CHAPTER 1
Review of Literature
1.1 ASTHMA; HISTORICAL PERSPECTIVE

The word asthma has been derived from Greek word “azein” meaning “panting” or “breathing hard”. Historically there has been enormous effort to define asthma based on understanding of its etio-pathophysiology. The heterogeneity in its symptoms makes its comprehensive definition difficult, and therefore, time to time, various workers and organizations have modified it to accommodate newer observations and findings. Literature suggests that for quite long time all the diseases leading to episodes of “difficulty in breathing” or what is referred to as etiologies where patients “pant for breath” were commonly referred to as asthma. It was probably in the late 19th century that “cardiac asthma” and “bronchial asthma” were identified/realized as being separate. Also, till late 19th century or early 20th century most of the definitions seem to be operational and there are rare descriptions or mentions of causes of disease or its pathogenesis, owing to, either lack of understanding of disease pathogenesis or confusions surrounding it. Early to middle of the 20th century witnessed further developments in the respiratory epidemiology where a number of obstructive lung diseases were characterized and differentiated from each other, asthma being one of them (http://genepl.qimr.edu.au/staff/davidD/asthma1.html). A feature unique to the asthma subcategory, among various obstructive lung diseases seems to be reversible airway obstruction. Currently, a number of asthma sub-types (atopic asthma, non-atopic asthma, eosinophilic asthma, aspirin sensitive asthma etc.) have been categorized based on etiological, pathological or symptomatological considerations. Also, there are classifications based on severity of the disease such as mild, moderate and severe asthma, primarily with a focus on efficient management strategies. It is not in the scope of this thesis to dive into detailed and critical analysis of definitions and classifications but it should be noted that efforts are ongoing to refine and improve both the definitions and classifications. We might witness changes in great detail or completely new strategies for classifications of asthma as some experts suggest doing away with the term “asthma” in favor of characterization of distinct disease processes that presents as asthma (Editorial, Lancet 2006). Incorporation of many dimensions of the disease to identify distinct patterns in large population data using multivariate analysis has been suggested (Haldar et al., 2008).
1.2 ASTHMA; SOME EPIDEMIOLOGICAL FACTS

According to some estimates asthma prevalence could be 300 million persons worldwide that is expected to grow up to 400 million persons by 2025 (Masoli et al., 2004; Locksley, 2010). Various reports put asthma prevalence in India to range between 2.3 % to 16.6 % (Jindal, 2007; Pal et al., 2009). There seems to be wide variations across geographical regions with respect to its prevalence even after discounting for the methodological issues. Urban school children cohort from Bangalore seems to have the highest prevalence while adults across the country have the lowest estimates. Gender and age seem to be important determinants of asthma similar to what has been observed globally. While boys seem to have higher prevalence than girls in early childhood the trend is nearly reversed during adulthood where prevalence seems to be higher among women. Also, lifestyle seems to be an important determinant, positive family history of asthma seems to be the most important factor. From a survey in Delhi involving urban and semi-urban population 11% of adults were found to be asthmatic. Nearly 60% of them had family history of asthma: atopy or positive family history of atopy was strongly associated with asthma (Gaur et al., 2006). Also, 70% of the asthmatics had allergic rhinitis (Gaur et al., 2006).

Although mortality seems to be very low for asthma there is high morbidity and it is a considerable disease burden in terms of healthcare expenditure, man days lost etc. (Locksley, 2010). In some societies it is estimated that nearly one in four emergency visits are due to asthma. There are no good estimates of health care expenditure from India but in USA alone it has been estimated that 20 billion/year is spent on asthma management (Locksley, 2010; Moorman et al., 2007).

1.3 ASTHMA DEFINITION AND SUBTYPES

From a clinical viewpoint a good definition should be operational and encompass observable and/or measurable phenotypes of disease that should respond to clinical therapy/management programmes. From research perspective a careful clinical phenotyping aids experimental designs to understand the disease pathogenesis which in turn is helpful to refine the definitions of the disease and its management/treatment/cure.
As mentioned earlier there are various organizations that issue definitions and guidelines for management of asthma. National Asthma Education and Prevention Program Expert Panel (NAEPP) from the United States. National Institute of Health is one of the most active organizations and in 1991 issued its first comprehensive guidelines for diagnosis and management of asthma (NAEPP, 1997). Asthma was defined as “A chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular, mast cells, eosinophils, T lymphocytes, macrophages, neutrophils, and epithelial cells. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment. The inflammation also causes an associated increase in the existing bronchial responsiveness to a variety of stimuli. Reversibility of airflow limitation may be incomplete in some patients with asthma.” In the latest NAEPP expert panel report 3, it is further simplified and defined as (NAEPP. 2007) “A common chronic disorder of the airways that is complex and characterized by variable and recurring symptoms, airflow obstruction, bronchial hyperresponsiveness, and an underlying inflammation. The interaction of these features of asthma determines the clinical manifestations and severity of asthma and the response to treatment.”

As mentioned earlier, beginning of 20th century saw different asthma subcategories being identified. Rackemann was the first to classify asthma as intrinsic and extrinsic asthma based on the aetiology (Rackemann, 1921). Intrinsic asthma refers to non-immune or non-atopic asthma while extrinsic asthma is the immune type or involving “atopic” or allergic hypersensitivity reactions (Romanet-Manent et al., 2002). Serum total IgE measurement has provided convenient tool for identification of atopic state in epidemiological studies (Johansson et al., 2001). At present, asthma is classified into number of subtypes such as allergic, non-allergic, exercise induced, nocturnal, occupational, and steroid induced asthma (http://www.aaaai.org/patients/allergic_asthma/types.stm).

The extrinsic or allergic subtype seems to be the most prevalent asthma subtype among these, particularly in Indian scenario (Gaur et al., 2006). Since the nature of the inducers is supposedly different among these subtypes, it is being
proposed that the underlying molecular mechanisms may be significantly different (Fahy, 2010; Moore et al., 2010). Probably, the understanding of the underlying pathogenic mechanism of allergic asthma subtype is better than the other subtypes. Since this thesis is based on atopic asthma, the subsequent discussions about disease pathogenesis is with a focus on this sub-category, although, it should be mentioned that some aspects may be common/shared among all.

1.4 ASTHMA; CLINICAL DIAGNOSIS

When patients complain of breathing difficulties, clinicians begin with patient’s medical history to ascertain if they are asthma cases. There are different guidelines, Clinical Practice Guidelines of Expert Panel Report-2, National Asthma Education and Prevention Program (NAEPP, 2002) is being discussed briefly.

1.4.1 Symptoms

Following are the primary symptoms that could lead to a clue towards asthma diagnosis followed by the physical examination of the patient.

- Coughing
- Chest tightness
- Shortness of breath
- Trouble sleeping because of breathing difficulty
- Being unable to take part in physical activities
- Wheezing, coughing or shortness of breath when exposed to certain substances such as pollen, tobacco smoke, cat dander or perfumes.

Eczema, hay fever, family history of asthma and/or atopic diseases are often associated with asthma. Although, not key indicators, they are taken into consideration for the clinical diagnosis.

1.4.2 Pulmonary Function Tests

Asthma diagnosis is done on the basis of spirometry in individuals identified along with the above mentioned indicators. Typically in spirometry, the maximal volume of air forcibly exhaled from the point of maximal inhalation (forced vital capacity, FVC) and the volume of air exhaled during the first second of the FVC (forced expiratory volume in 1 second, FEV1) are measured.
• **Airflow obstruction** is indicated by reduced FEV1 and FEV1/FVC values relative to reference or predicted values. A reduced ratio of FEV1/FVC (i.e., <65 percent) indicates obstruction to the flow of air from the lungs, whereas a reduced FVC with a normal FEV1/FVC ratio suggests a restrictive pattern.

Inhalation challenge tests or provocation test may be done to assess lung function on exposure to varying doses of aerosolized allergens, histamine or methacholine. Spirometry readings are taken before and after the inhalation challenge.

• **Reversibility** is indicated by an increase of ≥15 percent and 200 mL in FEV1 after inhaling a short-acting bronchodilator.

### 1.4.3 Additional Tests to Rule out Asthma Misdiagnosis

To rule out the possibility of the infections, large airway lesions, heart disease, or obstruction by foreign object, chronic obstructive pulmonary disease (COPD), pneumonia, bronchitis, pulmonary embolism and panic disorders, all of which may mimic asthma, following additional tests are required to be undertaken:

- Chest and sinus X-Ray
- Complete and differential blood count
- Computerized tomography (CT) scans
- Sputum induction and examination
- Gastroesophageal reflux assessment.

Additional non-invasive methodologies/technologies are being increasingly adopted into clinical practice for better asthma diagnosis. For example, exhaled breath condensate provides useful information of airway inflammation and airway lining fluid composition.

Assessment of the severity of the disease is also becoming common and there are guidelines that help such assessments and in turn management of therapies etc.
1.5 ASTHMA PATHOGENESIS

Traditionally asthma has been clubbed with allergic or atopic diseases. Allergic or atopic diseases are collectively referred to as "Disease of civilizations" due to increased prevalence in industrialized societies (Claude, 1966). Allergy is termed "atopic" or "strange" since common or supposedly "innocuous" environmental allergen are the triggers for allergic hypersensitivity reactions. This notion of common environmental allergens being "innocuous" or "not-dangerous", and body's immune reactions considered "hypersensitive" towards them, did have important implications for the perception of the disease. Since allergens were "harmless", mainly the host factors were considered to be the culprits in causing the "hypersensitivity" reactions. For example, according to "Hygiene Hypothesis" (Strachan, 1989) lack of microbial exposure during childhood might shape host immune system, in a way that predisposes individuals towards allergy. This hypothesis was mooted to explain the high prevalence of allergic diseases such as hay fever, atopic dermatitis, asthma etc. in industrialized societies. Another very strong theory envisages the role of regulatory T cells being mal-functioned in atopic asthmatics, the reasons for which are being investigated, again laying the focus on host factors. Important point to make here would be that from perspective of immunology, by designating allergen as being "harmless" we have missed useful details about "allergic hypersensitivity" reactions i.e. "why" and "how" allergens pose threat to the immune system. Multidisciplinary approaches/studies suggest that most of the immune disorders (infectious and others) are outcome of complex host-pathogen interactions. Therefore, a thorough understanding of disease pathogenesis is possible only if "host factors" and "pathogen factors" are included in the context.

Allergens are complex mixtures of bioactive entities and this has caught the fancy of the immunologists recently. Studies aimed to understand the mechanism of "allergenicity" are providing useful insights as to why allergen could be sensed as "danger signals" by our body's immune system (Locksley, 2010). Allergens may have the properties such as protease activity, longer stability etc., that may activate the immune system (Locksley, 2010). Molecular mimicry, a process whereby sequence similarity between foreign and host molecular factors induces or augments immune reactions, is considered to be another mechanism of allergic hypersensitivity (Locksley, 2010). House dust mite, a common allergen that induces IgE reactivity is
complex mixture of trypsin-like, chymotrypsin-like and serine proteases, fatty acid, lipid binding proteins, chitinase, papain like cystein protease and a MD2 molecular mimic besides other proteins (Thomas et al., 2002). Importantly, MD2 is constituent of toll like receptor signaling complex and this MD2 mimetic has been shown to be capable of augmenting TLR4 signaling (Trompette et al., 2009). Similarly compositions of several other common allergens like cockroaches etc., suggest that they may have deleterious effects on body and may elicit immune reactions in a way similar to other infectious agents (Locksley, 2010). As allergens are complex mixtures of several entities, they may induce various intersecting innate immune pathways leading to adaptive immune responses characteristic of allergic asthma. It is relatively new field and it would be interesting to see how these cocktails of natural allergens are emulated in experimental models of allergy and asthma.

In allergic asthma, aerosolized allergens gain entry into the conducting airways, evading muco-ciliary clearance. Lung has large surface area and mucosal surface due to extensive branching for efficient gas exchange. Lung epithelium is specialized for antigen surveillance by heterogeneous groups of antigen presenting cells that include dendritic cells, macrophages etc., (Locksley, 2010). Depending upon the nature of the pathogen/allergen or environmental triggers/stress immune reaction is elicited. Repeated allergen exposure and/or accompanying inflammation damages the airways and its normal functioning (Figure 1.1). There are two distinctive features of asthma; airway inflammation and airway remodeling (Bousquet et al., 2000; Cohn et al., 2004). Airway inflammation refers to the infiltration of immune cells in lungs and airway remodeling refers to the structural changes in the airway that leads to the narrowing of airway lumen and consequently inefficient lung function (Figure 1.1) (Homer et al., 2000; Roberts et al. 1997).

1.5.1 Airway Remodeling

It has been known for long that asthmatic airways have altered structure and architecture compared to normal subjects, the airway mass of asthmatics is significantly increased; which also varies between fatal asthmatics (50% to 300%) and nonfatal asthmatics (10-100%) (Elias et al., 1999; Maddox and Schwartz, 2002). Best described in context of tissue injury and wound healing, it involves two distinct processes; regeneration of parenchymal components that restore normal structure and
Figure 1.1: The two components of asthma; airway inflammation and airway remodeling. a) The interaction between the inflammatory cells and the local cells/tissue results in inflammation and remodeling of the airway. b) Continued or persistent inflammation and/or remodeling of the airway constrict the normal airways, making it difficult to breadth. (Adopted from a) Cohn et al., 2004; b) Google images; http://www.google.com/imghp).
function and replacement of damaged tissue by connective tissue (Elias et al., 1999; Maddox and Schwartz, 2002). The alterations in the structure involves airway wall thickening, sub-epithelial fibrosis, increased mucus production and goblet cell mass, myofibroblast hyperplasia, myocyte hyperplasia and hypertrophy and epithelial cell hypertrophy (Elias et al., 1999; Maddox and Schwartz, 2002). Airway remodeling leads to increased susceptibility to asthma exacerbations due to persistent airway hyperresposiveness (AHR) and increased mucus production (Bousquet et al., 2000; Elias, 2000; Maddox Schwartz, 2002). Some of the structural changes mentioned above were first described in postmortem sections from status asthmaticus victims in 1960s (Dunnill, 1960; Hamid and Tulic, 2009) and also described recently in patients with mild asthma and children with difficult to treat asthma (Payne et al., 2003), however, mechanisms involved in airway remodeling are poorly understood. Recent studies suggest that balance between matrix metalloproteinases and tissue inhibitors of metalloproteinases may have crucial role to play in the structural alterations (Hamid and Tulic, 2009). The issues related to airway remodeling are described in great details in these eloquent reviews (Hamid and Tulic, 2009; Hershenson et al., 2008; Maddox and Schwartz, 2002) for any further reference. Prior to further discussions, it is to be noted that airway remodeling events were considered to have late onset as a consequence of repeated airway inflammation. However, recently these events have been described in childhood asthmatics leading to the arguments that it may have early onset and might have independent regulation (Boulet, 2003; Payne et al., 2003). It appears that these structural changes may be initiated either due to interaction of inflammatory mediators with stromal cells or due to tissue injury encountered early in childhood as a result of infections etc., (Hamid and Tulic, 2009). Further, it has been argued that disease severity may be modulated by airway remodeling events (Hamid and Tulic, 2009; Minshall et al., 1997). Additionally, asthmatic phenotypes with minimal inflammatory cells infiltrates but with airway structural changes have also been described (Jenkins et al., 2003). In future, better understanding of airway remodeling mechanisms should provide better understanding of the disease.

1.5.2 Airway inflammation

Even though eosinophilic infiltration of the lung in patient who died of asthma was reported way back in 1908, inflammation was not considered to be the cause of
asthma (Cohn et al., 2004; Ellis, 1908). Advent of bronchial biopsy procedures around early 1980s provided the first evidence that even when patients were asymptomatic, inflammation in the airway was present. This was a significant breakthrough and subsequently bronchial biopsies, bronchoalveolar lavage, induced sputum etc.. added further evidences and it became established that chronic inflammation of the airway characterizes asthma (Cohn et al., 2004). Inflammation in the lungs of allergic asthmatics resembles or is similar to inflammatory responses to extracellular parasites or parasitic worms like helminthes etc., where Immunoglobulin E (IgE) producing B cells, T helper cell 2 or TH2 cells, mast cells, basophils and eosinophils play determining role (Figure 1.2) (Locksley, 2010). In the airway wall, mononuclear cells mostly CD4+ T cells and eosinophils are predominant while the airway lumen mucus is mixed with activated macrophages, lymphocytes, eosinophils and sloughed epithelial cells (Cohn et al., 2004; Laitinen et al., 1997). Mast cells, macrophages, plasma cells, neutrophils, eosinophils are variably increased in the airway of asthmatics where mast cells have shown to be having activated status as they appear degranulated in electron microscopy studies (Laitinen et al., 1997). Also, inflammation is seen mainly in the larger conducting airways of asthmatics; however, in severe form of the disease even the small airways could be inflamed (Barnes, 2008a).

Allergic hypersensitivity reactions are initiated and sustained through continuous interaction between the airway epithelium and the recruited antigen presenting cells that patrol the airway mucosa (Cohn et al., 2004). Current model envision engagement of adaptive immune responses through the innate immune machinery (Figure 1.2). Development of antigen specific memory T cells and antibody secreting plasma cells potentiates chronic inflammation upon repeated allergen exposure (Barrett and Austen, 2009; Lambrecht and Hammad, 2009; Locksley, 2010). Briefly, encounter of epithelium with allergens leads to production of thymic stromal lymphopoetins (TSLPs), IL-33 and IL-25 (Locksley, 2010; Swamy et al., 2010). Thymic stromal lymphopoetins aid dendritic cell maturation and their migration to the lymph nodes and its activation (Ziegler and Liu, 2006). It induces TNF superfam ily member OX40L in dendritic cells which in turn prime naïve CD 4+ T cells into IL-4-competent cells (Ito et al., 2005). These IL-4 competent T cells either differentiate into T follicular helper (Tfh) after migrating to the follicular zone in lymph nodes or enter the circulation, exiting through the draining lymph to
Figure 1.2: Mechanism of allergic airway inflammation characteristic of asthma. On contact with allergens, epithelium produces thymic stromal lymphopoietin (TSLP), IL-33 and IL-25, which is probably the beginning of inflammatory events leading to airway inflammation resulting in airway hyperresponsiveness and asthma. TSLP promotes dendritic cell migration and maturation which primes IL-4 competency of T helper (TH) cells. The IL-4 competent TH cells then move to the follicular areas of lymph nodes and mature as IL-4 secreting T follicular helper (Tfh) cells. The Tfh cell mediate isotype switching in B cells in the germinal centre reaction in lymph nodes which results in allergen specific production of IgE (IgG1 in mice). IgE binds to its high affinity receptors on mast cells and basophils facilitating their extended survival and activation. IL-33 promotes IL-4 secretion by the basophils and IL-33 and IL-25 mediate the release of IL-5, IL-6 and IL-13 from the IL-25R^ natural helper cells. Together the cytokines (IL-4, IL-5, IL-6, IL-13 etc) mediate the terminal differentiation and/or recruitment T helper-2 (TH2) cells to tissues. In the setting of high IL-4 and IL-13, macrophages attain alternate activation phenotype which further aids the allergic or TH2 inflammatory processes. Collectively these cytokines also enhance recruitment and function cells like eosinophils, mast cells, basophils etc., which release mediators like leukotrienes, histamine etc., resulting in sustained inflammation airway remodeling. These processes also generate memory responses that facilitate more rapid responses upon repeated stimulations or allergen challenge. (Adapted from Locksley, 2010)
complete maturation as TH2 cells (Locksley, 2010; Reinhardt RL et al., 2009). Follicular helper T cells mediate isotype switching in B cells in germinal center reaction and these B cells then produce antigen specific IgE (Reinhardt et al., 2009). Immunoglobulin E binds to high affinity IgE Fc immunoglobulin receptors (FcsRI) that are highly expressed on mast cells and basophils (Ying et al., 1998). This provides a mechanism for activation through antibody crosslinking resulting in release of lipid mediators that leads to depressed lung function (Kinet, 1999). Mast cells could also be activated by IL-33 to secret vasoactive amines, lipid mediators, chemokines and cytokines and may contribute to the anaphylactic reactions (Pushparaj et al., 2009). Interleukin-33 also activates basophils to release a number of cytokines particularly IL-4 (Valent, 2009). Both IL-33 and IL-25 activate Il-25R⁺ lymphoid cells that remain poorly characterized and referred as natural helper cells, to produce IL-5, IL-6 and IL-13 (Fort et al., 2001; Moro et al., 2009). These cytokines promote the differentiation and/or recruitment of TH2 cells to the tissue and the mediators released from the TH2 cells further augments the allergic hypersensitivity reaction by promoting the activation and survival of the innate immune cells (Cohn et al., 2004; Locksley, 2010). In the setting of TH2 cytokines macrophages assume alternate activation status that may further promote allergic inflammation (Martinez et al., 2009). In whole, a positive feedback loop is established as a result of interaction between these diverse cell types and the mediators released by them. The inflammatory environment in the allergic airway is referred to as TH2 environment since it is believed that CD4⁺ T Helper cells 2 have significant role to play in initiation and perpetuation of the disease (Figure 1.2) (Cohn et al., 2004; Locksley, 2010; Robinson et al., 1992).

1.6 ASTHMA; DYSREGULATION OF T HELPER (TH) CELL DEVELOPMENT AND FUNCTION

1.6.1 TH1/TH2 imbalance in asthma

Depending upon the nature of antigen/stress and signals from the innate immune machinery CD4⁺ T helper cells could differentiate into different subsets with specialized functions (Hoebe et al., 2004; Medzhitov, 2008; Medzhitov, 2010). Identification of TH (T helper) cells that could induce antibody mediated responses and TI (T inflammatory) cells that elicited inflammatory reactions at the site of
antigen injection was the beginning of such characterizations (Janevway, 2002; Kim et al., 1985). Later, these subsets were renamed based on their cytokine production profiles (Mosmann et al., 1986). TH cells were referred to as T helper 2 or TH2 cells and TI cells were referred to as T helper 1 or TH1 cells (Janevway, 2002). Higher IFNG production is the characteristic feature of TH1-cells while TH2 cells have high IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 production (Abbas et al. 1996; Ho and Glimcher, 2002; Mosmann et al., 1986). It was also reported that there is reciprocal regulation between these two subsets; i.e. each of them suppresses the development of other while promoting their own development (Murphy and Reiner, 2002). A number of such subsets such as TH3, TH17 etc. have been reported and named based on their cytokine production profiles and functions (Mosmann et al., 2009).

The balance between the TH1 and TH2 subsets and their mediators seem to be critical for asthma pathogenesis (Elias et al., 2003). What causes such imbalance is a matter of intense research with some interesting theories being proposed (Cohn et al., 2004; Umetsu et al., 2002). For example, lack of microbial exposure during early childhood resulting in failure to shift the immune responses towards TH1 which are TH2 type at birth has been suggested, as immunological basis of “hygiene hypothesis” (Strachan, 1989; Umetsu et al., 2002). Also, it has been proposed that reduced microbial exposure may reduce immune suppression mechanisms, which control TH1/TH2 balance and lead to disorders like asthma (Umetsu et al., 2002). Additional mechanisms inducing tolerance to allergen have been described, failure and/or dysregulation of which may induce asthma (Tournoy et al., 2006). Accordingly, it has been proposed that down-modulation of allergen induced TH2 responses may be via development of allergen specific TH1 responses or development of allergen specific regulatory T cells (Bluestone and Abbas, 2003; Ling et al., 2004; O’Garra and Vieira, 2004, Tournoy et al., 2006). Contrary to the role of TH2 cytokines in pathogenesis of asthma, TH1 cytokines have been found to have a protective role and bring about suppression of the harmful effects induced by TH2 cytokines (Cohn et al., 2004; Locksley, 2010, Tournoy et al., 2006). Normally lymphocytes constitute a very small proportion of total leukocyte population in the lung, however, in airway of asthmatics their proportion is significantly increased and these are predominantly TH2 cells (Cohn et al., 2004; Locksley, 2010, Robinson et al., 1992). Even during the periods of quiescent disease, TH2 cytokines are produced which otherwise should die down in
absence of antigenic exposure (Cohn et al., 2004). Persistence of TH2 cells in the airway may be as a consequence of enhanced generation from naïve T cell precursors due to genetic and epigenetic control. Increased recruitment and/or proliferation of effector/memory CD4+ T cells or their reduced elimination may be other reasons for their persistence in the airway (Cohn et al., 2004).

1.6.2 Molecular mediators of TH1, TH2 cell development

Understanding the molecular biology of naïve CD4+ T cells commitment to TH1 and TH2 lineages is an area of intense research (Murphy and Reiner, 2002; O'Shea JJ and Paul, 2010; Rautajoki et al., 2008). The micro-environment in the vicinity of the naïve TH-cells, antigenic dose, co-stimulators on antigen presenting cells (APCs), genetic modifiers and non-cytokine have significant role in deciding whether CD4+ T cells would assume TH1 or TH2 effector phenotype (Murphy and Reiner, 2002). The influence of the cytokine environment on TH1/TH2 polarization has been best characterized among these. Current models envision transcriptional and epigenetic changes in cytokines and transcription factor genes that are brought about by the intrinsic factors co-operating with extrinsic activation signals (Murphy and Reiner, 2002). It is well understood that IFNG and IL-12 provide important signal for TH1 responses. Antigen activated NKT cells might produce IFNG that act through signal transducer and activator of transcription-1 (STAT1) and together with T cell receptor (TCR) signaling, induce marked expression of T-bet by naïve T cells (Murphy and Reiner, 2002; Szabo et al., 2000; Szabo et al., 2002). Augmented T-bet expression acts in TH1-cell commitment by remodeling the IFNG locus to an active status, leading to its increased production (Mullen et al., 2001; Mullen et al., 2002). T-bet also induces increased expression of IL-12RB2 (Mullen et al., 2001; Afkarian et al., 2002) subunit making them responsive to IL-12 which signals through STAT4 and may further augment IFNG production as well as promote expression IL-18R on TH1-cells (Robinson et al., 1997; Yang et al., 1999). Signaling through IL-18 opens up alternative pathway of IFNG production and IL-12 and IL-18 might synergistically induce maximum IFNG production (Neillors et al., 2001; Robinson et al., 1997; Takeda et al., 1998; Yang et al., 1999).

TH2-cell development has been proposed to occur in response to extrinsic source of IL-4 or in absence of inhibitory signal from the innate immune cells
IL-4 signals through STAT6 and in vitro stimulation of naïve T cells with IL-4 coupled with signals through TCR leads to GATA3 up regulation that induces heritable remodeling of the IL-4 locus, characteristic of the fully differentiated TH2-cell lineage (Ansel et al., 2006). GATA3 has a transcriptional auto-activating property that leads to massive GATA3 up regulation; this pathway has been suggested to be effective as initial source of IL-4 in in-vivo conditions (Ouyang et al., 2000; Lee et al., 2000a). Other lineage specific (such as c-MAF) and non-specific (such as NFAT and AP1) transcription factors may control acute IL-4 transcription (Murphy and Reiner, 2002). Absence of inhibitory signals from innate immune cells, possibly absence of IL-12 and IFNG production allows TH2-cell development through establishment of positive feedback loop involving IL-4 and GATA3 (Murphy and Reiner, 2002). Alternatively, some allergens have been shown to induce IL-4 production from basophils that might act as APCs (Perrigoue et al., 2009; Sokol et al., 2009; Yoshimoto et al., 2009). Also, mast cells have been suggested to be early sources of IL-4 (Brown et al., 1987; Plaut et al., 1989; Le Gros et al., 1990; Kullberg et al., 1996). Furthermore, it has been reported that STAT6 independent pathway may induce TH2-cell differentiation since ectopic expression of GATA3 in STAT6 T cells has been shown to induce full TH2-cell development (Finkelman et al., 2000; Jankovic et al., 2000; Ouyang et al., 2000). Alternative pathways of GATA3 expression have been suggested that may be under positive regulation of CD28 and Inducible co-stimulator (ICOS) signaling (Greenwald et al., 2002). Therefore, like T-bet in TH1 lineage commitment, GATA3 seems to have central role in TH2-cell development in both STAT6 dependent and independent manner. GATA3 modulate TH2 cell differentiation by inducing TH2 cytokine production, promoting selective growth of TH2 cells and inhibition of TH1 cell specific factors (Murphy and Reiner, 2002).

Once decision for commitment to one lineage is taken, subsequently each of these subsets tends to stabilize themselves and prevent the development of the other. For example, IFNG promotes TH1 cell development while suppressing the development of TH2 cells. TH2 cells express the both the IFNGR receptor subunits IFNGR1 or IFNGRA and IFNGR2 or IFNGRB while TH1 cells lack IFNGRB (Tao et al., 2000). Both the subunits are required for IFNG signaling and interaction of IFNG with complete receptor generates anti-proliferative signals on TH2 cells. A more
detailed description of the auto-regulation and cross regulation is to be found in chapter 4 (Section 4.1.1.1).

1.6.3 Suggestive involvement of TH1/TH2; cue from animal and human studies

Animal models/studies have provided useful data for suggestive involvement of TH1/TH2 cells and their mediators in allergic asthma. Over expression of IL-4, IL-5, IL-9, IL-13 using lung specific Clara cell 10 kDa (CC10) promoter resulted in allergic inflammatory features including eosinophilia and increased mucus production. Transgenic mice over expressing IL-13, IL-5, IL-9 resulted in collagen deposition in the airway and AHR suggesting that chronic expression of TH2 cytokines could induce airway remodeling features (Lee et al., 1997; Rankin et al., 1994; Temann et al., 1998; Zhu et al., 1999). From studies in interleukin-4 knockout mice or IL-4^{-/-}, it has been concluded that IL-4 is essential for development of ovalbumin induced airway eosinophilia and airway hyperresponsiveness and IgE production (Brusselle et al., 1995). Another study provides suggestive role of IL-5 in asthma pathogenesis (Foster et al., 1996). Ovalbumin sensitization and challenge does not lead to airway hyperresponsiveness in IL-5 knockout (C57BL/6) mice as opposed to wild type mice, while reconstitution of IL-5 production restores eosinophil influx to the airways. In IL-4Ralpha^{-/-} and STAT6^{-/-} knockout mice TH2 development is impaired and allergic inflammation is not induced (Akimoto et al., 1998; Cohn et al., 1999a; Kuperman et al., 1998). Transgenic mice expressing dominant negative mutant form of GATA3 in inducible and T cell specific fashion have also demonstrated the involvement of TH2 cells in allergic asthma (Zhang et al., 1999). Additionally, induction of TH2 cells in respiratory tract with inhaled antigens stimulated allergic inflammatory responses with eosinophil, AHR and mucus hypersecretion (Cohn et al., 1999a). Several other asthma models have demonstrated that TH2 cells and factors are essentially for asthma causation (Bates et al., 2009; Kips et al., 2000).

As mentioned earlier TH1 cells and their mediators have been proposed to have a protective role, although, contrasting data also exist (Hansen et al., 1999; Holtman et al., 1996; Randolph et al., 1999). Mice deficient in T-bet or T-bet^{-/-} mice have increased effector/memory CD4 T cells in their lungs (Finotto et al., 2002). Naive T-bet^{-/-} mice have increased TH2 cytokines IL-4, IL-5 and IL-13 in BAL (Bronco-alveolar lavage) samples and production of TH1 cytokine IFNG is reduced.
These mice spontaneously develop eosinophilic airway inflammation, mucus metaplasia, airway collagen deposition, myofibroblast hypertrophy and AHR (Finotto et al., 2002). It has been shown that inhibition of TH2 cell development and a shift towards TH1 results in reduction in asthma (Ray and Cohn, 1999; Cohn, 2004). Also TH1 cells or IFNG producing TH1 cells inhibit TH2-induced eosinophilia, mucus production and AHR (Cohn, 2004). These effects may be effector functions of IFNG rather than direct inhibition of CD4+ T cells (Blyth et al., 2000; Cohn et al., 1999b; Hofstra et al., 1998; Huang et al., 2001; Iwamoto et al., 1993; Renz et al., 1994). Contrarily, increased TH1 cells have been identified in the respiratory tract of some asthmatics and some studies have also demonstrated that TH1 cells enhance pulmonary inflammatory responses and AHR (Corrigan et al., 1988; Cohn et al., 2004). Others have also demonstrated that TH1 cells can recruit and augment TH2 cells in absence of specific antigen raising a possibility that TH1 cells may support allergic inflammation (Stephens et al., 2002). In mice, inhaled antigen activated TH1 cells in respiratory tract to induce neutrophil-predominant inflammatory response (Cohn et al., 1997; Cohn et al., 2004). In light of these contrasting data it has been suggested that the timing of TH1 cell activation in the setting of background of ongoing TH2 mediated allergic inflammation may be vital determinant (Cohn et al., 2004). Alternatively, TH1 driven neutrophil predominant inflammation and asthma may represent different sub-category of asthma where nature of antigen may vary (Locksley, 2010).

In brief, both the TH1 and TH2 cells have pleiotrophic effects modulating multiple features relevant to asthma pathogenesis. Interleukin-4 is mainly responsible for driving naïve CD4+ T cells towards TH2 profile and IgE class switching in B cells (Li-Weber and Krammer, 2003). Eosinophil differentiation in the bone marrows and its survival is modulated by IL-5 (Kouro and Takatsu, 2009). Interleukin-9 modulates mast cell differentiation and survival (Reinaud et al., 1995) while IL-13 mediates goblet cells differentiation and mucus production (Kondo et al., 2002, Wills-Karp, 2004b). Interleukin-4 and IL-13 also induce alternative activation status in macrophages which subsequently produce proteases and enzymes involved in matrix remodeling (Martinez et al., 2009). Together, the TH2 cytokines are also responsible for the epithelial and smooth muscles manifestation of the disease leading to decreased lung function (Locksley, 2010). IFNG on the contrary has been shown to bring about suppression of
TH2 cytokine release, inhibition of effector cell recruitment to the site of inflammation, induction of apoptosis in T cells, eosinophils and mucus storing cells, blockage of IgE isotype switch in B cells etc., (Teixeira et al., 2005).

At this point, a brief discussion on adequacy of the TH1/TH2 model, for asthma causation, would be appropriate. For last 15-20 years, subsequent to the inception of this model, much of the focus and energy has been directed towards understanding this bias. In fact much of the emphasis of the novel drug discovery has been on modulation of this pathway (Holtzman, 2003). In light of newer observation, it seems that even if one considers the allergic asthma subtype, TH2 bias as sole explanation seems to be over-simplistic. For example, some studies have demonstrated that antigen specific TH2 cells are not sufficient for initiating the asthmatic phenotype in experimental models of allergy (Stephens et al., 2002). Also, treatments aimed at selective blockade of TH2 pathways, has not yet proven to be efficacious in asthma (Holtzman, 2003; Grayson and Holtzman, 2002). To put things into perspective, for a multifactorial disease involving multiple cells types and tissues, putting a lot of emphasis on single cell type and the mediators released by it, may only be lacking in finer details. For example, tissue inflammation is immune response to infection and/or injury that ensures survival under stressful conditions. All the living organisms have developed inflammatory responses to restore order and bring about homeostasis. Number of mediators have been described that traffic different cells at the site of infection and injury, and also their subsequent clearance, once the pathogen or invaders have been taken care of. Each of the cell types and tissues has to undergo certain changes at the level of transcription and translation to meet the demands of inflammation. For example, the inflammatory cells have to enhance their longevity to release mediators that helps in clearing the pathogen or aiding the repair of tissue injury, at the same time these must respond to the exit signals or be cleared once their job is over. In systematic analysis of systemic inflammation, as a result of endotoxin challenge in humans, Calvano et al (Calvano et al., 2005), describe a number of changes at the level of transcription in leukocytes. The leukocyte response to acute systemic inflammation involves widespread suppression, at the level of transcription, of the mitochondrial energy production and protein synthesis machinery. The network based analysis lead to the identification of a number of functional modules. Clearly, human disease phenotypes are manifestation of defects
in multiple functional modules that are interrelated in physiological regulatory systems. In asthma, therefore, involvement of multiple cell types indicate that there must be multiple functional modules and dysregulation of one or more of them may be critical for disease manifestation. Accordingly, a reductionist, linear definition would not be ideal for complex disease like asthma. Several lines of evidences involving microarray and genetic studies reinforce these views and would be discussed subsequently (Kumar and Ghosh, 2009).

1.7 GENETIC COMPONENT(S) OF ASTHMA

It has been known for years that diseases like asthma, hay fever and eczema runs into families. Cooke and Vander (Cooke and Vander Veer, 1916), had for the first time, undertook a comprehensive study to see if there was any consistent dominant or recessive mode of inheritance, which was not to be found as we know from our current knowledge. Van Arsdel and Motulsky (Van Arsdel and Motulsky, 1959) epidemiological study in 6000 students of Washington's university further reinforced the family history factor where they had shown that 58 percent of offspring were allergic if both the parents were allergic, while only 20 percent were allergic if only one parent was allergic and only 6 percent offspring were allergic if none of the parents were allergic. Heredity appears to contribute significantly in asthma, conferring susceptibility or resistance, affecting the severity or progression of disease, and interacting with environmental influences (Zaas and Schwartz, 2003).

Asthma is not inherited as a single-gene disorder and shows a complex pattern of inheritance (Alford et al., 2004; Lilly, 2005). Reasons that might explain the deviation from simple patterns of inheritance include the following: (1) Genetic heterogeneity - different disease alleles might exist in different individuals; (2) Epistasis - more than one gene in each individual might interact to produce the disease phenotype, may exert additive effects, or act synergistically; (3) Incomplete penetrance - interaction with the environment might lead to incomplete penetrance; and (4) Phenocopy – development of disease owing to environmental factors alone (Anderson and Cookson, 1999; Altman et al., 2001; Hoffjan and Ober, 2002). In contrast to single-gene disorders, genes that predispose to asthma usually do not contain mutations that lead to a gross aberration in function (Reich and Lander, 2001).
Most often these are variants of normal genes, the evolutionary advantages of which remain obscure.

The evidence for an inherited susceptibility to asthma and allergy/atopy includes segregation analysis, twin studies and the demonstration of susceptibility loci in association and linkage studies of asthma traits (in humans and in mouse models). There are now numerous studies of the pattern of inheritance of asthma, rhinitis, allergic dermatitis, and serum IgE levels and these have clearly shown that the familial concordance is at least partly due to shared genes (Anderson and Cookson, 1999).

Twin studies comparing disease frequency in monozygotic (MZ) vs dizygotic (DZ) twins provide further evidence for genetic contribution to a disease. MZ twins are identical in all their genes, while DZ twins share, on the average, half their genes. In both twin types, in most cases, the twins would be exposed to the same environmental factors. Therefore, the difference in disease prevalence between MZ and DZ twins would be because of genetic factors. In a large series of 7,000 same-sex twin pairs, the concordance (the likelihood of disease in the other twin if one of the twins has the disease) of asthma in MZ twin pairs was 19%, while it was 4.8% in DZ twins (Edfors-Lubs, 1971). Other twin studies have also shown significant differences in MZ vs DZ twins, providing evidence for genetic contribution to atopy and asthma (Hopp et al., 1984; Koeppen-Schornerus et al., 2001; Los et al., 2001).

1.7.1 Mode of Inheritance

Several studies have noted parent-of-origin effects (imprinting) in the inheritance of atopy. In particular, some, but not all, studies have demonstrated an increased risk of atopy in children whose mothers were atopic (Moffatt and Cookson, 1998). A maternal effect has also been noted in some of the molecular genetic studies of asthma and atopy (Cookson et al., 1992; Daniels et al., 1996). A parent-of-origin effect has also been demonstrated in a mouse model that suggests that a major gene that modulates the susceptibility to ozone-mediated lung inflammation is imprinted (Prows et al., 1997). The mechanism of this phenomenon in humans remains obscure. Hypotheses include: immunological interactions between the mother and the child in utero and postpartum; genetic imprinting; and bias in the populations studied.
Testing and estimating familial aggregation of a disease consist of comparing rates of disease in relatives of individuals with the disease (known as case probands) with rates of disease in relatives of individuals without the disease (known as control probands). Relative risk ($\lambda$) is the prevalence of the disease in first-order relatives of the affected individual compared to its prevalence in the general population. The higher the value of $\lambda$, the greater is the genetic contribution to the disease. For asthma, the prevalence in the general population is 4 to 5%, while the prevalence in first-order relatives is 20 to 25%, yielding a $\lambda$ of 4 to 5. This compares to a $\lambda$ of 15 for type I diabetes and 3.5 for type II diabetes (Sandford et al., 1996). Heritability ($h^2$) provides a measure of the relative importance of transmissible genetic effects in the overall phenotypic variation. Heritability for atopy and asthma has been estimated to be around 0.4 to 0.6, that is, the relative contribution of genetic factors to atopy and asthma is estimated to be 40 to 60% (Morton, 1996).

1.8 APPROACHES TO IDENTIFY ASTHMA ASSOCIATED GENES

Population genetic studies like association studies and linkage studies have played major roles in identification of several causative genes for most of the complex disorders including asthma. Other popular approaches include animal models, mostly murine models of asthma, and microarray approaches where differential profiling of transcripts of thousands of genes could be undertaken simultaneously. In trying to understand disease processes, information about genetic variation is critical for understanding how genetic and functional variations are related (Yan et al., 2002). The Human Genome and HapMap projects have added fresh impetus to disease gene identification (Lander et al., 2001). The ability to genetically map complex disorders has been facilitated by the technological improvement in identifying and genotyping polymorphic DNA markers. Traditionally used microsatellite repeats, are being taken over by single-nucleotide polymorphisms (SNPs), the most frequently observed genetic polymorphisms. Each individual has approximately 10 million SNPs that are common in a given population. This extensive human variability accounts for the tremendous genetic diversity in populations and the genetic uniqueness of individuals. Recent advances have made genotyping of SNPs simpler and cost effective in a way that whole genome could be covered. It has become possible to genotype millions of SNPs in a single reaction involving same amount of genomic DNA as much as it was used earlier for genotyping single polymorphism.
1.8.1 Linkage analysis: Genome wide scans

Genetic linkage is a very powerful tool that has aided identification of genes involved in various diseases. Initially designed to find genes responsible for simple Mendelian diseases, currently, it finds common application in identification of genes involved in complex diseases like asthma. Genetic linkage occurs when two chromosomal loci are physically located close to each other, and passed together as a single unit. Linkage analysis, examines whether a disease phenotype is inherited jointly with a genetic marker locus, thereby indicating that disease locus is physically located close to marker locus. It relies on using family based data; family structure information and precisely defined phenotypic data of the families where there is segregation of the disease, and genetic marker data for the individuals in the family. Two main strategies are followed to perform linkage analysis: a) Model-based or parametric linkage analysis and b) Model-free or non-parametric linkage analysis.

1.8.1.1 Model-based or parametric linkage analysis

Logarithm of the odds or LOD score ($Z(0)$) method is the traditional linkage analysis method originally proposed by Morton in 1955 (Morton, 1955). It is the logarithmic (log base 10) ratio of the likelihood of the probability that a disease and marker loci are linked to the probability that disease and marker loci are unlinked (Kruylik et al., 1996). The LOD scores of each family are added together; for simple Mendelian trait $Z(0) > 3$ is viewed as strong evidence in favor of linkage and equivalent to odds of 1000:1 in favor of linkage (Ott J., 1974). A LOD score of 3 is equal to a $\chi^2$ of 13.8 (approx.) with one degree of freedom that translate to a $p$-value of 0.0001 (approx.) (Xu et al., 1998). These tests require that the genetic model of the disease be specified that includes: (i) mode of inheritance (e.g. recessive, dominant, co-dominant, etc.); (ii) degree of penetrance (i.e. probability of disease in the presence of the gene mutation); (iii) degree of phenocopies (i.e. probability of disease in the absence of the gene mutation); and (iv) frequency of the mutation in the population and (v) number of genetic loci (Alison, 2005). Because of these requirements, this method best suit simple Mendelian diseases. For complex disorders the genetic model of the disease are not generally known. To circumvent that problem segregation analysis has been developed that determines the genetic model of disease. Parametric linkage analysis is a powerful approach if the disease model is correctly specified (Alison, 2005).
As per the guidelines of Lander and Kruglyak, four levels of genome-wide linkage significance have been suggested, though it is suggested that confirmed linkage occurs only when the results are replicated in an independent study sample (Lander and Kruglyak, 1995).

1.8.1.2 Model-free or Non-parametric linkage analysis

Non-parametric linkage analysis methods are also commonly referred to as model-free methods since no prior information about the genetic model of the disease is required. This method relies on assessment of proportion of alleles shared among pair of relatives. The first method was developed by Penrose (Penrose, 1935) that examined allele sharing between sibling pairs as a test of linkage. These initial methods examine alleles shared identical-by-state (IBS) among sibling pairs i.e. siblings carrying same allele at the same locus (Alison, 2005). Later on Haseeman and Elston developed method that examine alleles shared identical-by-descent (IBD) implying that these allele pairs have been transmitted to each individual in the related pair from a common ancestor: in the sibling pair, either from the father or mother of the sibling pair (Haseeman and Elston, 1972). Currently there are methods developed that assess linkage not only among sibling pairs but among various sets of relative pairs in a pedigree (Alison, 2005). These methods determine whether the extent of sharing is greater than the sharing that would be expected by chance alone. Thus, linkage between a marker locus and a disease susceptibility locus is likely when a significant increase in the sharing of a genetic marker's alleles, in affected relative pairs is observed across multiple families. If there is a susceptibility gene located somewhere in the genome and shared by affected individuals (IBD), markers physically close to this region will be transmitted along with the disease allele. Furthermore, if the region is shared among affected individuals more frequently than is expected by random segregation, it may harbor the disease gene. The methods applying this approach include the affected sib-pair method (ASP) using sib-pairs or nuclear families and the affected pedigree member method (APM) using extended families (Alison, 2005).

No requirements of priori genetic model make these methods very popular, since for most of the complex disorders, the underlying genetic model is unknown (Alison, 2005). Also, segregation analysis that is used to determine the genetic model
for use in parametric linkage analysis has its own limitations. However, non-parametric linkage analysis model have less power when compared to the parametric linkage studies. Moreover, various methods that use information from different set of relative have their own advantages and disadvantages which have to be taken into consideration when one looks for appropriate method for a given data (Alison, 2005; Krulyak et al., 1996).

1.8.1.3 Linkage studies and positional cloning

There are generally two main approaches to linkage studies: candidate region approach and genome wide scans. In candidate region approach, based on some priori evidence either from cytogenetic analysis or other linkage studies, investigation can be performed to specific regions of the genome. The other approach is genome wide scans where markers throughout the genome are assessed for presence of linkage to the disease. Since primary goals of linkage studies are identification of novel genes and pathways, genome wide scans are more popular and candidate region approaches have been mostly used to validate the findings of one population in other populations.

Most of the linkage studies utilize microsatellite repeats, which are mostly dinucleotide CA repeats (although GATA repeats and others are also used) as genetic marker data. In a typical genome-wide scan, all members of affected families are typed for markers spaced at ≈5 cM intervals across the entire genome (Weissenbach et al., 1992). Linkage analysis of all markers in a genome-wide scan results in a large number of statistical tests being performed.

Linkage peaks identified in genome-wide linkage studies are usually quite large and contain hundreds of genes. An important challenge, therefore, remains to identify the actual causal gene(s) and causal variant(s) for any given locus (Boehnke, 1994). The most common and powerful approach is that of association testing (candidate gene) as discussed below. The alternative approach, positional cloning, involves systematic testing for association of a dense set of markers in the suggestive region, a process termed as fine mapping (Sinha and Luo, 2007). Although it is possible that the causal genetic variant is included in the set of variants/markers tested, the premise for this approach is that one or more of the variants tested will serve as a proxy for the causal variant. This is because genetic variants that are in relatively close proximity to one another often are inherited together, a phenomenon
known as linkage disequilibrium (LD; this approach is sometimes referred to as LD mapping). LD mapping has been established as an important tool for delineating genes involved in complex disorders. In fact, LD mapping has been used as a powerful tool to perform finer mapping of regions identified by genome-wide scans or in the identification of the genes of minor effect in complex disorders (Abdurakhmonov and Abdukarimov, 2008). Although LD over longer distances have also been observed, the statistical signal from LD acts over distances much shorter (≤ 60 approximated kb) than that from linkage and therefore a much higher marker density is required. This approach is most effectively carried out in a hierarchical fashion whereby once a significant association signal is detected, additional markers in the vicinity are tested in an attempt to refine the signal (hopefully down to a single gene).

The genome-wide screens are non-biased and are independent of any hypothesis about specific genes (Wills-Karp and Ewart, 2004). Even though, these screens suffer from the problems of being costly, labor-intensive and lack of power for statistical calculations (as large multi-generation families from homogeneous populations are required for these studies), the susceptibility genes identified using this approach have been more reliable (Lander and Schork, 1994). These genes may lie in the biochemical pathways involved in asthma but have no direct implication, or may be of unknown function. In addition to this, the regions identified in these scans are generally large chromosomal regions that contain several genes and finer mapping and positional cloning of genes within these narrowed regions is required.

1.8.1.4 Identification of human asthma genes

Several genome-wide scans have been conducted so far and regions on almost every chromosome have been linked to asthma or the associated phenotype. Several asthma susceptibility genes have been identified by these approaches. Most consistently replicated linkage to asthma have been obtained for the chromosomal locations 2p, 2q, 3q, 5q, 5p, 6p, 12q and 13q (Bouzigon et al., 2007; Li et al., 2007a; Webb et al., 2007; Hersh et al., 2007; Celedon et al., 2007; Kurz et al., 2006; Brasch-Andersen et al., 2006; Pillai et al., 2006; Kurz et al., 2005; Blumenthal et al., 2004; Raby et al., 2003; Koppelman et al., 2002; Laitinen et al., 2001; Lee et al., 2000b; Kimura et al., 1999; Wijst et al., 1999; Ober et al., 1998). However, only a few of these reports have met the accepted criteria for significant genome-wide linkage (i.e.,
LOD score ≥ 3.7 and p > 0.00000). The most replicated chromosomal regions include:

- **2p** [cytotoxic T-lymphocyte associated protein 4 (CTLA4)];
- **3q21.3** [including chemokine receptor gene cluster (linked with atopy)];
- **5q23-31** [cytokine gene cluster including interleukin IL4, IL5, IL13];
- Uteroglobin related protein (UGRP);
- Cluster of differentiation 14 (CD14);
- **6p24-21** [major histocompatibility complex (MHC) and Tumor necrosis factors (TNF)];
- **11q13-21** [high affinity receptor for IgE (FceRIα), Clara Cell secretory protein 10 (CCL10)];
- **12q21-24** [stem cell factor (SCF), Interferon-G (IFNG) and signal transducer and activator of transcription-6 (STAT6)];
- **13q12-14**, **16q21-23**, **17q11.2-q12** [containing chemokine cluster and the gene for iNOS] and **19q**. However, some which are highly statistically significant but have not been replicated in different populations are also worth to be considered.

The chromosomal regions that have been identified contain a cluster of genes: the functional role of many of these in asthma physiology is known while for some we are clueless or their functional significance is appearing slowly. Few observations with respect to linkage regions are worth mentioning here. A number of genome screens have been carried out for other immune disorders that have genetic basis. These studies have identified regions that are shared between different diseases. Asthma, for example, shows consistent linkage to the MHC loci on short arm of chromosome 6 (Moffatt et al., 2001) as with many other diseases. The linkage region for asthma overlaps with other inflammatory and autoimmune diseases as well. These are type I diabetes, ankylosing spondylitis, multiple sclerosis and arthritis (Cookson, 2002). This indicates that the susceptibility to different diseases might be influenced by individual genes in various forms or polymorphic alleles. Alternatively, disease susceptibility might be modified by cluster of genes, as in case of MHC loci, which have multiple effects on immune responses.

**ADAM33** (A disintegrin and metalloprotease, chromosomal location 20q13) was the first asthma gene identified by this approach (Van Eerdewegh et al., 2002). It is a complex molecule whose expression is largely restricted to mesenchymal cells including fibroblasts and smooth muscles, indicating that it may be involved in the remodeling of the airway tissues rather than in the immunological aspect of the disease. It is a member of a family of genes that encode membrane-anchored zinc-dependent metalloproteinases that are implicated in cell-cell interactions, cell fusion and cell signaling. Subsequently, several genes, namely, **DPP10** (Dipeptidyl peptidase...
10, chromosome 2q14), PHF11 (chromosome 13q14) and GPRA (chromosome 7q14), have been identified following the positional cloning approach.

DPP10 (dipeptidyl peptidase 10) is thought to modulate the activity of many chemokines and cytokines that regulate the inflammatory process. It is also expressed on central neurons, indicating that it may be playing an important role in regulating the neuronal control of airway smooth muscle tone (Allen et al., 2003).

PHF11 [plant homeodomain (PHD) finger protein-11] (Zhang et al., 1999b), contains two zinc fingers that are thought to be important in chromatin-mediated transcriptional regulation. Although, the exact role of PHF11 is not known in asthma pathogenesis, its expression on unactivated T-cells provide a clue towards its involvement in regulation of T-cell activation and differentiation.

Another identified gene that codes for an orphan G-protein coupled receptor named GPRA (Laitinen et al., 2001), showed distinct distribution of protein isoforms between bronchial biopsies from healthy and asthmatic individuals. Interestingly, GPRA mRNA was significantly up regulated in the mouse lung subsequently after antigen challenge in the sensitized versus non-sensitized mice, implying that GPRA might be involved in the pathogenesis of asthma and other IgE-mediated diseases.

The gene for the chemoattractant receptor expressed on TH2 cells (CRTH2), the receptor for prostaglandin D2 (Spik et al., 2005), has also been suggested to be a strong candidate gene for asthma due to its differential expression at the sites of allergen challenge. The prostaglandin D2 receptor gene (PTGDR) is expressed on the surface of mast cells and eosinophils, cells that generate the effector molecules of asthma pathogenesis.

Recently, SPRS8 (splicing factor, arginine/serine-rich 8) present at 12q24.33, has been detected as novel asthma associated gene in Dutch families. SFRS8 regulates the splicing of CD45, a protein which, through alternative splice variants, has an essential role in activating T cells (Brasch-Andersen et al., 2006).

### 1.8.2 Identification of genes in murine model of asthma

Several groups have performed genome-wide screens of various asthma associated traits in murine models of asthma because of their various advantages (De
Sanctis et al., 1995; De Sanctis et al., 1999; Ewart et al., 1996, Ewart et al., 2000, Zhang et al., 1999b). The advantages associated with the use of murine models include: reduction in genetic heterogeneity, greater control of the phenotype to be studied, ability to control environmental exposures and the ability to manipulate the murine genome through selective breeding strategies and gene-targeting approaches (Wills-Karp and Ewart, 2004a). All these advantages provide considerable power to the study of complex genetic traits in the murine models. Several of the genetic regions identified by these screens have also been linked to asthma traits in human studies (De Sanctis et al., 1995). For example, in a genome-wide screen for asthma associated quantitative trait loci in mice model of allergic asthma (Zhang et al. 1999b), a suggestive linkage was obtained in region on chromosome 11 with ΔP<sub>enh</sub>, which shows syntenic homology to human chromosome 17 and harbors genes for various chemokines and iNOS. Further, TIM1 (T-cell immunoglobulin and mucin-domain containing molecules) and C5 (complement factor 5) have been discovered using this strategy and are thought to be involved in TH2 cell differentiation process through as yet undefined mechanisms. Recently, Li X et al. have identified prostaglandin-endoperoxide synthase-1 (Progs1) and mannose receptor C-type 1 (Mrcl) genes as two new positional candidate genes for allergen-induced airway hyperresponsiveness through comparative sequence analysis and mRNA expression studies of mouse strains with genetically mediated airway responsiveness (Li et al., 2007a).

The linkage studies have motivated researchers to look beyond the classical paradigm of asthma pathogenesis. For example, linkage of atopy and asthma with DPP10, Fillagrins and GPRA, which are found to be expressed in terminal differentiating bronchial epithelium, indicate that we need to understand the role of epithelium in mounting immune responses, as it provides the first line of physical barrier or defense against the invading foreign particles. It also appears from recent studies that it is very important in mediating innate and adaptive inflammatory responses.
1.8.3 Association studies; candidate gene and genome wide

While linkage analysis is arguably powerful method for identifying rare, high-risk alleles in asthma, and is an unbiased search of the entire genome without any preconceptions about the role of a certain gene, many consider genetic association analysis to be the best method for identifying genetic variants related to common complex diseases (Collins et al., 1997; Risch N and Merikangas K, 1996) as it is more focused, hypothesis-driven. Like linkage studies either candidate gene/region association testing or genome wide association (GWA) testing could be performed. In candidate gene studies, genes of known function are chosen, as their function implicates them in asthma pathophysiology. Thus, it allows researchers to investigate the validity of an “educated guess” about the genetic basis of a disorder. Advent of technologies that could simultaneously multiplex and genotype millions of single nucleotide polymorphisms (SNPs), have enabled association analysis spanning the whole genome. Genome wide association studies have narrowed the disadvantage that association studies had over linkage studies; with respect to novel gene and pathway identification. However, due to economical and other considerations such as sample size etc., candidate gene approach is more popular.

Association analyses are generally model free, or nonparametric, so need not to assume a mode of inheritance, thus is more useful for diseases like asthma, where mode of inheritance is uncertain. Unlike linkage analysis, where markers are identified, association studies determine whether or not a specific allele within a marker is associated with disease. Association studies can be conducted in a group of randomly selected patients and controls as well as in small families or affected sibling pairs. The controls for population-based/case-control studies are often derived from the population that shares ethnic or geographic similarities with the cases. Alternatively, family-based controls are used in family based studies, wherein the control subjects are selected from families of affected individuals (probands) [Transmission disequilibrium test (TDT) and/ family based association test (FBAT)]. Case-control studies suffer from chance of high false positive or type I error while family based association studies suffer from type II error or chances of false negative association.

A statistical significant association between a variant and a disease phenotype can be either due to the fact that the disease allele may (i) truly affect the gene function by altering the amino acid sequence or by modifying splicing, transcriptional
properties, mRNA stability, post translational modifications and may thereby directly affect risk, (ii) the marker(s) showing significant association may be in linkage disequilibrium (LD) with the true-disease causing variant.

The first critical step in conducting candidate gene studies is the choice of a suitable candidate gene that may plausibly play a relevant role in the process or disease under investigation. Since asthma is a complex disorder and a multitude of candidate genes may be studied due to the involvement of complex biochemical pathways leading to the associated responses, most of the candidate gene studies have focused on areas of the genome that have shown linkage to asthma in genome-wide scans and also contain important immune response genes (Holloway and Koppelman, 2007; Bosse and Hudson, 2007; Park et al., 2006; Malerba and Pignatti, 2005; Ghosh B et al., 2003; Hakonarson and Halapi, 2002; Noguchi and Arinami, 2001; Illig and Wjst, 2002, Vercelli D. 2008). Broadly, in asthma, candidate genes could categorized into several ways such as genes involved in TH1-TH2 balance (IL-4, IL-5, IL-12B, IFNγ, IL-13, IL-4R, STAT6, STAT4, GATA3, T-bet etc.), cytokine/cytokine receptor genes (IL4, IL5, IL9, IL17, IL10, IL-21R, IL-4R, IL-5R, IL-13R, CD14, etc.), genes involved in innate immune recognition (TLR2, TLR4, TLR6, TLR10, NOD1, NOD2, IL10, TGFB1, STAT3, MHC class II molecules, PDGER2, etc.), signal transduction genes (STAT6, STAT4, STAT3, FceR1g, NFκB1, etc.), genes of metabolic pathways (LTC4S, ALOX5, ARG1, VDR, etc.), genes of oxidative stress (nNOS, eNOS, iNOS, GST, NAT2, etc.), genes involved in effector cell trafficking (CCR3, CCR5, CCR2, RANTES, Eotaxin, CRTH2, MCP1, CXC3CR1, etc.), airway remodeling genes (TGF-β1, ADAM33, DPP10, UGRP1, etc.), genes expressed on epithelial genes (CCL5, CCL11, CCL16, CCL24, CCL26, DEFB1, etc.), genes involved in mucus overproduction (hCLCA1, MUC5, etc.).

Most of the genes implicated from the etiology of asthma stem from candidate gene studies. Candidate gene association studies are quick to perform and the cost involved is low. However, unlike linkage studies, there is no chance of identifying novel gene or pathway. As mentioned above genome wide association studies have sort of filled the gap between association studies and the linkage studies. ORMDL3, a member of a gene family that encodes trans-membrane proteins anchored in the endoplasmic reticulum, was found to be associated with the risk of childhood asthma.
using genome-wide association studies (Moffatt et al., 2007). ORM family of proteins mediate sphingolipid homeostasis (Breslow et al., 2010) and have been shown to regulate endoplasmic reticulum mediated calcium signaling and cellular stress (Cantero-Recasens et al., 2010). There are other applications of genome wide SNP data such as identification of copy number polymorphisms and extension of GWA data to gene expression phenotypes.

1.8.4 Gene Expression Studies

Microarrays are often used as a high-throughput tool for the identification of differentially expressed genes, and they have been used to study animal models of allergen-induced asthma as well as human samples as it allows surveying thousand of genes in parallel (McClintock, 2002). Although this technology is recent and number of issues remains unresolved, unlike traditional experimental approaches, a microarray experiment is certain to generate novel data if the designing of the experiments is done carefully (Schena et al., 1998). Using both human subjects and animal models a number of studies have been undertaken that have identified novel genes or pathways that play important role in asthma pathogenesis and have therapeutic potentials. These studies have either utilized asthmatic tissues or cell types that have been implicated in asthma. Animal models have been used extensively as sufficient quantity of tissue samples could obtained with ease and the uncontrolled genetic and environmental confounding factors could be minimized. Zou et al. used arrays containing 40,000 human cDNA probes to analyze lung gene expression in a monkey model. They identified 149 genes whose expression was changed by at least 2.5-fold at 4, 18, or 24 h after inhalation of Ascaris suum antigen, or 24 h after treatment with IL-4 (Zou et al., 2002). In another study, 6.5% (300 genes) of the tested transcriptome were altered in the asthmatic lung, which were called as ‘asthma signature genes’ (Zimmermann and Rothenberg, 2006).

Karp and colleagues (Karp et al., 2000) provided an outstanding example of the combined use of microarray and genetic mapping data for asthma-gene discovery and identified C5 (complement factor 5), which mapped to one of the previously defined loci on chromosome 2. By additional experiments, they found correlations between the C5 genotype and airway responsiveness in larger groups of mice and also demonstrated the effects of C5 on the production of IL-12. Similarly, Mastuda et al.
(Mastuda et al., 2005) identified Tenascin-C (TNC) to be upregulated in microarray studies. Tenascin-C is an extracellular matrix glycoprotein that is a histopathological subepithelial marker used to monitor the severity of asthmatic disease and response to drugs. The TNC appears to be the susceptibility locus because LD mapping around the TNC gene (30 kb upstream and 20 kb downstream) with 48 SNPs found no other genes in the same linkage disequilibrium block. Further, the 44513A/T SNP represented a coding amino acid substitution (Leu1677He) in the Fn-III-D domain of an alternative spliced region of the gene and was predicted by computer modelling to cause instability in a β-sheet domain of the protein (Mastuda et al., 2005). Undoubtedly, microarrays had identified large numbers of genes that are differentially expressed in asthma models but there is a clear need for creative strategies to allow investigators to identify which of these genes play key roles in asthma pathophysiology.

Well it can be said with certainty that genetic studies have contributed significantly to understanding of asthma pathogenesis. The inconsistencies observed with respect to replication of genes observed in genetic studies, the alleles specifically involved and the direction of their effect still puzzles investigators. Segregation analysis suggests that the genetic structure of asthma consists of a relatively small number of moderate effects, as opposed to a large number of more diminutive effects. It has been suggested that due to issue of non-replication genetic association studies have acquired bad reputation. The pervasive failure to replicate has been suggested to be due to studies being underpowered. Methodological issues, such as sample size, study designs and analysis etc. have been highlighted to some of the other reasons. Such issues are not common to asthma but to majority of the complex disorders. Increasing sample size to achieve sufficient power seems to have caught up recently; however merely increasing the sample size may not solve the issue. The underlying biology of the complex disorders like asthma should teach geneticists to improvise in future. Some emerging concepts are being discussed briefly.

1.9 INFLUENCE OF ETHNIC BACKGROUND IN ASTHMA

Population studies in U.S.A alone, have repeatedly found differences in asthma prevalence (Boudreaux et al., 2003a; Boudreaux et al., 2003b; Fagan et al., 2001), morbidity (Perrin et al., 1989) and mortality (Grant et al., 2000) between the
Black and White populations. Such differences have been largely attributed to socio­
-economic factors and its correlates (Boudreaux ED et al., 2003a). While the evidence
for genetic basis of asthma is clear (CSGA, 1997; Holbeerg et al., 1996), information
about the contribution of genetic factors to race/ethnicity differences in asthma is still
not clear (Feldman C et al., 1993). There are two schools of thoughts, one which
believe that such differences are due to genetic difference between race/ethnic groups
(Chakravarti, 1999, Lester et al., 2001) and the other which opposes it arguing that
racial categories are social categories and not biological categories (Foster and Sharp,
2004; Cooper et al., 2003).

Both genome wide scans and candidate gene approaches have indicated that
asthma susceptibility loci are different in ethnically different populations (CSGA,
1997; Lester et al., 2001). Also, there are reports of population specific
responsiveness to certain drugs in ethnically different populations (Choudhry et al.,
2005; Drazen et al., 1999b). Albeit, population genetic studies have provided great
variations within ethnic/racial groups, differences between them are significant
especially between five major racial groups from different continents (Bowcock et al.,
1994; Dean et al., 1994; Risch et al., 2002). This leads us to evaluate the relevance of
population/ethnicity based data in understanding the mechanism of asthma
pathogenesis and also in administration of therapeutics. We have to realize that while
dealing with asthma we are dealing with heterogeneous disease caused due to
complex interaction of genes and environment.

1.10 PHENOTYPIC HETEROGENEITY

As discussed throughout the test, like other complex disorder asthma is a
heterogeneous disease. It has variable age of onset; there are different stimuli which
trigger it; it can present itself as mild throughout its history or may be severe and life
threatening; and it is associated with many intermediate phenotypes such as IgE,
eosinophilia, allergic rhinitis etc. and these could be assorted in any combination
(Guerra and Martinez, 2008; Vercelli, 2008). The emerging consensus is that asthma
is unlikely to be a single disease, rather a collection of phenotypes. Whether these
phenotypes are result of single pathological mechanisms or have different etiologies
remains to be understood. For this, as discussed earlier, refinement of the phenotypic
characterization has to be undertaken and search for determinant of those initiated
(Chanock et al., 2007). Clinically, either of the two components i.e. airway remodeling and airway inflammation could lead to airway hyperresponsiveness. Airway remodeling and airway inflammation could be triggered by various stimuli, each having distinct and overlapping features leading to compromised lung function. It could be argued that lack of standardized phenotypic characterization may contribute to the observed inconsistencies, especially since the distinct phenotypes may represent involvement of distinct genetic and molecular pathways to an extent (Kumar and Ghosh, 2009).

1.11 GENE-ENVIRONMENT INTERACTIONS

In complex disorders gene-environment interactions modify the impact of a given gene on complex phenotypes. Various epidemiological studies support the view that allergic inflammation phenotypes are influenced by the environment (von Mutius, 2004; Vercelli, 2008). Animal experiments do demonstrate that gene-environment interactions do account for the proportion of the phenotypes variance more than genetic or environmental factors could account for individually (Valdar et al., 2006). Interactions involving genes involved in immunity and pathogen products provide suitable examples of interaction effects (Vercelli, 2008). Many genetic studies have studied the role of $CD14\,-\,159CT$ polymorphisms which is a functional polymorphism and associated with allergic inflammation (Le Van et al., 2001). These studies have reported protective (Jones et al., 2002; Leyaert et al., 2006), disease promoting (Ober et al., 2000; Woo et al., 2003) as well as no effect (Kabessch et al., 2004; Kedda et al., 2005) for the same alleles in different population. In fact opposite effects, for the same polymorphisms, have been reported, within the same population (Vercelli, 2008). Gene-environment seems to explain the discrepancies (Vercelli, 2008). In European population, the influence that $CD14\,-\,159CT$ polymorphism has on IgE and asthma, seems to be modulated by environmental exposure; varying in response to cat and dog exposure versus stable animals. $CD14$ genotype effects on allergen sensitization, eczema and wheezing seems to be dependent on amount of domestic endotoxin exposure (Vercelli, 2008). Polymorphisms in $17q21$ confer higher risk in early onset asthma and the risk increases further when there is exposure to environmental tobacco smoke in early life (Bouzigon et al., 2008). This region contains four genes all of which could have potential role in asthma pathogenesis (Bouzigon et al., 2008). The conclusion that one may draw from these observations
are that a given polymorphism may be associated with disease or be protective against it in context of environment. It would not be unreasonable to argue that various gene-environment interactions may explain some of the discrepancies of non-replication. Guerra S. et al rightly point out that we have to take a paradigm shift and design studies that take into account multiple factors that could be partners in bringing about disease pathogenesis (Guerra and Martinez, 2008).

1.12 GENE-GENE INTERACTIONS

Asthma is a multigenic disorder and is greatly influenced by environmental factors, as we have seen in our earlier discussions. Therefore testing for a single gene or single factor for accurate prediction of disease outcome is an unjustified expectation (Beaudet AL, 1998; Evans et al., 2001; Holtzman and Marteau, 2000). In fact, analyzing for a single loci for traits that are controlled by multiple loci, there is considerable loss of power, magnitude of which depends upon the underlying genetic model (Knapp et al., 1994). In a linkage study involving three ethnic groups from America, Xu et al., report significant increase in LOD score for several loci in their gene-gene interaction analysis (Xu et al., 2001). For example, evidence of linkage at 5q31 increase from LOD score 0.98 to 3.21 when analysis was conditioned upon linkage at lp32 (Xu et al., 2001). Other loci such as 12q22, 8p23, 15q13 also showed increased LOD score when their analysis was conditioned upon loci that were showing marginal signals in their independent analysis. These results were also complemented by affected sib-pair two locus analysis (Xu et al., 2001). Multiple genes that reside in these regions could have additive or synergistic effect in modulating asthma pathogenesis. Similarly, a case-control study in the Dutch population suggested significant gene-gene interaction between S478P SNP of IL-4Ra and −1111 promoter SNP of IL13 genes with BHR (p= 0.003). Individuals with the risk genotype for both genes were at almost five times greater risk for the development of asthma compared to individuals with both non-risk genotypes (P= 0.0004) (Howard et al., 2002). Several other studies in asthma and other complex disorders suggest that gene-gene interaction studies could enhance disease outcome prediction when, concurrently, genes from a pathway or interacting pathway are selected (Yang et al., 2003; Moore and Williams, 2005).
Current analytical tools have limitations with regard to number of parameters (genetic/environmental etc.) that could be included in interaction analysis since increase in parameters result in increase in dimensionality of the data. Traditionally, logistic regression analyses have been performed to identify interacting partners but they do have limitations since only parameters having independent primary effect could be tested for interactions. Approaches such as multifactor dimensionality reduction (MDR) etc. are non-parametric tests that could identify interaction even in the absence of independent primary effects (Moore and Williams, 2005) and are becoming very popular for performing gene-gene and gene-environment interactions. It is expected that in future low cost genotyping along statistical tools that handle high dimensional data would revolutionize this field.

1.13 COPY NUMBER VARIATION/POLYMORPHISMS

The genomic variation in the human genome ranges from single nucleotide variation to large microscopically detectable variations that have also been shown to be associated with many disorders (McCarroll and Altshuler, 2007). The advancement in the genotyping technology have led to identification of structural variation that fall in between these two extremes, known as copy number variations (CNVs) (McCarroll and Altshuler, 2007). Currently all genomic variations larger than 1kb of DNA are termed as structural variations. Structural variants could lead to change in gene dosage in case of deletion or duplication etc. or without any change in gene dosage as in inversions or balanced translocation (McCarroll and Altshuler, 2007). Initially identified in case of sporadic disorders, inherited CNVs have been reported and associated with many infectious and immunological disorders like. HIV, systemic lupus erythomatous, lupus glomerulonephritis etc. (McCarroll and Altshuler, 2007). Therefore it is postulated that CNV has a significant contribution in the regulation of the immune system. Some CNVs might predispose to increased disease susceptibility. Case-control and family-based association tests has established that low copy number of Complement Component C4 is a risk factor against SLE Susceptibility in European Americans (Yang et al., 2007). Further, an association between low FCGR3β copy number and autoimmune glomerulonephritis has also been reported in SLE. (Aitman et al., 2007). A low copy number of CCL3L1 (Gonzalez et al., 2005) and DEFB4 genes (Fellermann et al., 2006) have also been associated with increased susceptibility to AIDS and Crohn disease, respectively. All these CNVs are expected to play a
significant role in asthma pathogenesis. Various issues related to identification and analysis of copy number polymorphisms are being debated and under modification (Scerer et al., 2007). However, it has generated enthusiasm among geneticists as it has potential to explain gene dosage changes in some of the complex disorders (Carter, 2007; Conrad and Hurles, 2007; Sebat, 2007). Asthma like other complex disorders should certainly benefit from this field and more and more genetic components could be identified.

1.14 EPIGENETICS

Epigenetics, the term, that refers to heritable characters other than those encoded in the DNA sequence, play major role in gene-expression (Egger et al., 2004). Epigenetic silencing, which is mediated by DNA methylation, histone modifications and small RNAs, is influenced by both genetic and environmental factors (Egger et al., 2004). These epigenetic changes could also be inherited transgenerationally influencing disease susceptibility (Miller and Ho, 2008). Epigenetic studies have potential to demonstrate the gene expression changes that occur during disease processes, as mentioned earlier, the epigenetic changes accompanying T helper cell differentiation towards TH1 or TH2 have been described (Ansel et al, 2003). Methylation changes in the promoter and intronic regions of IL-4 gene has been shown to modulate the production of IL-4 (Lee et al., 2002). Similarly hypermethylation in the IFNG gene leads to higher production of IL-4 due to suppressed production of IFNG (Jones and Chen, 2006). These two genes are critical modulators of TH1/TH2 balance and play vital roles in asthma pathogenesis. Also, it has been shown that untreated subjects with asthma possess higher levels of histone acetyltransferase (HAT) and lower levels of histone deacetylase (HDAC) in bronchial biopsies which get reversed upon steroid administration (Ito et al., 2002). Similar observations have also been made for COPD which has many feature common to asthma (Cosio et al., 2004). Parent of origin effect has also been noted wherein polymorphisms inherited from a particular parents (father or mother) influence the disease susceptibility of the offspring (Miller and Ho, 2008). In this regard maternal prenatal environment seems to play vital role in bringing about gene expression changes in the offspring (Miller and Ho, 2008). Many epidemiological studies point towards critical role that prenatal and early postnatal environmental exposures could play in bringing about asthma pathogenesis (Miller and Ho, 2008). For asthma which
has variable time of onset it has been proposed that certain epigenetic changes during adulthood could also influence the disease onset and progression (Miller and Ho, 2008). Micro RNAs (miRNAs) have emerged as critical players of gene regulation, post-transcriptionally and post-translationally and could be key mediators of epigenetic regulation (Chuang and Jones, 2007). It is worthwhile to note that a number of miRNAs have been shown to have critical role in immunity (Taganov et al., 2007). Till now, nearly 300 miRNAs have been identified and each of them could target hundreds of genes (Krek et al., 2005). Recent development of technologies that enable high-throughput/genome-wide detection of epigenetic changes should bring out more data relevant to asthma and related phenotypes. It should be vital to know how genetics, environmental factors and epigenetics regulate each other and in turn the molecular events that underlie complex diseases such as asthma.