Lung (2015) 193:739–747
DOI 10.1007/s00408-015-9753-3

IL-10 and *IL-17F* Promoter Single Nucleotide Polymorphism and Asthma: A Case-Control Study in South India

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Received: 20 March 2015 / Accepted: 15 June 2015 / Published online: 25 June 2015

Abstract

Background Several studies have assessed the association between *IL-17F* and *IL-10* promoter polymorphisms and asthma, but the results were conflicting. Furthermore, few studies have evaluated the association of cytokine polymorphisms with asthma and its clinical phenotypes.

Objective This study was conducted to evaluate the association of *IL-10* (interleukin 10) and *IL-17F* (interleukin 17F) promoter polymorphisms (rs1800871, rs1800896 and rs1889570) with asthma and its clinical phenotypes including severity, atopic status, spirometric parameters, and response to treatment in south Indian population. A substudy was conducted to assess cytokine levels in subjects with different gene variants.

Methods *IL-10* and *IL-17F* polymorphisms were genotyped in 419 asthmatic patients and 393 controls using Mass ARRAY.

Results Our results showed an association between *IL-10* SNPs and mild asthma. No association was found with any of three SNPs in moderate to severe asthma. Comparison of genotype distribution of *IL-17F* rs1887570 AA variant among atopic and non-atopic patients showed significant difference ($p = 0.024$). Correlation analysis of *IL-10* and *IL-17F* SNPs to clinical variables showed a positive correlation between *IL-17F* rs1887570 AA and number of allergen sensitized ($rs = 0.142$, $p = 0.004$). Significant improvement in lung function was observed after 2 months of ICS (Inhaled corticosteroids) and LABA (long acting β2 agonist) treatment in all subjects with no statistically significant difference among SNPs variants. Cytokines levels were similar in different SNP variants.

Conclusion We observed an association between *IL-10* rs1800871 and rs1800896 SNPs and mild asthma, as well as *IL-17F* rs1887570 AA variant and number of allergens sensitized.

Keywords Asthma · *IL-10* · *IL-17F* · Single nucleotide polymorphism · Promoter · Atopic status

Introduction

Asthma is the most heterogeneous respiratory disease with a growing prevalence in resource-rich and resource-poor countries. It is associated with airway hyper-responsiveness, reversible airway obstruction, and allergen-specific IgE production [1, 2]. Growing body of evidence suggests the role of genetics in etiology of asthma through interactions with environmental factors [3]. More than 100 genes have been associated with asthma in candidate gene studies, positional cloning, or linkage studies [4].

IL-10 was first identified in 1989 as cytokine synthesis inhibitory factor with a down-regulatory effect on inflammatory responses. The *IL-10* gene is located on chromosome 1 both in human (1q31-32) and murine genomes and is consisted of 5 exons and 4 introns [5]. It inhibits the production of several cytokines, chemokines, chemokine

Electronic supplementary material The online version of this article (doi:10.1007/s00408-015-9753-3) contains supplementary material, which is available to authorized users.

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receptors, and inflammatory enzymes [6]. *IL-10* has a protective role in asthma. While, low levels of *IL-10* have been associated with asthma [7, 8], overexpression of *IL-10* in regulatory T cells can suppress airway hyper-responsiveness [9].

IL-17F is located on chromosome 6p, a region which is associated with asthma in several genome studies. Furthermore, gene expression studies have identified *IL-17F* as a novel candidate gene in asthma-related studies [10, 11]. The gene-encoding human *IL-17F* is 7742 bps in length, consisting of three exons and two introns. It is expressed in basophils and activated mast cells, mononuclear cells, T cells clones, and $CD4^+$ cells, a group of cells which are involved in the pathogenesis of asthma [12, 13]. *IL-17F* is overexpressed in the asthmatic patients airways where the level of overexpression is correlated with the severity of the disease [14]. In asthmatic patients, the overexpression of *IL-17F* induces airway neutrophilia, pulmonary mucus hyper-secretion, and goblet cell hyperplasia [7].

As cytokine expression is genetically controlled at transcription level, polymorphism in promoter region can affect cytokine expression level by changing the binding site of the transcription factors. As *IL-10* −1082A/G polymorphism is located within a negative putative ETS (E26 transformation-specific or E-twenty-six) -like transcription factor binding site and the −819C/T polymorphism is located within a putative positive regulatory region, these polymorphisms are considered as significant loci to investigate for their association with asthma which is affected by the expression level of this cytokine [15]. Several studies have shown the association between these polymorphisms and asthma [16, 17]. An association between *IL-10* −1082G allele and asthma has been indicated in East Asian population including that of north India [17, 18]. Although the role of *IL-17F* overexpression in asthma and its severity has been established well, very few studies have investigated the association of this cytokine polymorphism with asthma, with none from India. Among the identified *IL-17F* promoter polymorphisms, rs1889570 polymorphism has shown the highest minor allele frequency in European Americans (MAF: 0.348), African Americans (MAF: 0.375), and East Asians (MAF: 0.362) [19, 20].

Although several studies have investigated the association between *IL-10* and *IL-17F* polymorphisms and asthma, very few ones have evaluated the association of these polymorphisms with asthma clinical phenotypes. Furthermore, population diversities are noticeable and specific genetic markers exist for each population. This study was conducted to evaluate the association of *IL-10* (rs1800871, rs1800896) and *IL-17F* (rs1889570) promoter polymorphisms with asthma and its clinical phenotypes including severity (GINA), atopic status, spirometric parameters, and response to treatment with ICS and LABA in south Indian population for the first time. A sub-study was conducted to assess these cytokine levels in subjects with different *IL-10* and *IL-17F* variants.

Methods

Subjects

This study included 419 asthmatic patients and 393 non-asthmatic controls. While the asthmatic subjects were recruited from "Allergy, Asthma and Chest Center," India, age and gender-matched controls were selected from the general population. We included controls who had no history of asthma or other respiratory diseases, were non-smokers, and showed normal lung function in Pulmonary Function Test (PFT).

Spirometry to verify asthma in patients was performed according to ATS (American Thoracic Society) standards [21]. Diagnosis of asthma and its severity was performed according to the current Global Initiative for Asthma (GINA) guidelines. Asthmatic patients were classified into three groups: mild persistent, moderate persistent, and severe persistent [22]. This study was approved by the ethical committee of the University of Mysore, with Institutional Human Ethical Committee numbered IHEC-UOM No.80 PhD/2012-13, and accordingly informed written consent was obtained from all subjects.

DNA Extraction and SNP Genotyping

Two milliliter of venous blood was collected from the participants and stored in EDTA tubes at −20 °C for further analysis. DNA was extracted using Promega Wizard genomic DNA purification kit. The test procedure was conducted according to the manufacturer's guidelines. Three promoter polymorphisms [*IL-10* −819C/T (rs1800871), *IL-10* −1082A/G (rs1800896) and *IL-17F* −1507G/A (rs1889570)] were selected for genotyping using Mass ARRAY technique (eTable 1 in Electronic Supplementary Material).

Comparison of Serum Cytokine Levels in Subjects with Different *IL-10* and *IL-17F* SNPs

Serum levels of *IL-10* and *IL-17F* were assessed in 44 asthmatic and 44 non-asthmatic subjects [23]. Commercial ELISA kits (Ray Biotech Inc., USA) were used to measure serum levels of *IL-10* and *IL-17F*. The test procedure was performed according to the manufacturer's guidelines. *IL-10* and *IL-17F* serum levels were compared among subjects with different *IL-10* and *IL-17F* SNPs.

Association of *IL-10* and *IL-17F* SNPs to Different Clinical Variables

Total serum IgE was measured using ELFA (Enzyme-Linked Fluorescent Assay) method (Minividas, France). In skin prick test (SPT), sensitization to 36 common allergens (HollisterStier Allergy, USA) was tested where the wheal size of 3 mm greater than saline control was assumed as positive [24]. Severity of sensitization was specified by comparing the largest allergen wheal size with that of histamine as positive control. Severity of sensitization was tested according to Aas et al. (1973) where allergen wheal diameter of greater than histamine is graded as 4^+ [25].

Comparison of FVC (Forced Vital Capacity) and FEV1 (Forced Expiratory Volume in 1 s) Improvements Following Two Months of ICS and LABA Treatment in Patients with Different *IL-10* and *IL-17F* SNPs

232 asthmatic patients were followed up after 2 months of ICS and LABA treatment. The improvement in FVC and FEV1 was compared among subjects with different *IL-10* and *IL-17F* SNPs. The rest of 187 patients were referred to their family practitioner and were not available for follow-up.

Statistical Analysis

Hardy–Weinberg equilibrium (HWE) was calculated using FINETTI program [26]. The statistical power of the study was confirmed by Quanto (http://hydra.usc.edu/gxe) software V.1.2.4 [27]. Genotype and allelic frequencies were assessed using Chi-square, Odds Ratio (OR), and 95 % Confidence Interval (CI). Further analysis was performed by assessment of genotype frequencies under dominant, recessive, and additive genetic models [28]. Haplotype–disease association was assessed using Haploview software V.4.0 [29]. The association between SNPs and clinical variables was tested using SPSS software V.19 (SPSS, Inc, USA). As multiple comparisons were carried out on the same sample, Bonferroni correction was applied and $P < 0.016$ was considered as significant.

Results

Demographic characteristics and mean of the spirometric variables of the study population are presented in Table 1. *IL-10* (rs1800871, rs1800896) and *IL-17F* (rs1889570) promoter polymorphisms were genotyped in 419 cases and 393 controls. All the SNPs were in Hardy–Weinberg equilibrium in both the cases and controls (Table 2). Our results on power analysis showed the desirable power of 80 % with the relative risk ratio of 1.33 for *IL-10* rs1800871 and *IL-17F* rs1889570 SNPs, and 1.39 for *IL-10* rs1800896 SNP. Assessment of genotype and allele distributions of *IL-10* and *IL-17F* SNPs showed no significant difference between asthmatics and controls (Table 2). On subgroup analysis based on asthma severity, *IL-10* rs1800871 SNP in both homozygous and heterozygous conditions as well as allelic distribution and *IL-10* rs1800896 SNP in homozygous condition as well as allelic distribution were significantly different in mild asthmatics as compared to controls, indicating an association (Table 3). There was no association with any of three SNPs in moderate to severe asthma. Comparison of *IL-10* and *IL-17F* genotypes frequencies between asthmatics and controls under dominant, recessive, and additive genetic models showed nonsignificant differences (Table 4). On haplotype analysis, our results showed no significant difference in *IL-10* haplotypes frequencies in asthmatics as compared to controls (eTable 2 in Electronic Supplementary Material). The association of *IL-10* and *IL-17F* SNPs to their serum levels showed no significant difference in cytokine serum levels in subjects with different *IL-10* and *IL-17F* SNPs (eTable 3 in Electronic Supplementary Material). Comparison of genotype distribution of *IL-17F* rs1887570 AA variant among atopic and non-atopic patients showed a significant difference ($p = 0.024$, eTable 4 in Electronic Supplementary Material). Comparison of the number of allergens sensitized among atopic patients with different *IL-17F* rs1887570 genotypes showed a significant difference ($p = 0.008$, eFig. 1 in Electronic Supplementary Material). The least (47 patients) and the most (247 patients) amount of sensitization was shown to helminthosporium and house dust mite mixed allergens, respectively. Correlation analysis of SNPs to different clinical variables showed a significant correlation between *IL-17F* rs1889570 SNP and the number of allergens sensitized (Table 5, rs = 0.142, $p = 0.004$). Significant improvement in lung function was observed after 2 months of ICS and LABA treatment with no statistically significant difference between SNPs variants (Table 6).

Discussion

One of the major goals of SNP studies is to perceive the genetic basis of human phenotype variation, especially of complex diseases [30]. Identification of asthma-associated genetic variants may be clinically useful for identification of the patients at risk [31]. Our results showed an association between *IL-10* rs1800871 and rs1800896 SNPs and mild asthma in south Indian population (Table 3). There was no association with any of the three SNPs in moderate

Table 1 General characteristics of the study population

	Controls	Cases
Age		
≤40 years	231 (58.8 %)	247 (58.9 %)
>40 years	162 (41.2 %)	172 (41.1 %)
Sex		
Male	200 (50.9 %)	213 (50.8 %)
Female	193 (49.1 %)	206 (49.2 %)
Smoking status		
Smoker	0 (0 %)	49 (11.7 %)
Non-smoker	393 (100 %)	370 (88.3 %)
Family history of asthma		
Yes	0 (0 %)	189 (45.1 %)
No	393 (100 %)	230 (54.9 %)
Severity		
Mild persistent	–	108 (25.8 %)
Moderate persistent	–	170 (40.6 %)
Severe persistent	–	141 (33.6 %)
Sensitization level		
No sensitization	–	40 (9.5 %)
≤Histamine	–	216 (51.6 %)
>Histamine	–	163 (38.9 %)
Mean ± SE of total serum IgE	–	668.45 ± 39.21
Pulmonary function test	Pre-bronchodilator	Pre-bronchodilator
Mean ± SE of FVC [%]	88.35 ± 10.59	69.88 ± 18.93
Mean ± SE of FEV1[%]	88.76 ± 9.84	66.57 ± 20.58
Mean ± SE of FEV1/FVC %	104.18 ± 7.35	97.48 ± 13.77
Mean ± SE of PEF [L/s]	92.66 ± 19.61	71.94 ± 23.13

IgE immunoglobulin E, *SE* standard error of mean, *FVC* forced vital capacity, *FEV1* forced expiratory volume in 1 s, *PEF* peak expiratory flow

to severe asthma. Comparison of IL-*17F* rs1887570 AA genotype distribution among atopic and non-atopic patients showed significant difference ($p = 0.024$, eTable 4 in Electronic Supplementary Material). Comparison of the number of allergens sensitized among atopic patients with different *IL-17F* rs1887570 genotypes showed a significant difference ($p = 0.008$, eFig. 1 in Electronic Supplementary Material). Correlation analysis of *IL-10* and *IL-17F* SNPs to clinical variables showed a correlation ($rs = 0.142$, $p = 0.004$) between *IL-17F* SNP and the number of allergen sensitized (Table 5). No difference was observed when serum cytokines levels were compared between subjects with different *IL-10* and *IL-17F* SNPs (eTable 3 in Electronic Supplementary Material). Significant improvement in lung function was observed after 2 months of ICS and LABA treatment with no statistically significant difference between SNPs variants (Table 6).

Most of the interest in *IL-10* has focused on the promoter region to illustrate the variation in *IL-10* levels by altering the binding site of the transcription factors [32]. Our results showed an association between *IL-10* rs1800871 and rs1800896 polymorphisms and mild asthma, where a significant difference was noticed in T and G allele frequencies in controls than mild asthmatics (Table 3). No association was found with *IL-10* SNPs in moderate to severe asthma. Several studies have investigated the association between *IL-10* promoter polymorphism and asthma (eTable 5 in Electronic Supplementary Material), but the results are inconsistent [17, 18]. While some have reported the association between *IL-10* polymorphism and asthma [33, 34], others have reported negative results [35–37]. Such discrepancies are because of differences in patient demographics, sample size, environmental factors, genetic background, study design, asthma definition, gene–gene and gene–environment interactions.

We observed an association of both *IL-10* SNPs with mild asthma, but not with moderate and severe asthma. This observation should be confirmed in other studies. Unlike this study, most of the studies on *IL-10* SNPs and

Table 2 Hardy–Weinberg equilibrium test, genotype, and allelic distributions of *IL-10* and *IL-17F* SNPs in asthmatic patients and healthy controls

Gene	SNP	Total no. of samples		Minor allele frequency		Genotype	Controls	Cases	OR (95 % CI)	*P* value	HWE *p* value	
		Controls	Cases	Controls	Cases						Controls	Cases
IL-10	rs1800871	393	419	0.452	0.437	CC	126	138	1 (referent)	–	0.088	0.263
						CT	178	195	1.00 (0.73–1.37)	0.998		
						TT	89	86	0.88 (0.60–1.29)	0.520		
						C	430	471				
						T	356	367	0.94 (0.77–1.15)	0.544		
IL-10	rs1800896	393	419	0.222	0.241	AA	238	244	1 (referent)	–	0.879	0.478
						AG	135	148	1.07 (0.80–1.43)	0.655		
						GG	20	27	1.32 (0.72–2.41)	0.372		
						A	611	636				
						G	175	202	1.11 (0.88–1.40)	0.380		
IL-17F	rs1889570	393	419	0.530	0.508	GG	85	107	1 (referent)	–	0.743	0.263
						AG	199	198	0.79 (0.56–1.12)	0.183		
						AA	109	114	0.83 (0.56–1.22)	0.348		
						G	369	412				
						A	417	426	0.92 (0.75–1.11)	0.371		

SNP single nucleotide polymorphism, *OR* odds ratio, *HWE* Hardy–Weinberg equilibrium, *IL-10* interleukin 10, *IL-17F* interleukin 17F

asthma have not assessed the association of *IL-10* SNPs with different subgroups of asthma severity (GINA), possibly containing a heterogeneous severity groups. Reports suggest different pathogenesis mechanisms, pathways, and cytokine profiles in mild as compared to severe asthma [38]. There is a need for further analysis of our subjects with different asthma severities to understand the pathogenetic mechanism of *IL-10* in mild asthma.

The −1082 A allele is associated with reduced *IL-10* levels, whereas the −1082G allele is associated with elevated *IL-10* levels [39]. Lack of association between *IL-10* promoter polymorphism and its serum levels (eTable 3 in Electronic Supplementary Material) may show that these promoter sites does not affect *IL-10* expression, both alleles are codominantly expressed and there are other genes which regulate *IL-10* expression [40]. The presence of serum inhibitors, disparate assay sensitivities, ethnic discrepancies in impact of *IL-10* polymorphism on its expression level [41] and possibility of false negatives due to small sample size are other factors to explain our nonsignificant results.

Comparison of the *IL-10* SNPs among atopic and nonatopic patients showed nonsignificant differences (eTable 4 in Electronic Supplementary Material). Our results are in line with other studies showing no difference between atopic and non-atopic asthma with respect to *IL-10* promoter polymorphisms [35, 37]. Correlation analysis of *IL-10* SNPs to clinical variables showed nonsignificant results (Table 5). Karjalainen et al. [42] found no association between *IL-10* polymorphism and lung function in asthmatic patients. As lung function is a complex trait, a couple of genes may not cause enough variation to change the spirometric parameters significantly [32].

IL-17F induces the expression of several proinflammatory cytokines, chemokines, and growth factors which are involved in leukocyte activation and airway remodeling in patients with asthma [43]. No association was found between *IL-17F* promoter polymorphism and asthma in our study population (Tables 2, 3). Our results are in line with the results of Kawaguchi et al. [20] who found no association between *IL-17F* rs1889570 polymorphism and asthma. Overall variation in *IL-17F* expression cannot be solely because of the variation in the promoter region. Polymorphisms in the gene located upstream in the inflammatory pathway may change *IL-17F* expression as opposed to SNPs within *IL-17F* itself.

Comparison of *IL-17F* serum level among subjects with different *IL-17F* variants showed nonsignificant differences (eTable 3 in Electronic Supplementary Material) showing that this promoter polymorphism may not affect the *IL-17F* expression or other genes may regulate the expression of this cytokine [44]. Comparison of IL-*17F* rs1887570 AA variant among atopic and non-atopic patients showed a significant difference ($p = 0.024$, eTable 4 in Electronic

Table 3 Genotype and allele frequency distributions of *IL-10* and *IL-17F* SNPs among controls (CT) and patients with mild asthma (MI), moderate asthma (MO), and severe asthma (SE)

Gene	SNP	Genotype	Controls ($n = 393$)	Cases ($n = 419$)			OR (95 % CI)			p value		
				MI	MO	SE	MI/CT	MO/CT	SE/CT	MI/CT	MO/CT	SE/CT
IL-10	rs1800871	CC	126	11	43	53	1 (referent)	1 (referent)	1 (referent)	–	–	–
		CT	178	42	87	65	2.70 (1.34–5.45)	1.43 (0.93–2.20)	0.87 (0.57–1.33)	0.005	0.102	0.517
		TT	89	55	40	23	7.08 (3.51–14.28)	1.32 (0.79–2.19)	0.61 (0.35–1.08)	<0.0001	0.289	0.088
		C	430	64	173	171						
		T	356	152	167	111	2.87 (2.07–3.97)	1.17 (0.90–1.50)	0.78 (0.59–1.03)	<0.0001	0.237	0.085
IL-10	rs1800896	AA	238	54	104	86	1 (referent)	1 (referent)	1 (referent)	–	–	–
		AG	135	42	60	46	1.37 (0.87–2.16)	1.02 (0.69–1.49)	0.94 (0.62–1.43)	0.174	0.930	0.781
		GG	20	12	6	9	2.64 (1.22–5.74)	0.69 (0.27–1.76)	1.24 (0.55–2.84)	0.013	0.433	0.601
		A	611	150	268	218						
		G	175	66	72	64	1.54 (1.10–2.15)	0.94 (0.69–1.28)	1.03 (0.74–1.42)	0.011	0.685	0.881
IL-17F	rs1889570	GG	85	29	47	31	1 (referent)	1 (referent)	1 (referent)	–	–	–
		AG	199	48	75	75	0.71 (0.42–1.20)	0.68 (0.44–1.06)	1.03(0.63–1.69)	0.196	0.090	0.895
		AA	109	31	48	35	0.83 (0.47–1.49)	0.80 (0.49–1.30)	0.88(0.50–1.54)	0.538	0.364	0.656
		G	369	106	169	137						
		A	417	110	171	145	0.92 (0.68–1.24)	0.90 (0.69–1.16)	0.94 (0.71–1.23)	0.579	0.394	0.637

SNP single nucleotide polymorphism, *OR* odds ratio, *IL-10* interleukin 10, *IL-17F* interleukin 17F

Table 4 Comparison of genotype frequencies between controls and asthmatic cases by dominant, recessive, and additive models

Gene	SNP	Model	Genotype	Controls	Cases	OR (95 % CI)	p value
IL-10	rs1800871C/T	Dominant	CC/CT+TT	126/267	138/281	1.04 (0.78–1.40)	0.790
		Recessive	CC+CT/TT	304/89	333/86	0.88 (0.63–1.23)	0.463
		Additive	CC	126	138		
			CT	178	195	1.00 (0.73–1.37)	
			TT	89	86	0.88 (0.62–1.26)	0.763
IL-10	rs1800896A/G	Dominant	AA/GA+GG	238/155	244/175	0.91 (0.69–1.20)	0.500
		Recessive	AA+GA/GG	373/20	392/27	0.78 (0.43–1.41)	0.409
		Additive	AA	238	244		
			GA	135	148	1.07 (0.80–1.43)	
			GG	20	27	1.23 (0.66–2.30)	0.643
IL-17F	rs1889570G/A	Dominant	GG/GA+AA	85/308	107/312	1.17 (0.84–1.63)	0.341
		Recessive	GG+GA/AA	284/109	305/114	1.03 (0.75–1.40)	0.866
		Additive	GG	85	107		
			GA	199	198	0.79 (0.56–1.12)	
			AA	109	114	1.05 (0.76–1.46)	0.405

SNP single nucleotide polymorphism, *OR* odds ratio, *IL-10* interleukin 10, *IL-17F* interleukin 17F

Table 5 Haplotype analysis of *IL-10* rs1800871 and rs1800896 SNPs among asthmatic patients and healthy controls

Haplotype	Haplotype Frequency	Case: Control Ratio	χ^2 value	*p* value
TA	0.442	0.437: 0.447	0.161	0.687
CA	0.326	0.322: 0.331	0.133	0.715
CG	0.229	0.240: 0.217	1.268	0.260

χ^2 chi-square value

Table 6 Mean concentration of *IL-10* and *IL-17F* in different genotypes of the corresponding genes variants

SNP	Genotype	Controls	Cases	Mean ± SEM of *IL-10* in controls (pg/ml)	Mean ± SEM of *IL-10* in cases (pg/ml)	Mean ± SEM of IL-17F in controls (pg/ml)	Mean ± SEM of *IL-17F* in cases (pg/ml)
rs1800871	CC	10	22	17.20 ± 2.40	3.36 ± 0.59	325.35 ± 60.06	3754.75 ± 796.07
rs1800871	CT	25	16	13.61 ± 2.14	2.63 ± 0.68	195.92 ± 27.17	2634.33 ± 737.79
rs1800871	TT	9	6	16.18 ± 4.95	2.24 ± 1.23	190.79 ± 49.38	5068.06 ± 2390.31
P value				0.200	0.522	0.143	0.525
rs1800896	AA	29	22	15.34 ± 2.10	3.61 ± 0.66	210.77 ± 28.54	3030.18 ± 759.57
rs1800896	AG	13	16	15.23 ± 3.04	2.65 ± 0.56	254.66 ± 48.09	3535.82 ± 908.45
rs1800896	GG	2	6	7.42 ± 0.35	1.28 ± 0.78	222.87 ± 34.24	4667.13 ± 1937.42
P value				0.279	0.204	0.679	0.323
rs1889570	GG	9	11	15.24 ± 4.75	3.31 ± 0.87	272.32 ± 70.23	3321.57 ± 1091.41
rs1889570	AG	19	19	16.82 ± 2.71	2.80 ± 0.63	225.58 ± 37.32	3508.51 ± 903.75
rs1889570	AA	16	14	12.56 ± 1.88	2.83 ± 0.76	195.73 ± 30.63	3711.66 ± 1078.40
P value				0.458	0.830	0.748	0.869

SNP single nucleotide polymorphism, *SEM* standard error of mean, *OR* odds ratio, *IL-10* interleukin 10, *IL17F* interleukin 17F, *pg/ml* pictogram per milliliter

Table 7 Correlation analysis of *IL-10* and *IL-17F* SNPs to various clinical variables

	IL-10 rs1800871	*IL-10* rs1800896	*IL-17F* rs1889570
Asthma duration	rs = 0.010, $p = 0.844$	rs = 0.002, $p = 0.975$	rs = 0.025, $p = 0.614$
Smoking status	rs = 0.036, $p = 0.464$	rs = 0.089, $p = 0.069$	rs = 0.060, $p = 0.223$
Severity	rs = −0.031, $p = 0.521$	rs = − 0.085, $p = 0.084$	rs = − 0.025, $p = 0.614$
Sensitization level	rs = 0.007, $p = 0.889$	rs = − 0.011, $p = 0.815$	rs = 0.081, $p = 0.098$
Number of allergens sensitized	rs = 0.024, $p = 0.629$	rs = 0.001, $p = 0.980$	rs = 0.142, $p = 0.004$
Total serum IgE	rs = 0.050, $p = 0.383$	rs = 0.022, $p = 0.698$	rs = − 0.064, $p = 0.258$
FVC [%]	rs = 0.040, $p = 0.419$	rs = 0.062, $p = 0.205$	rs = − 0.040, $p = 0.411$
FEV1[%]	rs = 0.017, $p = 0.727$	rs = 0.076, $p = 0.120$	rs = − 0.012, $p = 0.802$
FEV1/FVC %	rs = − 0.087, $p = 0.074$	rs = 0.079, $p = 0.105$	rs = 0.064, $p = 0.192$
PEF [L/s]	rs = − 0.022, $p = 0.657$	rs = 0.062, $p = 0.178$	rs = − 0.023, $p = 0.642$
Reversibility in FVC	rs = 0.073, $p = 0.134$	rs = 0.076, $p = 0.122$	rs = 0.019, $p = 0.700$
Reversibility in FEV1	rs = 0.019, $p = 0.692$	rs = 0.061, $p = 0.215$	rs = 0.006, $p = 0.900$

rs Spearman correlation coefficient. *p* value of <0.05 is considered as significant

IgE immunoglobulin E, *FVC* forced vital capacity, *FEV1* forced expiratory volume in 1 s, *PEF* peak expiratory flow

Supplementary Material), where the most number of allergens sensitized were observed in atopic patients with *IL-17F* rs1887570 AA variant. Correlation analysis of *IL-17F* rs1889570 variant to clinical variables showed a correlation (rs = 0.142, $p = 0.004$, Table 5) between *IL-17F* SNP and the number of allergen sensitized. Interestingly,

Table 8 Comparison of FVC and FEV1 improvements following 2 months of ICS and LABA treatment in patients with different *IL-10* and *IL-17F* SNPs variants

Gene	SNP	Genotype	Cases ($n = 232$)	Mean ± SE of FVC (mL) improvements	95 % CI lower and upper limits	Mean ± SE of FEV1 (mL) improvements	95 % CI lower and upper limits
IL-10	rs1800871	CC	74	398.91 ± 42.04	(315.13–482.70)	387.03 ± 40.99	(305.34–468.72)
		CT	112	383.75 ± 41.23	(302.04–465.46)	386.79 ± 40.66	(306.22–467.35)
		TT	46	408.04 ± 62.57	(282.02–534.06)	411.52 ± 55.22	(300.29–522.75)
p value				.936		.931	
IL-10	rs1800896	AA	134	379.40 ± 35.24	(309.69–449.11)	380.15 ± 31.05	(318.72–441.57)
		AG	83	394.46 ± 45.36	(304.22–484.69)	393.98 ± 48.46	(297.58–490.37)
		GG	15	512.67 ± 107.65	(281.79–743.55)	483.33 ± 112.33	(242.40–724.26)
p value				.492		.631	
IL-17F	rs1889570	GG	59	437.80 ± 63.77	(310.14–565.45)	396.10 ± 58.33	(279.33–512.87)
		AG	112	384.02 ± 37.11	(310.47–457.56)	391.70 ± 34.37	(323.59–459.80)
		AA	61	367.70 ± 45.58	(276.53–458.88)	387.70 ± 51.30	(285.10–490.31)
p value				.611		.993	

SNP single nucleotide polymorphism, *SE* standard error of mean, *FVC* forced vital capacity, *FEV1* forced expiratory volume in 1 s, *CI* confidence interval, *IL-10* interleukin 10, *IL-17F* interleukin 17F

comparison of the number of allergens sensitized among atopic patients with different *IL-17F* rs1887570 genotypes showed a significant difference ($p = 0.008$, eFig. 1 in Electronic Supplementary Material). The positive correlation between the number of allergens sensitized and *IL-17F* rs1889570 variants suggests the role of *IL-17F* in atopic sensitization. *IL-17F* was first established in BALF of atopic patients on stimulation with ragweed allergen [13].

Inhaled corticosteroids are the most common medications to control asthma. It is likely that the response to ICS treatment has a genetic background [45]. Our results showed significant improvement in lung function after 2 months of ICS treatment with no statistically significant difference between SNPs variants (Table 6). Lack of association between *IL-10* and *IL-17F* SNPs and spirometric parameters can be because of the complex genetic basis of response to ICS treatment or not including other functional SNPs which may be associated in our population (Tables 7, 8).

To the best of our knowledge, this is the first report on the association of asthma and *IL-10* promoter polymorphism in south Indian population and on the association of asthma and *IL-17F* promoter polymorphism in India. Taking a desirable sample size, collection of control samples from the general population and assessment of various clinical variables are other advantages of this study. Our study has several potential limitations. Unlike the controls, the cases of our study were recruited from the hospital and not from the general population. Some of the subgroup analysis performed did not have sufficient sample size in the subgroups.

In conclusion, our results showed an association between *IL-10* SNPs and mild asthma and *IL-17F* rs1887570 AA with number of allergens sensitized. Further large scale investigations along with in vitro functional experiments are required to assess the potential roles of these polymorphisms in asthma.

Acknowledgments The authors thank all the study participants, "Allergy, Asthma, and Chest center" staff and the Chairman, Department of Zoology, University of Mysore.

Conflict of interest The authors have no conflict of interest to declare.

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