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A preliminary study on the association of single nucleotide polymorphisms of interleukin 4 (*IL4*), *IL13*, IL4 receptor alpha (*IL4Rα*) & Toll-like receptor 4 (*TLR4*) genes with asthma in Indian adults

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***Background & objectives*: Interleukin 4 (*IL4*) and *IL13* genes are believed to be responsible for inflammation of the airways in asthmatics. These share a common receptor component called *IL4Rα* which is another potentially important candidate gene linked to asthma phenotypes. Another gene Toll-like receptor 4 (*TLR4*) might affect the incidence or progression of asthma through the expression of proinflammatory genes. Several single nucleotide polymorphisms (SNPs) in *IL4*, *IL13*, *IL4Rα* and *TLR4* have been reported to be linked to asthma or related phenotypes in several ethnic populations using linkage studies and association studies. However, the results have not been consistent. We investigated five SNPs (C-589T and C-33T of *IL4*, G+2044A of *IL13*, A+1902G of *IL4Rα*, and A+896G of *TLR4*) in patients with adult onset asthma to evaluate their role in manifestation and severity of asthma.**

***Methods*: Adult (>18 yr of age) patients with asthma (n=100) and healthy controls (n=50) were included in the study. Genotyping was performed using sequenom MassARRAY technology.**

***Results*: The mutant alleles of the C-589T and C-33T SNPs in the promoter region of *IL4* were present in 4 per cent patients with asthma but absent from the control group suggesting that the variations in *IL4* may contribute to asthma occurrence. The SNPs of other genes were seen in both controls and patients.**

***Interpretation & conclusions*: The results suggest the possible association between the genetic distribution of C-589T and C-33T SNPs of *IL4* with asthma in Indian adults.**

Key words Allele - asthma - cytokine - genes - interleukin - SNPs - TLR4

Asthma is a complex disease characterized by reversible airway obstruction and chronic airway inflammation and is associated with a number of intermediate phenotypes such as elevation of the total serum IgE and airway hyper-responsiveness[1]. Multiple studies have been carried out to investigate the causes and the risk factors to developing asthma. It has been suggested that interactions among multiple genes and

environmental factors increase asthma susceptibility. A variety of environmental factors such as allergens, airborne pollutants, tobacco smokes, and viruses are shown to influence the development of allergic diseases including asthma[2].

Genome-wide linkage studies and candidate gene approaches have been used to identify asthma susceptibility genes. Several loci linked to asthma or related phenotypes have been reported using genome-wide linkage studies[3,4]. Human chromosome 5q31-33 is among the regions that has shown linkage to asthma[5]. This region contains cytokine cluster and harbour genes for the T-helper Type 2 (Th2) cytokines such as interleukin 4 (IL4) and IL13. Both these cytokines have been implicated in the pathogenesis of asthma and their associations with asthma and atopy have been reported[6,7]. Two single nucleotide polymorphisms (SNPs) in the promoter region of *IL4* (C-589T and C-33T) and one variant in the fourth exon of *IL13* (G+2044A) have been identified in relation to asthma phenotypes[7-10]. *IL4* and *IL13* also share a receptor component, the α chain of the IL4R (IL4Rα), which is an essential component of both the IL4 and the IL13 signal transduction pathway. The *IL4Rα* is located on chromosome 16q12 and is another potentially important candidate gene linked to asthma[11]. Several *IL4R* polymorphisms have been shown to be associated with a higher risk of atopic asthma. The Q576R variant (G1902A) in exon 12 of the *IL4Rα* was found to be associated with a higher risk of atopy, atopic asthma and variation in IgE levels[12].

Toll-like receptor 4 (TLR4) belongs to the TLR family and is a part of the endotoxin receptor complex which recognizes endotoxin and activates the innate immune system through the expression of proinflammatory genes[13]. TLR function might be involved in the development of asthma phenotypes. An A896G polymorphism in the fourth exon of the *TLR4* is shown to alter the extracellular domain of this receptor and confers hyporesponsiveness to endotoxin in human[14]. The association studies which have been carried out in several ethnic groups to find out the associations of these polymorphisms with asthma or related phenotype have not shown consistent results. In this study, we analyzed five SNPs (C-589T and C-33T of *IL4*, G+2044A of *IL13*, A+1902G of *IL4Rα*, and A+896G of *TLR4*) to determine the involvement of these SNPs in the manifestation and severity of asthma in Indian adults.

Material & Methods

Subjects: The study included 100 adult patients with asthma aged more than 18 yr and 50 non asthmatic controls. The asthma patients were selected consecutively from patients attending a tertiary care asthma center (Allergy, Asthma, and Chest Center, Mysore, India), during 2009-2010. Asthma in the index adult was diagnosed according to Global Initiative for Asthma (GlNA) guidelines[15] with reversible airway obstruction of 12 per cent and 200 ml improvement in forced expiratory volume in 1 sec (FEV1) after inhalation of salbutamol. Spirometry was performed according to American Thoracic Society Guidelines[16]. Patients were categorized based on Global Initiative for Asthma (GINA) guidelines[15] to different asthma severity groups that included 15 patients with mild asthma, 30 patients with moderate and 55 patients with severe asthma. Non asthmatic controls had no history of asthma and were selected randomly from the general population of Mysore. The patients and controls who had other chronic respiratory symptoms were excluded from the study.

The study was approved by the Institutional Human Ethical Committee (IHEC) of the University of Mysore, and informed written consent was obtained from all cases and controls who participated in this study.

SNP genotyping: Five SNPs were selected for this study; two SNPs in the promoter region of *IL4* (C-589T and C-33T), one SNP in *IL13* (G+2044A), one SNP in *IL4Rα* (A+1902G), and one SNP in the *TLR4* (A+896G). The SNP details and sequence data were obtained from NCBI database using the unique accession numbers (*www.ncbi.nlm.nih. gov*). Genomic DNA was extracted from blood using the DNA isolation kit for mammalian blood (Roche, USA, catalogue number: 11667327001, version October 2008) following the manufacturers' instructions. Genotyping of the five SNPs was performed using sequenom MassARRAY technology (Sequenom®, San Diego, CA, USA) at Vimta Labs Ltd in Hyderabad, India. It consisted of Sequenom-iPLEX® Gold SNP genotyping platform with SpectroCHIP® and MALDI-Time of Flight (TOF) Mass spectrometer.

Statistical analysis: Genotypes were tested for Hardy-Weinberg equilibrium in patients and controls separately. Differences in the distribution of genotypes and alleles between groups were estimated by univariate statistical analysis (Chi-square test) on SPSS 18.0. (SPSS Inc., Chicago, IL, USA).

Results & Discussion

The demographic profile of the patients and controls is presented in Table I. Schematic representation of the *IL4*, *IL13*, *IL4Rα* and *TLR4* showing their structural organization and the location of the genotyped SNPs are shown in Fig. 1.

In this study, the homozygous mutant genotype (TT) of both the -589T and -33T SNPs in the promoter region of *IL4* was present in four per cent patients with asthma. These two mutations were absent from the control group (Table II). The normal genotype (CC) of C589T SNP was seen in 65 per cent of the patients and 72 per cent of the controls. The heterozygous genotype (CT) of C-589T SNP was present in 31 per cent patients and 28 per cent controls. In case of C-33T SNP in the proximal promoter region of *IL4*, the normal genotype (CC) was seen in 68 per cent of the patients and 78 per cent of controls. The heterozygous genotype (CT) of C-33T SNP did not show a significant difference between the asthmatics and the controls (Table II).

The SNP genotype and allele frequencies of C-589T, C-33T, G+2044A, A+1902G and A+896G in patients and controls are provided in Table II. No significant differences of genotypes were observed in G+2044A, A+1902G and A+896G SNPs. Dominant alleles were more frequent than the recessive alleles in all the five SNPs of the four genes in this study

Table I. Demographics and clinical characteristics of thestudy population in Mysore

	Patients	Controls (%)
Age (yr)		
20-30	27	17 (34)
31-40	20	13 (26)
41-50	30	7 (14)
>50	23	13 (26)
Gender		
Male	45	21 (42)
Female	55	29 (58)
Family history of asthma	69	13 (26)
Severity		
Mild	15	-
Moderate	30	-
Severe	55	-
Total	100	50

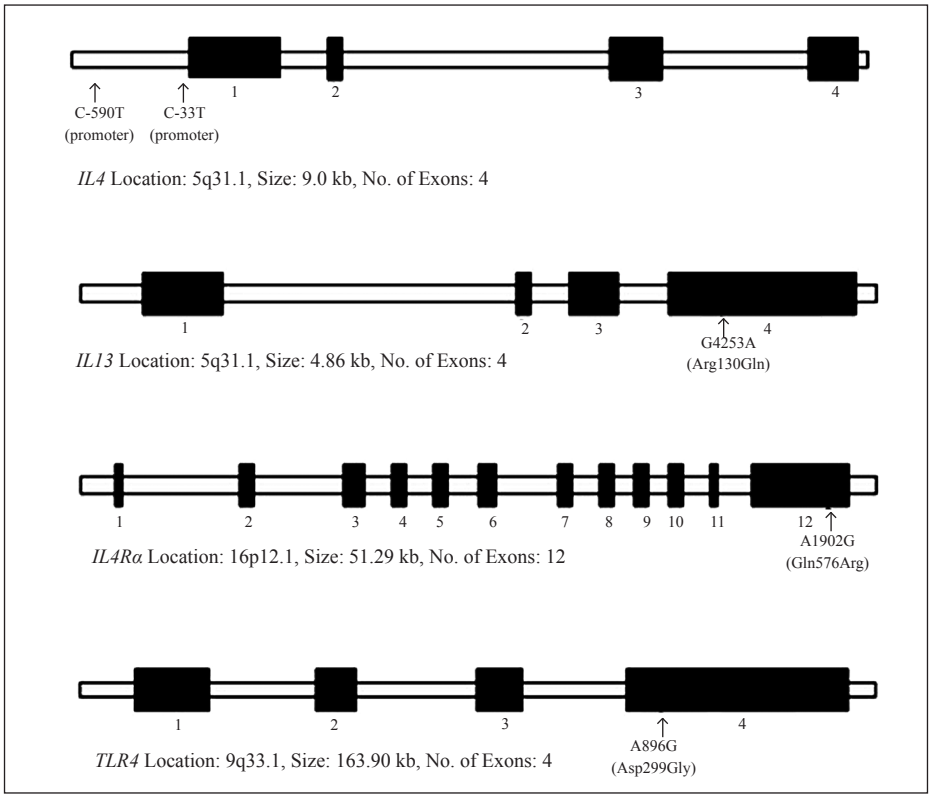

Fig. 1. Schematic representation of interleukin 4 (*IL4*), *IL13*, *IL4Rα* and Toll-like receptor 4 genes (*TLR4*) showing their structural organization (filled boxes represent the exons) and the location of the SNPs genotyped in this study.

Table II. Genotype and allele frequency distributions of five SNPs of *IL4, IL13, IL4Rα* and *TLR4* in all asthmatic patients (AAP), mild asthmatics (MIA), moderate asthmatics (MOA), severe asthmatics (SA) and controls (CON)

SNP genotype	CON N=50	AAP N=100	MIA N=15	MOA N=30	SA N=55
IL4 (C-589T)					
CC	0.72	0.65	0.67	0.54	0.71
CT	0.28	0.31	0.27	0.43	0.25
*TT	0.00	0.04	0.06	0.03	0.04
Allele frequency					
C	0.86	0.80	0.80	0.75	0.84
T	0.14	0.20	0.20	0.25	0.16
IL4 (C-33T)					
CC	0.78	0.68	0.67	0.60	0.73
CT	0.22	0.28	0.27	0.37	0.23
*TT	0.00	0.04	0.06	0.03	0.04
Allele frequency					
C	0.89	0.82	0.80	0.78	0.85
T	0.11	0.18	0.20	0.22	0.15
IL13 (G2044A)					
GG	0.90	0.89	1.0	0.90	0.85
GA	0.00	0.03	0.0	0.03	0.04
AA	0.10	0.08	0.0	0.07	0.11
Allele frequency					
G	0.90	0.90	1.0	0.91	0.87
A	0.10	0.10	0.0	0.09	0.13
*IL4Rα (*A1902G)					
AA	0.50	0.61	0.60	0.60	0.62
AG	0.40	0.33	0.27	0.37	0.33
GG	0.10	0.06	0.13	0.03	0.05
Allele frequency					
A	0.70	0.77	0.73	0.78	0.78
G	0.30	0.23	0.27	0.22	0.22
TLR4 (A896G)					
AA	0.58	0.55	0.67	0.50	0.54
AG	0.40	0.43	0.33	0.47	0.44
GG	0.02	0.02	0.0	0.03	0.02
Allele frequency					
A	0.78	0.76	0.83	0.73	0.76
G	0.22	0.24	0.17	0.27	0.24

*Denotes the presence of mutation in patients and not in controls

(Table II). After comparing the genotype frequency distribution of all the SNPs between each subgroup of asthma severity and the controls, no significant differences was observed. The intergroup comparisons of genotype frequency of the five SNPs in the three subgroups of asthma severity also did not show any significant differences (data not shown). Distribution in percentage of normal homozygous, heterozygous and mutant homozygous genotypes of the five SNPs in mild, moderate and severe asthma is shown in Fig. 2.

We had previously measured the serum concentrations of IL13 and interferon gamma (IFN-γ) for the same group of patients[17]. Increased serum concentration of IL13 was observed in the homozygous mutant genotype of the five SNPs. Also in the control group, the homozygous recessive genotype of IL13 showed a dramatic increase in its serum concentration (data not shown). No significant effect was seen between different genotypes of the five SNPs and the serum concentration of IFN-γ.

In our previous study *IL4* C-589T SNP was investigated in Mysore, where higher allele frequency of the homozygous mutant genotype (TT) of -589T *IL4* was reported in patients with severe asthma (7%) compared to that of the controls (2%), but the difference was not significant[18]. The interesting result of this study was the presence of the homozygous mutant allele (T allele) of both C-589T and C-33T SNPs in the promoter region of *IL4* in 4 per cent of the patients. Both the mutations were absent in the control group. Transcription of *IL4* has been shown to be regulated by multiple promoter elements. It has also been reported that polymorphisms in the promoter region of *IL4* correlates with enhanced IL4 activity that is higher binding of transcription factors and increased transcription[19].

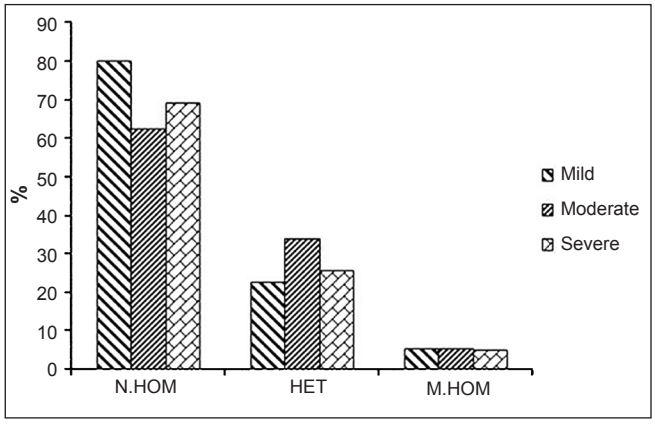

Fig. 2. Distribution of normal homozygous (N.HOM), heterozygous (HET) and mutant homozygous (M.HOM) genotypes of five SNPs of *IL4*, *IL4R, IL13* and *TLR4* in mild, moderate and severe asthmatic patients.

The limitations of this study were small sample size and selection of the patients from a tertiary care centre. Therefore, the results may not be reflective of the general population. Therefore, it is important to replicate this study in a larger sample size. Taken together, our data suggest the possible association between the genetic distribution of C-589T and C-33T SNPs of *IL4* with asthma in Indian adults. It would however, appear to exclude a role for Arg130Gln variation in *IL13* and Q551R variation in *IL4Rα* in the overall susceptibility to asthma. This study also confirms the previous observed lack of association of *TLR4* polymorphism (Asp299Gly) with asthma[20]. Further studies in larger populations should examine the role of these polymorphisms in the development and severity of asthma phenotypes, in order to generalize these results.

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