SECTION III

SUMMARY
• Asthma is a chronic inflammatory disease of the airways, accompanied with enhanced airway hyperresponsiveness, airflow limitation, recurring incidents of wheezing, breath shortness, chest tightness and coughing, especially at night or early morning.

• Around 300 million people worldwide have asthma, and it is predicted that this number will rise to 400 million by 2025, as countries become more urbanized.

• According to the Indian council of medical research report, the overall prevalence of asthma was 2.05% in 2012. It has been predicted that by 2020, India will have one third of the world’s asthmatic population.

• Sensitization to indoor allergens including house dust mite and cockroach has been contributed to the susceptibility, severity as well as morbidity of asthma.

• A significant inconsistency in both the susceptibility and prevalence of asthma has been noticed among different gender and age groups. The incidence of asthma is about 2 fold higher among boys as compared to girls; however among adults, asthma incidence is more among females than males. Furthermore, the advancing age is considered as one of the most important risk factors in adult patients with asthma.

• Obesity is considered as an important risk factor in the development of asthma and its severity. Obesity is associated with increased inflammatory responses, poorer disease control, more physical activity limitations, acute exacerbations, frequent wheezing, decreased quality of life, higher hospitalization rate as well as decreased FEV1 and FVC measurements in patients with asthma.

• Though environmental factors can induce the susceptibility to asthma, both familial and twin studies suggest the impact of genetic factors in asthma through the interaction with the environmental triggers. 18 genomic regions and > 100 genes and have been associated with asthma.
The inflammatory responses in asthma are mediated by a variety of anti-inflammatory (IL-10) and pro-inflammatory (IL-17F, IL-33) cytokines, where the low expression of anti-inflammatory IL-10 and high expression of pro-inflammatory IL-17F and IL-33 cytokines in sera has been associated with asthma in different ethnicity groups, with no reports from south India.

As cytokine expression is controlled at transcription level, promoter SNPs may change cytokine expression level by altering the binding position of the transcription factors. An association between \textit{IL-10} (rs1800871 and rs1800896) and \textit{IL-17F} (rs1889570) SNPs and asthma was found in different populations, with no reports from south India.

Though both candidate gene studies and GWAS have specified a large number of asthma-associated SNPs, difficulties in identifying the epistatic effects and the need for screening a huge number of SNPs has shed light on bioinformatic tools such as PolyPhen 2, Phyre 2 and SIFT as additional tools to costly laboratory experimentations to reduce the number of SNP screens by discriminating between neutral and functional SNPs.

Taking all that into account, current study was carried out to investigate the association between age, gender, BMI, allergen sensitization and the severity of asthma; to assess the association between \textit{IL-10} and \textit{IL-17F} promoter SNPs and asthma; to conduct in silico studies on nonsynonymous SNPs in \textit{IL-17F} as asthma candidate gene; to measure serum levels of IL-10, IL-17F and IL-33 in patients with asthma; and to assess the correlation between \textit{IL-10} and \textit{IL-17F} SNPs and their serum levels.

A total of 2436 doctor-diagnosed asthmatic patients (419 children and 2017 adults) were recruited from “Allergy, Asthma and Chest Center”, Mysore. Diagnosis of asthma and its severity was performed according to GINA guidelines. Spirometry to verify asthma was conducted according to American Thoracic Society standards. The study was approved by the ethical committee of
the University of Mysore, and accordingly informed written consent in both English and local language was taken from all the participants.

- A total of 393 age and gender-matched nonasthmatic healthy controls were collected from the general population in Mysore and surrounding areas. Spirometry to confirm absence of asthma was conducted in all the subjects. Only nonsmoker individuals with no family history of asthma were included.

- Comparison of the spirometric parameters between the cases and controls showed a significant difference, where a significant decrease in the spirometric parameters was observed in asthmatic patients as compared to nonasthmatic controls.

- Assessment of asthma duration among asthmatic patients showed that most of the patients, especially those with severe asthma have the asthma duration of > 1 years. Evaluation of the family history of asthma showed that most of the patients, particularly those with severe asthma have a family history of asthma.

- Chest symptoms were observed in all the patients with different severity of asthma. Following chest symptoms, nasal symptoms are the most common symptoms in patients with different severity of the disease.

- Among different asthma triggers, dust exposure was the most frequent complication among the patients with different severity of asthma.

- Assessment of total IgE serum level among patients with different severity of asthma showed that most of the patients have a total IgE serum of above normal level (> 120 kUA/l). Furthermore, Evaluation of E and AEC levels among the asthmatic subjects with different severity of the disease showed above normal levels of E (> 6%) and AEC (>440 Cells/mm³) in most and all the patients, respectively.
• An association was found between gender and age of the patients and severity of asthma disease. While in children the most number of patients with severe asthma were males, in adult group, most number of patients with severe asthma were females. Furthermore, assessment of age range among adult patients with different severity of asthma showed that the most number of patients with severe asthma are in the age group of 51-60 years old, showing the association between the advancing age and severity of asthma disease.

• The results on the correlation of BMI to the severity of asthma showed a significant positive correlation, where the most number of overweight and obese patients belonged to the severe group in both asthmatic adults as well as children.

• In skin prick test, house dust mite and cockroach were two allergens to which asthmatic patients showed the most amount of sensitization, where the severity of sensitization was correlated with the severity of the disease. Assessment of the number of positive allergens and the sensitization level in the study subjects showed high level of sensitization in the study subjects, particularly those with severe asthma.

• Correlation analysis of the clinical variables to the severity of asthma disease showed a significant correlation between severity of asthma and age, childhood/adulthood status, asthma duration, BMI, total serum IgE, number of allergens sensitized, sensitization level and spirometric parameters.

• *IL-10* (rs1800871 and rs1800896) and *IL-17F* (rs1889570) SNPs were genotyped in a total population of 419 asthmatic patients and 393 nonasthmatic controls using MassARRAy technique. All three SNPs were in HWE in both asthmatic patients and nonasthmatic controls and the study showed the desirable power of 80% for all the SNPs.

• This study revealed an association between *IL-10* rs1800871 and rs1800896 SNPs and mild asthma in south Indian population, where a significant difference
in T and G variants frequencies was detected in nonasthmatic controls when compared to the patients with mild asthma. There was no association with any of \textit{IL-10} SNPs in patients with moderate and severe asthma.

- Comparing the genotype frequencies of \textit{IL-10} SNPs between atopic and nonatopic asthmatic patients revealed nonsignificant differences.

- No association was observed between \textit{IL-17F} promoter variant and asthma in the subjects of this study.

- Comparing the genotype frequency of \textit{IL-17F} rs1889570 AA SNP between atopic and nonatopic subjects revealed a significant difference, in which the most amount of sensitization was shown in atopic subjects with AA genotype. Interestingly, correlation analysis of \textit{IL-17F} SNP to various clinical parameters revealed a significant positive correlation between \textit{IL-17F} rs1889570 SNP and the number of positive allergens. Furthermore, comparing the number of positive allergens between atopic subjects with different \textit{IL-17F} variants showed a significant result.

- Comparing \textit{IL-10} and \textit{IL-17F} genotypes distributions between the cases and controls under different genetic models revealed nonsignificant results.

- Assessment of \textit{IL-10} haplotype frequencies between asthmatic patients and non asthmatic controls showed nonsignificant results.

- The results of this study showed significant improvement in lung function performance following two months of inhaled corticosteroid treatment in all subjects, with no significant difference among different SNPs variants.

- The conformational and functional impacts of 10 nonsynonymous \textit{IL-17F} SNPs (rs763780, rs141798304, rs200163061, rs376671742, rs373228601, rs11465553, rs146083682, rs144576902, rs368500268, and rs2397084) was predicted using Phyre2, PolyPhen2 and SIFT softwares.
• The results on structural variation analysis revealed mutant proteins having conformational changes in the number of atoms, bonds, H-bonds, helices, strands and turns as compared to wild type.

• Functional variation prediction of IL-17F SNPs in PolyPhen2 software predicted five SNPs (rs763780, rs141798304, rs200163061, rs376671742, and rs373228601) as benign, two SNPs (rs11465553 and rs146083682) as possibly damaging and three SNPs (rs144576902, rs368500268, and rs2397084) as probably damaging.

• The results on functional variation prediction in SIFT software predicted 6 SNPs (rs763780, rs141798304, rs146083682, rs200163061, rs376671742, and rs373228601) as tolerated and four SNPs as deleterious (rs144576902, rs11465553, rs368500268, and rs2397084).

• Serum levels of IL-10, IL-17F and IL-33 were evaluated in a total of 44 asthmatic patients and 44 nonasthmatic healthy controls using ELISA method. A significant decrease in IL-10 serum level and a significant increase in IL-17F and IL-33 serum levels were observed in asthmatic patients as compared to nonasthmatic controls.

• On subgroup analysis based on the severity of the disease, a significant difference in all three cytokines serum levels was observed between each of asthma severity groups and nonasthmatic controls. Furthermore, comparison of the cytokines serum levels among patients of different severity groups showed a significant difference in IL-17F serum level across the severity groups.

• Comparison of the cytokine serum levels among subjects of different gender, age group and BMI showed that while neither asthmatic cases nor nonasthmatic controls show a significant difference in their own groups, a significant difference in cytokines levels was observed between the cases and control groups.
Further assessment of cytokines serum levels between asthmatic subjects with either normal or high levels of total serum IgE, and AEC showed no significant difference, nevertheless, a significant difference in cytokines serum levels was observed in asthmatic subjects with different E levels.

Correlation analysis of cytokine serum levels to different clinical variables showed that serum levels of IL-17F is associated negatively to FVC and FEV1 and positively to FEV1 reversibility and the number of positive allergens.

On spirometric follow-up, following two months of inhaled corticosteroid and Long Acting β2 Agonist treatment, asthmatic patients with higher serum levels of IL-10 showed a significant improvement in FEV1 % predicted, while asthmatic subjects with higher serum levels of IL-17F and IL-33 showed a poorer response to the same therapy.

No difference was obtained when serum levels of IL-10 and IL-17F was assessed in individuals with different IL-10 rs1800871 and rs1800896 and IL-17F rs1889570 promoter SNP variants.

The strengths of the current study includes: 1) collection of age and gender-matched control samples from the general population, 2) performing PFT in all controls samples to verify the absence of asthma, 3) diagnosis of asthma and its severity according to GINA guidelines, 4) performing spirometry in all the cases according to ATS standards, 5) having a desirable sample size for demographic as well as SNP association studies, 6) assessment of the actual cytokines serum levels due to selection of ICS/LABA treatment naïve subjects, 7) investigating several clinical parameters, 8) conducting follow-up studies in both cytokines serum analysis and SNP association study in order to assess the impact of cytokine level as well as promoter polymorphism on response to ICS/LABA treatment, and 9) assessment of cytokine profile and SNP association studies in a population of south India for the first time.
• Limitations of the present study includes: 1) assessment of cytokines serum levels in a small sample size, 2) selecting a few SNPs for the association studies, and 3) not having a desirable sample size in some of the subgroup analysis of SNP association study.

• Final conclusions of the present study includes: 1) the association of advancing age, male gender in childhood, female gender in adulthood, and obesity to the severity of asthma, 2) association of IL-10 rs1800871 and rs1800896 SNPs and mild asthma and lack of association of IL-17F rs1889570 SNP and asthma, 3) showing conformational and functional variations in mutant IL-17F proteins as compared to the wild type 4) association of lower level of IL-10 and higher levels of IL-17F and IL-33 with asthma, and 5) lack of correlation between IL-10 and IL-17F SNPs and their serum levels.

• Future perspectives includes: 1) expanding the demographic studies of asthma in a larger sample size including more clinical parameters, 2) further in depth investigations on cytokine biology using a wider cytokine profile, and 3) performing whole genome, exome and transcriptome analysis which may give a better understanding of asthma genetics.