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ASSOCIATION OF -590C/T INTERLEUKIN-4 GENE PROMOTER POLYMORPHISM WITH ATOPIC ASTHMA IN SOUTH INDIAN PEOPLE

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Abstract: Interleukin-4 (IL-4) is a key cytokine involved in the development of atopy and asthma. The IL-4 promoter region would seem to be the most likely site for polymorphism. Our study was to identify polymorphisms within the promoter region of IL-4 (–590C/T) and check their association with atopic asthma in a group of south Indian population. Polymerase chain reaction based restriction analysis was performed in DNA samples of 56 atopic asthma patients and 42 healthy control subjects of equivalent gender, age, and ethnicity. The genotypic frequency of the IL-4 promoter was significantly greater among patients than controls (p=0.044). A positive association between the IL-4 -590 TT genotype and elevated levels of IgE was confirmed in the study population (p=0.05) with an increased risk for the development of atopic asthma. These results suggest correlation between genetic variability at the promoter of IL-4 gene (-590C/T) and occurrence of atopic asthma.

Keywords: Atopy, Asthma, Il-4 Gene Promoter, genotypic frequency, Single-Nucleotide Polymorphism, yu787 Total IgE.

INTRODUCTION

Allergic asthma is a multifactorial disease, influenced by genetic and environmental factors, characterized by bronchial hyper responsiveness, presence of IgE antibodies to inhalant allergens and enhanced total serum IgE levels (Cookson and Moffatt, 2004). Interleukin-4 (IL-4), which is produced by T-cells and mast cells, is the key cytokine involved in the development of atopy, required for switching of B-cells to IgE production and also promoting eosinophils, chemotaxis and adherence (Brown and Hural, 1997). Asthma and atopy are related conditions caused by a complex gene-environmental interaction (Leung et *al*., 2001) which does not exhibit classical Mendelian patterns of inheritance. Efforts to identifying potential genes associated with asthma have been carried out in various laboratories and because of the complexity and heterogeneity of asthma it has been a daunting task (Van der Pouw Kraan *et al*., 1998). More than 118 genes have been associated with asthma or atopy (Ober and Hoffjan, 2006), of which 54 genes have been replicated in follow-up studies by different investigators suggesting a possible association between asthma or atopy and the specific gene.

IL-4 gene has been mapped to chromosome 5q31 where asthma and atopy have also been linked. The *IL-4* gene is an attractive candidate gene for atopy. Genetic polymorphisms at loci encoding *IL-4* and *IL-4* receptor influence the activity of these genes or their products and are associated with genetic predisposition to atopy and/or elevated serum IgE (Marsh *et al*., 1994). Mutation in the *IL-4* receptor was associated with enhanced signaling activity and was more common in patients with atopy (Hershey *et al*., 1997).

Single nucleotide polymorphisms (SNPs) located in the promoter or coding regions of cytokine genes result in differential cytokine secretion due to altered transcriptional activation. The most common *IL-4* gene promoter variant is the single nucleotide polymorphism (SNP) – 590C/T (NCBI Entrez SNP rs2243250), previously described to be involved in functional gene modification (Rosenwasser, 1995). Such a functional polymorphism in the promoter region of *IL-4* gene may alter IL-4 levels and thereby influence the IL-4 dependent events which determine disease progression. Based on this hypothesis, the association and functional significance of the *IL-4* -590T polymorphism in pulmonary tuberculosis was reported (Luoni *et al*., 2001).

The spanning position between 522 to 774 regions in *IL-4* gene sequence is the promoter region of this gene. The polymorphism within the promoter region of *IL-4* gene seems to correlate with *IL-4* gene transcription. *IL-4* genotype consists of CC, CT and TT. The T- allele may be considered as the severity indicator of asthma.

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Based on the reported associations between susceptibility to asthma and *IL-4* polymorphisms (especially the -590C/T SNP), the aim of this study was to further extend analysis of this candidate gene to evaluate whether the -590C/T SNP polymorphism is a factor contributing to differential genetic susceptibility to atopic asthma in the south Indian population.

MATERIALS AND METHODS

Study subjects:

A total of 98 individuals, 56 patients with atopic asthma and 42 healthy subjects without symptoms or history of asthma or atopy and with negative skin prick tests were recruited from south Indian population. Lung function was measured by spirometry and asthma was diagnosed by physicians according to the ATS (American Thoracic Society) criteria and classified following the GINA (Global Initiative for Asthma) guidelines. Skin prick tests were performed according to EAACI (European Academy of Allergy and Clinical Immunology) recommendations. All participants and volunteers were Indian residing in south India. All experimental procedures were conducted in accordance with the principles set forth in the Declaration of Helsinki. The purpose of the research and the experimental protocols were explained to all participants, and their prior written informed consent was obtained.

Clinical protocol:

Ten milliliters of blood were obtained by veinpuncture from all the participants. Five milliliters of the whole blood were centrifuged for 5 minutes at 5000 rpm to isolate the serum. Total serum IgE quantitated using a standard antibody sandwich ELISA protocol.

DNA preparation and genotyping:

Genomic DNA was extracted from 5 ml of EDTA peripheral blood leukocytes by the standard method (Maniatis *et al*., 1982). The quality of DNA was checked through 0.8% agarose (Sigma, USA) gel electrophoresis, and quantification was done on a UV spectrophotometer (Specgene Ltd, UK). Synthetic oligonucleotides were acquired from Genetix, France. Primers (Forward (*AW41A*) 5'-ACTAGGCCTCACCTGATACG-3', Reverse (*AW41B*) 5'-GTTGTAATGCAGTCCTCCTG-3' for promoter region of IL-4 gene were used as described by Walley and Cookson (1996). Amplification was done in an automated thermocycler (BioRad PTC100, USA). The cycling conditions were 95°C for 3 min followed by 35 cycles at 94°C for 1 min, 57°C for 1 min and 72°C for 1 min, and then a final extension for 5 min at 72°C. The amplified 252-bp PCR fragment was then digested by addition of 1 unit of *BsmFI* (Fermentas, Germany) and incubated for 3 hrs at 65°C to determine the genotypes by the restriction pattern. *BsmFI* recognizes the restriction sequence GGGAC↓, present only in amplified DNA fragments without the polymorphic allele (-590C). This yielded two fragments of 192 and 60 bp in the presence of the wild-type sequence and a single fragment of 252 bp in the presence of the mutation. These products were visualized on a 3% ethidium bromide-stained agarose gel.

Statistical analysis:

All the analyses were performed with SPSS for Windows, Version 11.0 (SPSS Inc., Chicago, IL). The effect of polymorphisms on disease susceptibility were determined using Fisher's exact test and the characteristics of patients and controls were evaluated by comparing clinical findings using the Student's t-test. A two-tailed p value of $p < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows the demographic data for the study groups. There were no statistically significant differences between the patients and control groups ($p > 0.05$) as regarding sex. Out of 56 patients with atopic asthma, 35(62.5%) females and 21 (37.5%) males, showed relatively more prevalence of allergic asthma. Sixty percent of the patients were in the age group of 22- 40 years, the mean age being 32 years. FEV_1 values of >80%, 60-80% and <60% were used to categorize mild, moderate and severe disease, respectively. Serum levels IgE were detectable in all asthmatic subjects and in most healthy controls which showed a positive correlation with each other in the subjects.

Table I: Characteristics of patients and controls.

Clinical profile	Patients	Controls
N	56	42
Age (mean, range)	32.3(14-65)	35.6(18-65)
Sex (female: male)	35:21	22:20
Current smokers (%)	11(19.6)	10 (23.8)
FEV_1% predicted	83.02± 19.01*	98.01 ± 17.11*
FVC% predicted	79.05± 0.70*	91.0 ± 11.03*
IgE serum level(IU/mL)	87± 21.84*	66.28±19.6*

* Mean ± SD, N = number of tested individuals

The genotype distribution of *IL-4* gene promoter –590 in atopic asthma patients and in controls (P=0.044) was significantly different from those expected based on the Hardy–Weinberg equilibrium. The genotype distributions showed greater number of homozygous TT (65.38 %) in patients than in controls (28.57%) and wild type C allele (54.76%) and CC genotype (38.09%) was predominant in the control group as shown in Table 2. Total Serum IgE Levels by -590 *IL-4C/T* genotypes in both patients and control group was analyzed. A significant difference between total IgE among the three genotypes was observed with the greatest variation. The homozygous TT genotype shows a high level of total IgE in patients; obviously control group displays homozygous CC

genotype (Table 3). This indicates that the high serum total IgE levels of the patients in the study were largely due to the presence of the TT genotype at the -590 position of the IL4 gene.

Table II: Genotype and allele frequencies at -590 *IL*-4C/T gene promoters in patients and controls.

Genotypes/ Alleles		Patients(n=56)	Controls(n=42)	OR (95% CI)
Genotypes	CC	10(19.23%)	16(38.09%)	
	CT	12(23.07%)	14(33.33%)	0.729 (0.694-0.765)
	TT	34(65.38%)	12(28.57%)	0.302 (0.2856-0.319)
	P value	0.044		
Alleles	C	32(30.76%)	46(54.76%)	
	T	80(76.92%)	38(45.23%)	0.330 (0.14 -0.35)
	P value	0.22		

Table III: Distribution of Total Serum IgE Level among -590 *IL*-4C/T Genotypes of patients and controls

IgE level (IU/ml)	Genotype	Frequencies	
		Patients	Controls
< 1000	TT	0.492	0.264
	CT	0.294	0.312
	CC	0.214	0.424
> 1000	TT	0.607	0.240
	CT	0.261	0.233
	CC	0.132	0.527

DISCUSSION

Interleukin - 4 gene and nearby markers located on chromosome 5 to atopy and asthma (Noguchi *et al*., 1998; Kabesch *et al.,* 2003). Association of total serum IgE levels to the IL-4 gene locus has been reported previously in different population studies (Postma *et al*., 1995; Xu *et al*., 1995; 2005; Amirzargar, *et al.,* 2009) has shown that those polymorphisms within the promoter region of IL-4 gene may be coupled with enhanced *IL-4* activity; a C to T transition at position -590 in the *IL-4* promoter. Data on the correlation between T–590C and bronchial asthma are controversial (Gervaziev *et al*., 2006).

Unambiguous genotypes for the -590 C/T promoter polymorphism in the IL-4 gene were attained for 56 patients with atopic asthma and 42 control subjects in this study. Our results have shown that *IL-4*-590T allele may be a risk factor for the development of asthma and atopy in the south Indian population. These results support earlier association studies of this polymorphism with asthma (Abe *et al*., 1992) and atopy (Borish *et al.,* 1994; Arai *et al*., 1989; Hosseini-Farahabadi, *et al*., 2007). This was in disagreement with a north Indian study which reported negative association of asthma and IL-4 gene polymorphism (Binjanzadeh *et al*., 2010).

The significantly high serum total IgE levels among individuals carrying the T allele may be attributed to the presence of the SNP at the 5'- flanking region of the IL-4 gene. The –590C/T polymorphism is located in one of the unique binding sites for the nuclear factor of activated T cell which plays an important role in the transcription of several cytokine genes and correspondingly the increased serum IgE levels and the occurrence of atopy (Hoey *et al*., 1995).

Similarly, previous studies have found associations between *IL-4*-590T and total serum IgE in American whites (Borish *et al*., 1994) but not in Australian and British whites (Arai *et al*., 1989). A weak association was detected between *IL-4*-590T and specific IgE to specific allergens. It was shown that *IL-4* -590T was associated with asthma but not with total and specific IgE levels in Japanese children (Abe *et al.,* 1992).

CONCLUSION

In conclusion, the -590C/T *IL-4* promoter polymorphism is a possible risk factor to the development of atopic asthma in south Indian population. Patients who carried T allele of -590C/T *IL-4* showed an increased risk of allergic asthma. Current study group consisted of a limited number of asthmatic patients and we consider that it would be beneficial to test this hypothesis in advanced studies with larger samples and multiple variables.

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