	120 (0.40)	0.08	1.46 (0.961–2.226)
G	80 (0.51)	80 (0.60)	0.087	0.684 (0.449–1.041)

^a*p* < 0.05.

were more frequent. These haplotypes were found to be highly significant at *p* < 0.00 (Table 4).

Frequencies of diplotypes of *IL-10*-592C/A and -1082A/G genotypes

On analysis, highly significant difference was found in diplotype frequencies of A/A-A/A and A/A-G/A in patients, whereas in controls positive association was found in the diplotype frequencies A/C-G/A, respectively (Table 5).

Discussion

Allergic respiratory disorders are associated with considerable patient morbidity, a diminution of productivity, and an increase in health care utilization. Urbanization, air pollution, and environmental tobacco smoke contribute more significantly to the pathogenesis of the disease.

Various routinely used markers with allergy, such as high serum total IgE, positive allergen-specific IgE, or skin-prick tests to common allergen, are in practice and none of these surrogates has high sensitivity and specificity (Pekkanen and Pearce, 1999; Pearce *et al.*, 2000; Remes and Pekkanen, 2002). It is challenging to identify an appropriate disease marker to understand the disease pathology.

The regulatory balance between Th1 and Th2 cytokines regulates the immune response in allergy. It has been reported that high levels of Th2 cytokines lead to elevated total serum IgE (Chung and Branes, 1999; Barnes, 2001). In the Macedonian and Chinese populations it was reported that *IL-10* cytokine gene polymorphisms have been associated with altered levels of cytokines that are susceptible to various autoimmune diseases, allergy, and allograft tolerance (Zhang *et al.*, 2002; Trajkov *et al.*, 2008).

IL-10 being an immunomodulatory cytokine is likely to be a candidate gene. It acts as a potent immunosuppressive cyto-

kine by downregulating the expression of Th1 cytokines and co-stimulatory molecules (Redpath *et al.*, 2001). A comparison of the distribution of *IL-10* polymorphism in the South Indian population with the published data in other populations revealed certain key differences (Chatterjee *et al.*, 2005).

In the current study we observed a significant association of A/A genotype of *IL-10*-592 gene and G/G genotype of *IL-10*-1082G → A gene with the disease.

In the case of *IL-10*-592C → A, the A/A genotype was associated significantly with the disease. Similar results were observed in asthmatics in a North Indian population (Chatterjee *et al.*, 2005) and allergic patients (women) in a French population (Guglielmi *et al.*, 2006), whereas aspirin-intolerant asthma and rhinosinusitis patients in Korean population (Kim *et al.*, 2009), bronchial asthma in Macedonian population, and atopic dermatitis in European population showed no association (Trajkov *et al.*, 2008; Lacy *et al.*, 2009).

In *IL-10* (-1082G → A), the G/G genotype was found to be significantly associated with the disease. Similar results were noted in asthma patients from a North Indian population, atopic dermatitis patients in a Korean population, and food allergy patients in a Japanese population (Chatterjee *et al.*, 2005; Shin *et al.*, 2005b; Campos *et al.*, 2008). In contrast to our study, this was not seen in bronchial asthma patients among adults and children (Macedonian and Chinese populations), or in atopic dermatitis (European population) (Zhang *et al.*, 2002; Trajkov *et al.*, 2008; Lacy *et al.*, 2009).

The allelic and genotype observations made by the current study, however, reveal a strong linkage between the two polymorphisms, which was evident when both alleles were present together in the A-A diplotype. Also a high frequency of C-A and C-G haplotypes in controls presents protection against allergy.

The A/A-G/A and A/C-G/G diplotypes of -1082G → A and -592C → A at the *IL-10* gene were associated significantly

TABLE 4. HAPLOTYPE FREQUENCIES OF *IL-10*, -592C/A AND -1082A/G, IN ALLERGIC PATIENTS AND HEALTHY CONTROLS

Haplotype	-592 C/A	-1082 G/A	Allergy <i>n</i> = 136 (%)	Controls <i>n</i> = 200 (%)	<i>p</i> -Value	OR value (95% CI)
1	A	G	56 (41.2)	72 (36.0)	0.338	1.244 (0.797–1.944)
2	A	A	44 (32.4)	16 (8.0)	0.000 ^a	5.500 (2.963–10.200)
3	C	A	21 (15.4)	64 (32.0)	0.001 ^a	0.388 (0.224–0.671)
4	C	G	15 (11.0)	48 (24.0)	0.003 ^a	0.393 (0.211–0.730)

^a*p* < 0.05.

TABLE 5. DIPTYPE FREQUENCIES OF *IL-10*, -592C/A AND -1082A/G, IN ALLERGIC PATIENTS AND HEALTHY CONTROLS

Diplo type	-592 C/A	-1082 G/A	Allergy n=68 (%)	Controls n=100 (%)	p-Value	OR value (95% CI)
1	A/A	G/G	9 (13.3)	5 (5.0)	0.05 ^a	2.89 (0.967–8.652)
2	A/C	G/G	2 (2.9)	17 (17.0)	0.005 ^a	0.148 (0.037–0.600)
3	C/C	G/G	2 (2.9)	6 (6.0)	0.36	0.475 (0.107–2.136)
4	A/A	A/A	6 (8.8)	2 (2.0)	0.04 ^a	4.72 (1.053–21.13)
5	A/C	A/A	2 (2.9)	2 (2.0)	0.69	1.485 (0.255–8.637)
6	C/C	A/A	2 (2.9)	4 (4.0)	0.717	0.727 (0.152–3.515)
7	A/A	G/A	30 (44.2)	10 (10.0)	0.000 ^a	7.105 (3.194–15.767)
8	A/C	G/A	6 (8.8)	35 (35.0)	0.000 ^a	0.180 (0.073–0.447)
9	C/C	G/A	9 (13.3)	19 (19.0)	0.325	0.650 (0.280–1.515)

^a*p* < 0.05.

with the disease and the results confer that the combined effect at the *IL-10* locus and its possible interactions may influence the susceptibility of disease.

Therefore we conclude that, in terms of sample size, genetic marker included, and haplotype, diplotype analysis of our study is the first of its type in allergic patients from South India. In the future it would be interesting to analyze further the *IL-10* locus and its possible interactions with other risk factors, which would help clinicians in early identification of disease.

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Disclosure Statement

No competing financial interests exist.

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