SECTION I

REVIEW OF LITERATURE ON ASTHMA
Definition of asthma

The Global Strategy for Asthma Management and Prevention guidelines describe asthma as 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 night time or early in the morning (Masoli et al., 2004). Asthma is the outcome of an improper T cell response to foreign allergens, resulting in bronchial inflammation, bronchial hyperresponsiveness and excessive mucus secretion. Airway inflammation is associated with epithelial cell activation, increased airway smooth muscle cell contraction and alterations in airway construction (James et al., 2012). Symptoms and airflow limitation may improve immediately or in response to treatment, and sometimes can be missing for weeks or months at once. In contrast, sometimes patients may show periodic exacerbations that can be life-treating and impose a considerable burden to both patients and society (www.ginasthma.org).

Asthma phenotypes

Asthma is a heterogeneous disorder with diverse underlying disease processes. Defined groups of demographic, clinical and/or pathophysiological features are usually named asthma phenotypes (Moore et al., 2010; Wenzel, 2012). In severe asthmatic patients, some phenotype-directed medications are accessible. Nevertheless, so far, no strong link has been established between particular pathological characteristics and specific clinical patterns or medication responses (Anderson, 2008). More investigation is required to realize the clinical value of phenotypic classification in asthma. Several phenotypes have been recognized as follows:

- Allergic asthma: This is the most simply recognizable asthma phenotype, which usually begins in childhood and is correlated with a previous and/or family history of allergic diseases like eczema, allergic rhinitis, or food or drug allergy. Assessment of the induced sputum of the patients prior to treatment regularly shows that eosinophilic airway patients with this phenotype of asthma typically respond fine to inhaled corticosteroid [ICS] therapy (www.ginasthma.org).
• Nonallergic asthma: Some adult patients have asthma which is not correlated with allergy. The sputum cellular profile of these patients may be neutrophilic, eosinophilic or include just a small number of inflammatory cells. Nonallergic asthmatic patients regularly respond less well to ICS treatment (www.ginasthma.org).

• Late-onset asthma: Some adult patients, especially women, show asthma for the first time in their adult life. Such patients are usually nonallergic, and regularly need higher doses of ICS or rather are resistant to corticosteroid therapy (www.ginasthma.org).

• Asthma with fixed airflow limitation: Some long-standing asthmatic patients show fixed airflow limitation which is believed to be because of bronchial remodeling (www.ginasthma.org).

• Asthma with obesity: Some obese asthmatic patients show significant respiratory symptoms along with little eosinophilic airway inflammation (www.ginasthma.org).

Clinical diagnosis of asthma

Medical history: A clinical diagnosis of asthma is recommended by symptoms like intermittent breath shortness, wheeze, cough, as well as chest tightness (Löwhagen, 2012). Intermittent symptoms following an allergen exposure, seasonal variability of asthma symptoms, along with a family history of asthma and allergic disease are also useful diagnostic clues. The symptoms patterns which strongly recommend an asthma diagnosis include variability; expedition by general irritants like smoke, fumes or exercise; exacerbating at night; and responding to proper asthma treatment. In patients with sensitization, asthma may get worse by seasonal increases in particular aeroallergens such as birch, grass and ragweed pollens (Canova et al., 2011). Cough-variant asthma is mainly frequent in children and is usually more challenging at night; assessments during the day time can be absolutely normal (Yuan et al., 2013). Physical
activity is an imperative reason for asthma symptoms in most asthmatic patients and in some patients it is the only reason.

**Physical examination:** The most common unusual physical finding is wheezing on auscultation, a finding which verifies the incidence of airflow limitation. Nevertheless, in some individuals with asthma, wheezing may be missing or only figured out when the patient breathe outs by force, even in the presence of prominent airflow limitation (Bateman et al., 2008).

Tests for diagnosis and monitoring

**Measurements of lung function:** Though the diagnosis of asthma is typically based on the incidence of distinctive symptoms, patients with asthma regularly have imperfect recognition of their symptoms and imperfect view of symptom severity, mainly if they have asthma for a long time (Killian et al., 2000); evaluation of symptoms like dyspnoea and wheezing by physicians can also be imprecise. For 5 yrs old patients, assessments of lung function to verify airflow limitation, and especially the manifestation of reversibility in lung function abnormalities, significantly improve diagnostic assurance. Quality control and sufficient guidance for patients on how to carry out the forced expiratory manoeuvre are substantial (Standardization of Spirometry, American Thoracic Society [ATS] guidelines, 1995). The amount of reversibility in FEV1 that specifies a diagnosis of asthma is commonly approved as 12% and 200 mL from the pre-bronchodilator value (Pellegrino et al., 2005). Nevertheless, the majority of patients with controlled asthma will not show reversibility at each measurement; especially those who are on treatment and as a result the test does not show enough sensitivity. Repetitive examination at different visits is recommended. As a lot of lung diseases may bring about decreased FEV1, a practical measurement of airflow limitation is considered as the ratio of FEV1 to FVC. The FEV1/FVC ratio is generally 0.75–0.80, and probably 0.90 in children. Lower values imply airflow limitation (Bateman et al., 2008).
Peak expiratory flow [PEF] examination using a peak flow meter is also a key aid to both diagnose and monitor asthma. Nevertheless, assessments of PEF are not identical with other assessments of lung function, like FEV1 in adults (Sawyer et al., 1998) or children (Paton, 2012), as values found with various peak flow meters differ and the assortment of predicted values is too broad. PEF assessments are also extremely shot dependent and quality is not good enough. Thus, assessments should constantly be compared with the patient’s own former best assessments (Reddel et al., 2004) by patient’s own peak flow meter. The former best assessment is regularly taken while the patient is asymptomatic and controlled. The words “reversibility” and “variability” refer to variations in symptoms associated with changes in airflow limitation which take place spontaneously or in response to therapy. The term reversibility is usually used for fast improvements in FEV1 (or PEF), considered within minutes following inhalation of a rapid-acting bronchodilator or more persistent improvement over days or weeks following an efficient controller therapy, such as inhaled glucocorticosteroids (Pellegrino et al., 2005). Variability is specified as increase or decline in symptoms and lung function happening over time. Variability can occur daily, monthly, or seasonally. Taking a history of variability is an important part of asthma diagnosis and involves a part of asthma control assessment (Bateman et al., 2008).

**Measurement of airway responsiveness:** In patients with consistent asthma symptoms, but with normal lung function, assessments of airway responsiveness to methacholine, mannitol, histamine or adenosine monophosphate exercise challenge may help in the diagnosis of asthma (Malmström et al., 2013).

**Measurements of allergic status:** With the potent association between asthma and allergic rhinitis, the incidence of allergy, allergic diseases, or allergic rhinitis especially, enhances the chance of asthma diagnosis in patients with respiratory symptoms. Confirming allergic sensitization in asthmatic patients through skin testing or assessment of serum specific immunoglobulin [Ig] E can help to make out risk factors which result in asthma symptoms in patients with asthma (Bateman et al., 2008; Cramer et al., 2014).
Classification of asthma severity

According to Global Initiative for Asthma [GINA] guidelines, the severity of asthma is subdivided into four categories based on the intensity of symptoms, airflow limitation and lung function variability: intermittent, mild persistent, moderate persistent, and severe persistent (Table 1.1). Classification of asthma based on the severity of symptoms is helpful when decisions are taken on the management of the disease at the early assessment of a patient. Nevertheless, it is essential to know that asthma severity includes both the severity of the disease and its responsiveness to therapy. Severity is not a constant feature of an asthmatic patient but may vary over months or years (Bateman et al., 2008).

Global burden of asthma

Masoli et al., (2004) and the GINA merged data from the phase 1 International Study of Asthma and Allergies in Childhood [ISAAC] study gathered in 1992–1996 and the European Community Respiratory Health Survey [ECRHS] in 1988–1994 to make global estimations of asthma burden. They estimated that 300 million people worldwide have asthma, and predicted that this number will rise to 400 million by 2025, as countries became more industrialized. In a consistent cross-sectional review by To et al., (2012), the prevalence of doctor diagnosed asthma differed extensively amongst the 70 involving countries, ranging from 0.2% in China to 21.0% in Australia. Making use of a less strict definition, the universal incidence of doctor-diagnosed asthma was 4.5%. The incidence of clinical asthma also differed extensively amongst the 70 participating countries, ranging from 1.0% in Vietnam to 21.5% in Australia, showing a 21-fold global discrepancy. The first five countries with the greatest incidence of clinical asthma were Australia (21.5%), Sweden (20.2%), England (18.2%), Netherlands (15.3%), and Brazil (13.0%). Making use of the least strict definition, the global incidence of wheezing asthma was 8.6%. The incidence of wheezing asthma showed a 15-fold discrepancy across the world, with the greatest incidence reported for Australia (27.4%), the Netherlands (22.7%), England (22.6%), Brazil (22.6%), and Sweden (21.6%). 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 (Jindal et al., 2009).

**Etiology of asthma**

Asthma includes a variety of heterogeneous phenotypes which vary in appearance, etiology, and pathophysiology. The risk factors involved in etiology of the asthma involve genetic, environmental, and host factors. Though in many of the asthma cases a family history of asthma is general, it is not still enough to develop asthma (Burke et al., 2003). The significant raise in the prevalence of asthma in the past years, and the geographic discrepancy in both incidence rates and degree of the raise hold up the assumption that environmental variations play an important role in related to asthma (Subbarao et al., 2009). Additionally, environmental risk factors can influence asthma in a different way at different courses of an individual’s life, and the related triggers may alter in due course. Short-term investigation of the possible triggers may propose a lower chance of asthma, while the same triggers may be related to a higher risk in case the follow-up is more extended. This model may be associated with overlap between diverse wheezing features in early childhood, just some of which continue asthma in future childhood and adulthood (Subbarao et al., 2009). As a result, the triggers of persistent asthma have been investigated at various ages including the prenatal, childhood and adulthood.

**Prenatal risk factors:** The risk factors that are involved in the prenatal age are multifactorial. Evaluation is intricate by the diversity of wheezing situations that might happen in childhood, just some of which develop to classical asthma (Tavendale et al., 2008). It includes prenatal tobacco smoke (Burke et al., 2012), diet and nutrition (Nwaru et al., 2014), stress (Kozyrskyj et al., 2007), antibiotic use (Stensballe et al., 2013) and mode of delivery (Almqvist et al., 2012).

**Childhood risk factors:** Childhood asthma triggers include breastfeeding (Dogaru et al., 2014), lung function (Haland et al., 2006), family structure (Strachan et al., 2015), socio-economic status (Davoodi et al., 2013), antibiotics and infections (Alm et al.,
2008), allergic sensitization (Siddiqui et al., 2012), exposure to environmental tobacco smoke (James et al., 2005), exposure to animals (Stoltz et al., 2013), gene - by - environment interactions (Subbarao et al., 2009), gender (Leynaert et al., 2012), obesity (Chen et al., 2013).

**Adult-onset asthma:** Adulthood asthma may have continued from childhood, may have arisen as a relapse of former childhood asthma or may be just adult-onset asthma without any symptoms in former life (Butland and Strachan, 2007). New-onset asthma in adulthood may have environmental basis with or without allergen sensitivity (Kogevinas et al., 2007). Adult-onset asthma risk factors include occupational asthma (Tarlo and Lemiere, 2014), smoking tobacco (Tamimi et al., 2012) or marijuana (Tetrault et al., 2007), air pollution (Andersen et al., 2012), obesity (Brumpton et al., 2013) and atopy (Ige et al., 2012).

**Genetics contribution in asthma**

Investigations to identify the genetic basis of a number of single-gene disorders have been successful. However, comparatively identification of the genetic contribution of complex genetic disorders like asthma and allergy is quite difficult, considering the role of multifactorial inheritance and major environmental contributors in etiology of such diseases (March et al., 2011). Five types of study designs are usually used to realize or better characterize the genetic basis of complex diseases: candidate gene association studies, genome-wide approaches, genome-wide linkage studies, genome-wide association studies [GWAS] and resequencing studies.

**Candidate gene association studies:** Most of the > 100 loci linked to the risk of asthma and allergy were first identified through candidate gene association studies (Ober and Hoffjan, 2006; Vercelli, 2008), beginning with the study on human leukocyte antigen [HLA] and allergies in the Blumenthal and Marsh laboratories during the 1970s (Marsh and Bias, 1975). Till 2007, polymorphism in > 30 genes had known to be associated with asthma and allergy in at least five independent association studies (Ober and Hoffjan, 2006; Vercelli, 2008). Candidate gene association studies are theoretically simple. The most frequent type of association study is the case-control study in which a
marker, allele or haplotype in cases is compared to the controls (Ober and Yao, 2011; Patnala et al., 2013). Other association studies are cohort and cross-sectional studies in which subjects are not chosen based on the disease status. In these approaches, an enrichment of the disease in subjects with a specific genotype at marker locus or haplotype is assessed. The effect size of the associated alleles, genotypes, or haplotypes is usually described by odds ratios [OR] in the case-control studies or by relative risks [RR] in the cohort and cross-sectional studies (Ober and Yao, 2011). As indicated by its name, gene selection in such studies is based on the identified function, or the chromosomal position in a linkage or next to a former association signal. Particular variants employed in the candidate gene studies can be considered on the basis of the function or earlier associations with the same or linked disease. Most of the genetic studies on asthma and allergic diseases are candidate gene studies in which genes are selected based on their specific function and possible contribution to the pathogenesis of diseases (Ober and Hoffjan, 2006; Vercelli, 2008). Therefore, such studies are greatly biased towards immune-related gene studies. Actually, the most important limitation of such studies is that it is restricted to the understanding of disease etiology and gene functions. The candidate gene study cannot, independently, identify exquisite pathways or genes.

**Genome-wide approaches:** Contrary to candidate gene association studies, other gene identification methods are genome-wide approaches in which whole genome region is considered with no previous hypotheses about the position of the most important genetic risk contributors. Therefore, genome-wide approaches are called either “hypothesis-free” or “hypothesis-generating” (Ober and Hoffjan, 2006). The most privilege of genome-wide approaches is that they can identify new genes and pathways contributed to the etiology of various diseases. The major limitation of this approach is the statistical burden which originates from the vast number of tests conducted and the consequential need for very big sample sizes to attain desirable statistical significance. Additionally, the association between the identified variants and disease pathogenesis is not clear all the time (Ober and Yao, 2011).
**Genome-wide linkage studies:** The first genome-wide study which was technically possible to conduct in large sample size was linkage studies. One of the main requirements of linkage studies is the accessibility to families with a minimum of two affected individuals. The main idea of linkage studies is co-segregation of the disease loci with the consequent disease within the families. Therefore, genome regions with susceptibility loci will be shared by affected individuals more frequently than what is expected by chance. The presence of linkage is shown by a likelihood ratio, or by a logarithm (base 10) of odds [LOD] score. Linkage studies are conducted in several families, and LOD scores are added across families. Generally LOD scores of +3, preferably more than 3.4, are needed to claim a significant linkage to a complex disease (Ober and Hoffjan, 2006; Ober and Yao, 2011). The main advantage of linkage studies is the requirement of comparatively few genetic markers. Furthermore, linkage studies represent regions or genes with several rare variants which give susceptibility to the disease, even though the particular variant is not the same among families. This is usually an unnoticed benefit of linkage studies, though identifying various rare variants which are attributing to a linkage peak is an overwhelming work. Other than the necessity of familial studies, linkage studies have other limitations, too. It has poor resolution in the sense that it discovers very expansive regions which may harbor hundreds of genes and has less power to identify risk variants with low impact on the disease risk (Ober and Yao, 2011).

**Genome-wide association studies:** Contrary to genome-wide linkage studies, GWAS has some major advantages which include outstanding resolution, high power to identify risk variants with low impact, and lack of requirement for familial studies (Bush and Moore, 2012). The GWAS expands the candidate gene study to incorporate genetic markers which tag all frequent genome variations. GWAS has become accessible during recent years as highly efficient genotyping approaches. Furthermore, with current access to the data from the HapMap project (Manolio and Collins, 2009), over 3 million genotypes can presently be assigned for many racial or ethnic groups using genotypes available from Affymetrix or Illumina platforms as well as haplotypes from the HapMap samples (Howie et al., 2009; Li et al., 2010). The results of meta-
analyses on asthma GWAS conducted in Europe (Moffatt et al., 2010) as well as USA (Torgerson et al., 2011) make the potency of this approach obvious. The major drawback of the GWAS is that it can mainly identify common risk variants, as the genotyping platforms usually contain frequent variants and has less power to identify associations with SNPs harboring low minor allele frequencies (<1%). Actually the risk alleles discovered by the GWAS approach include a minor portion of the possible genetic risk. Though several reasons may explain this observation, but the best explanation to the low predictive power of GWAS in identifying the risk variants is that rare variants, that cannot be identified by GWAS, have generally more effects on disease risk compared to the common ones, criticizing the “common disease-common variant theory” (Wang et al., 2005), one of the major justifications to conduct GWAS (Manolio and Collins, 2009; Li et al., 2010).

**Re-sequencing studies:** While GWAS has been frequently used in disease genetic studies over recent years, sequencing studies will take over the upcoming years. The current explosion of sequencing studies is owing to highly efficient, extremely parallel sequencing, called as Next Generation [NextGen] sequencing (Metzker, 2010). The basis of such studies is the assumption that rare variants account for a major part of the genetic risk in etiology of the common diseases, as they have more impact on disease risk compared to the common variants (Eichler et al., 2010). In fact, previous theoretical modeling on the etiology of the common diseases proposes that the susceptibility loci occurrence of multiple rare alleles are more probable compared to one or few common alleles (Pritchard and Cox, 2002). Such kind of allelic heterogeneity cannot be identified by traditional approaches such as GWAS. Furthermore, most of the common genotyping platforms investigate common variation, exclusively. On the contrary, identification of rare variants involves the re-sequencing of either genes or exomes or even whole genomes in a large sample size to identify rare variants that cannot be tagged by variants on classical genotyping platforms. Nowadays, many ongoing studies are being conducted to re-sequence the exomes of thousands individuals in order to identify rare variants that are more common in cases than controls. Nevertheless, it is not still clear whether the rare variants contribute to the major portion of the genetic risk.
in complex diseases such as asthma and allergy. With sequencing becoming less expensive, it is possible that it will replace current SNP genotyping platforms in the upcoming future (Ober and Yao, 2011).

**Cytokines in asthma**

Cytokines are typically extracellular signaling proteins that are produced by several cell types and act through particular receptors on target cells (Chung and Barnes, 1999). They play an important role in synchronization and persistence of the inflammatory response as they induce several pro-inflammatory responses in the airways of asthmatic patients. Airway inflammation is associated with infiltration of a panel of cells including T lymphocytes, eosinophils, macrophages, monocytes as well as mast cells into the airway walls (Walsh et al., 2010; Lauzon-Joset et al., 2014). Obviously, the chronic and acute inflammatory responses in asthmatic patients are the result of immoderate secretion of several types of cytokines which has been reported in allergen-induced asthma, viral infections, or throughout symptomatic asthma (Schuijs et al., 2013). It has shown that cytokines are involved both in initiation as well as maintaining the chronic inflammatory responses. It is not easy to categorize numerous cytokines which are potentially involved in etiology of asthma due to their pleiotropic features and overlapping characteristics. Nevertheless, with regard to the distinctive asthma abnormalities and to the perceptive of the asthma pathogenesis, they have been classified as:

(a) Lymphokines: interleukin-2 [IL-2], interleukin-4 [IL-4], interleukin-5 [IL-5].

(b) Pro-inflammatory cytokines: interleukin-1 [IL-1], tumor necrosis factor α [TNF-α], interleukin-6 [IL-6], granulocyte-macrophage colony-stimulating factor [GM-CSF], IL-17F, IL-33.

(c) Anti-inflammatory cytokines: IL-10, interferon gamma [IFN-γ], interleukin-12 [IL-12], interleukin-18 [IL-18].

(d) Chemotactic cytokines (chemokines): monocyte chemotactic protein-4 [MCP-4], macrophage inflammatory protein 1α [MIP-1α], eotaxin, interleukin-8 [IL-8].
(e) Growth factors: transforming growth factor α [TGF-α], fibroblast growth factors [FGFs], epidermal growth factor [EGF], insulin-like growth factors [IGFs] (Chung and Barnes, 1999).

A network of several cytokines is contributed to asthma and their involvement in asthma pathogenesis seems to be very disparate. Even though massive data on the biological features of cytokines and their potential contribution to asthma inflammatory responses are available these days, much remains to be understood about the role of cytokines in etiology of asthma (Chung and Barnes, 1999; Holgate, 2012).

**IL-10 and asthma**

Fiorentino et al., (1989) first illustrated a cytokine called as “cytokine synthesis inhibitory factor [CSIF]” which is secreted by T helper 2 [Th2] cells and prevents IFN production by T helper 1 [Th1] cells. Nowadays this cytokine is more known as IL-10. While a number of different immune cells secrete IL-10, macrophages are considered as the main source of IL-10 production. Several studies have revealed the crucial role of this cytokine in immunoregulation of asthma (Palomares et al., 2014; Stanic et al., 2015).

**Identification and structure:** IL-10 was first identified as cytokine synthesis inhibitory factor in 1989. It has shown that it inhibits the secretion of IFN-γ and other inflammatory cytokines in murine Th1 cells (Fiorentino et al., 1989). Though both Th1 and Th2 cells secrete IL-10, Treg cells are the major sources of T-cell-derived IL-10 in human system. The *IL-10* gene is located on chromosome 1 both in human (1q31-32) and murine systems (Kim et al., 1992). *IL-10* structure is very much conserved consisting of 5 exons and 4 introns, a feature which is the same in the majority of *IL-10* homologs. Human IL-10 has a molecular weight of 18 kilo Dalton [kD] and is produced as a homodimer bearing two subunits of 178 amino acids long (Singh et al., 1998). IL-10 protein is consisted of four conserved cysteine residues and forms six α-helices in its tertiary configuration (Akdis et al., 2011).

**Cellular sources and targets:** In human system, IL-10 is largely secreted by monocytes, T cells (mostly T regulatory cells [Tr]), B cells, dendritic cells, mast cells as
well as macrophages (Nagalakshmi et al., 2004). *IL-10* expression is regulated by two ubiquitously expressed transcription factors named Sp1 and Sp3 (Powell et al., 2000). Nevertheless, another part of *IL-10* expression regulation is due to existence of various copies of mRNA destabilizing motifs located within the 3’ untranslated region [UTR] of the *IL-10* mRNA (Powell et al., 2000). Such findings propose that in a lot of cells the *IL-10* is ubiquitously transcribed, while the actual *IL-10* secretion level depends upon post-transcriptional signals, too (Akdis et al., 2011). *IL-27* was introduced as an effective inducer of *IL-10* production in T cells (Stumhofer et al., 2007). Furthermore, the role of antigen presenting cells in regulation of *IL-10* expression is demonstrated where binding of histone deacetylase 11 protein to distal *IL-10* promoter region inhibits *IL-10* transcription (Villagra et al., 2009).

**Role in allergic diseases:** *IL-10* gives protection against allergic diseases. While it is constitutively produced in the airways of healthy individuals, its production is decreased in the airways of patients with asthma or allergic rhinitis. It has shown that T cell tolerance stimulated throughout immunotherapy process is due to elevated *IL-10* levels (Akdis et al., 1998). Particularly *IL-10*–producing Type 1 [Tr1] plays an important role in induction of allergen tolerance in human being (Jutel et al., 2003). It has clearly shown that allergen-specific Tr1 cells are prevailing in healthy individuals to avoid unnecessary immune responses to environmental triggers such as house dust mite, bee venom and food antigens. Both healthy and allergic individuals present three distinct allergen-specific T cell subsets (Th1, Th2 and Tr1) with different ratios (Akdis, 2006). An imbalance between Th2 and Tr1 cells can stimulate allergy development or recovery process. During allergen exposure, *IL-10*–producing Tr1 cells will be differentiated from both Th1 and Th2 cells. This results in suppression of the allergen-stimulated immune responses by Th1 as well as Th2 cells. This immunosuppressive response continues with allergen exposure and comes back to prior levels in two to three months (Meiler et al., 2008).

**IL-17F and asthma**

The pro-inflammatory role of IL-17F in asthma has been well described both in vitro and in vivo. IL-17F is highly expressed in asthmatics airways where the level of
overexpression is correlated with the severity of the disease (Kawaguchi et al., 2009). Furthermore, \textit{IL-17F} coding region variant (H161R) is reversely related to asthma, encoding an antagonist for \textit{IL-17F} wild type (Kawaguchi et al., 2006). Overexpression of murine \textit{IL-17F} has been linked to airway neutrophilia, proinflammatory cytokines induction, bronchial hyperreactivity as well as mucus hypersecretion. Therefore, \textit{IL-17F} may have a critical role in allergic inflammatory responses, and have imperative therapeutic implications in related to asthma (Kawaguchi et al., 2009).

**Identification and structure:** \textit{IL-17F} was discovered based on structural homology to \textit{IL-17A} (Kawaguchi et al., 2001; Starnes et al., 2001). Among \textit{IL-17} cytokine family, \textit{IL-17A} and \textit{IL-17F} demonstrate the highest degree of sequence homology. While other family members have been located on different chromosomes, synteny of \textit{IL-17A} and \textit{IL-17F} on mouse chromosome 6 proposes a common regulatory system. \textit{IL-17F} has two isoforms, a longer and a shorter isoform (ML-1) (Kawaguchi et al., 2001; Starnes et al., 2001). The structural characteristics of \textit{IL-17F} imply that it can make homodimers, where each monomer is covalently linked through a disulfide bond (Hymowitz et al., 2001). With the high sequence homology, having heterodimers of \textit{IL-17A} and \textit{IL-17F} monomers is not surprising (Hymowitz et al., 2001).

**Cellular sources and targets:** Both \textit{IL-17F} isoforms have been localized in activated memory T cells, later on named as Th17 cells (Harrington et al., 2005). While, the shorter isoform (ML-1) has been identified in blood basophils and mast cells as well, the longer isoform is produced by monocytes (Kawaguchi et al., 2001; Starnes et al., 2001). \textit{IL-17F} receptor (IL-17RA) has been localized in a variety of tissues such as lungs, liver, spleen and kidney (Yao et al., 1995). At cellular level, it is identified in T and B lymphocytes, epithelial cells, fibroblasts, vascular endothelial cells, marrow stromal cells as well as myelomonocytic cells (Moseley et al., 2003). In human, \textit{IL-17RC}, one of the main receptors in \textit{IL-17F} signaling pathway, is expressed in different tissues like heart, kidney, prostate, cartilage, liver, and muscle (Haudenschild et al., 2002).

**Role in allergic diseases:** Several reports suggest the potential role of \textit{IL-17F} in etiology of allergic asthma. The presence of ML-1 in allergen-induced T cells, mast
cells as well as basophils, and its increase following the allergen challenge in asthmatic subjects suggest the role of this cytokine in allergic inflammatory responses (Kawaguchi et al., 2001). Additional evidence comes from reports using mouse model of the asthma where IL-17F is stimulated in bronchial epithelial as well as infiltrating inflammatory cells following ovalbumin challenge (Suzuki et al., 2007). Interestingly, it has shown that *IL-17F*- deficient mice display malformed airway neutrophilia in response to the allergen challenge (Yang et al., 2008). Asthmatic mice models with IL-17F deficiency shows increased Th2 cytokine expression and eosinophil function. IL-17F seems to be involved in allergic diseases by recruiting neutrophils (Liang et al., 2007). IL-17F-induced expression of TGF-β in human endothelial cells may represent the role of this cytokine in airway remodeling, a phenomenon which is commonly observed in patients with severe asthma.

**IL-33 and asthma**

The effect of the IL-33/ST2 pathway has been associated with asthma susceptibility in mice. The number of IL-33–positive epithelial cells is significantly higher in bronchial epithelial cells of individuals with asthma (Kurowska-Stolarska et al., 2009). Furthermore, serum soluble ST2 [sST2] levels of patients with acute exacerbations of asthma are promoted as compared with nonasthmatic controls (Oshikawa et al., 2001). A number of asthma studies on mice have revealed the blockage of Th2 type inflammatory responses by either sST2 transfer or applying neutralizing antibodies (Baekkevold et al., 2003). IL-33 also contributes to mast cell activation in atopic asthma. Exogenous IL-33 administration in mice results in lymphocyte-independent airway hyperresponsiveness as well as goblet cell hyperplasia (Schmitz et al., 2005).

**Identification and structure:** In 2003, IL-33 was first identified by Baekkevold et al., (2003) as nuclear factor from high endothelial venules. It is a member of IL-1 cytokine family and an effective inducer of Th2 type responses through its receptor ST2 (also known as IL1RL1) (Schmitz et al., 2005). IL-33 protein has a b-trefoil structure with a molecular weight of 30 kD. It holds an extremely conserved N-terminal DNA-binding domain with a helix-turn-helix motif (Baekkevold et al., 2003). It is located in the
nucleus, linked to heterochromatin as well as mitotic chromosomes, and shows significant transcriptional inhibitor activities. Therefore, it is primarily located within the nucleus (Moussion et al., 2008). IL-33 is located on chromosome 9p24.1 in human, and 19qC1 in mouse model (Schmitz et al., 2005).

**Cellular sources and targets:** Although IL-33 is located within the nuclei of a variety of cells, epithelial (Préfontaine et al., 2010) and endothelial cells (Küchler et al., 2008) are considered as the main sources of IL-33. Therefore, like thymic stromal lymphopoietin [TSLP], IL-33 is considered as a mesenchymal-epithelial-derived cytokine, affecting both inflammatory as well as immune responses (Liu, 2006). IL-33 can promote IL-5 and IL-13 expression through in vitro-skewed Th2 cells that extremely secrete ST2 (Schmitz et al., 2005; Pecaric-Petkovic et al., 2009). Natural helper cells also produce ST2 and can express high levels of IL-5 as well as IL-13 on stimulation by IL-33 (Moro et al., 2010). Mast cells are other key targets of IL-33. Similar to the impact of IL-33 on mast cells and Th2 cells, IL-33 by itself can stimulate the expression of Th2 type cytokines as well as chemokines by basophils and induce cell adhesion and CD11b (Cluster of Differentiation molecule 11b) secretion by human and mice basophils (Pecaric-Petkovic et al., 2009; Schneider et al., 2009). In human, IL-33 can stimulate the expression of superoxide and IL-8, and provoke IL-5, IL-3 as well as GM-CSF-stimulated IL-8 secretion by eosinophils (Pecaric-Petkovic et al., 2009). IL-33 promotes the development of dendritic cells [DCs] from bone marrow cells (Mayuzumi et al., 2009). IL-33 provokes the expression of IL-6 by bone marrow-derived dendritic cells [BMDCs] and enhances the secretion of major histocompatibility complex [MHC] class II, CD40, CD86 as well as OX40 ligand on BMDCs cell surface (Rank et al., 2009).

**Role in allergic disease:** Daily intraperitoneal IL-33 treatment for a duration of one week leads to upregulation of IL-13, IL-4 as well as IL-5 in several tissues including the lungs, the spleen, the liver, and the thymus in vivo. It also induces eosinophilia, raises mononuclear as well as plasma cells numbers, and stimulates spleen enlargement with increased Ig A, IL-5, and IL-13 serum levels, while the expression of other cytokines such as TNF-α, IFNγ, IL-1a, IL-2 and IL-12 are not changed. It is also associated with
eosinophilic and monocyte infiltration, hypertrophy, mucus hypersecretion as well as media hypertrophy of lung arteries (Schmitz et al., 2005). IL-33 can stimulate airway inflammation in the mice lungs by amplifying macrophage activation in an IL-13 and IL-4Ra–dependent mode (Kurowska-Stolarska et al., 2009). IL-33–positive epithelial cells were considerably more in skin biopsy samples of individuals with atopic dermatitis. A significant sST2 secretion by bronchial alveolar epithelial cells is demonstrated in response to pro-inflammatory cytokines such as IL-1a, IL-1b, as well as TNF-a in a human endotoxemia inflammation model (Mildner et al., 2010).

In view of the above, several questions can be put forth:

- Whether genetic or environmental triggers prevail in susceptibility to asthma?
- Can age and gender of the patients affect the severity of asthma?
- Is there any association between BMI and asthma disease?
- Is allergic sensitization affecting the severity of asthma?
- Is there any association between interleukins promoter SNPs and asthma?
- Can in silico studies help us to estimate the effect of genetic variations in asthma-associated genes?
- Is there any difference in cytokines serum levels in asthmatic patients as compared to nonasthmatic controls?
- Can promoter polymorphisms in asthma-associated genes affect the expression level of these genes?

Taking all that into account and in order to answer some of the mentioned questions, this study was taken up. In the following sections the findings are presented and discussed.
<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
<th>FEV1 or PEF</th>
<th>PEF or FEV1 variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermittent</td>
<td>Symptoms less than once a week</td>
<td>≥ 80% pred</td>
<td>&lt; 20%</td>
</tr>
<tr>
<td></td>
<td>Brief exacerbations</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Nocturnal symptoms not more than twice a month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild persistent</td>
<td>Symptoms more than once a week but less than once a day</td>
<td>≥ 80% pred</td>
<td>20–30%</td>
</tr>
<tr>
<td></td>
<td>Exacerbations may affect activity and sleep</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nocturnal symptoms more than twice a month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate persistent</td>
<td>Symptoms daily</td>
<td>60–80% pred</td>
<td>&gt; 30%</td>
</tr>
<tr>
<td></td>
<td>Exacerbations may affect activity and sleep</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nocturnal symptoms more than once a week</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Daily use of inhaled short-acting $\beta_2$ agonist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe persistent</td>
<td>Symptoms daily</td>
<td>≤ 60% pred</td>
<td>&gt; 30%</td>
</tr>
<tr>
<td></td>
<td>Frequent exacerbations</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequent nocturnal asthma symptoms</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Limitation of physical activities</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FEV1 or PEF ≤ 60% predicted</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PEF or FEV1 variability &gt; 30%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** FEV1 = Forced Expiratory Volume in one second, PEF = Peak Expiratory Flow.