Chapter 3

Review of Literature
3.1. Asthma: Overview

“According to the Global Initiative for Asthma Guidelines (GINA 2002), asthma is defined as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role”. These conditions are variable and usually reversible in asthma (Louhelainen et al., Int J Chron Obstruct Pulmon Dis. 2008; Barnes et al., Nat Rev Immunol., 2008). Each of the mechanisms of asthma has multiple underlying potential causes. Therefore a fixed *modus operandi* for managing asthma leads to many patients being insufficiently diagnosed and wrongly treated, as many of the underlying mechanisms are left undiscovered or unaddressed (Hunt J, Am J Respir Crit Care Med., 2007). This has led to the suggestion that asthma is a syndrome comprising of different sub-types that share a common general phenotype of episodic reversible bronchoconstriction, but may be mechanistically different.

![Players in Asthma pathogenesis](Image)

**Figure 2: Players in Asthma pathogenesis** (Holgate S, Discovery medicine, 2010).

As per the definition of NIH, “Asthma is a disorder that causes the airways of the lungs to swell and narrow, leading to wheezing, shortness of breath, chest tightness, and coughing.” Involvement of multiple cell-types results in final etiology with asthma as
an outcome. Till recently it was believed that asthma was a Th-2 dominated disorder with high levels of IL-4, IL-5 and IL-13, along with implications of few cytokines and chemokines in asthma etiology. Chemokines are important in the pathogenesis of allergic inflammation, as they are potent leukocyte chemoattractants, cellular activating factors, and histamine-releasing factors. Theotaxin subfamily of chemokines and their receptor CC chemokine receptor 3 are in particular very important and have roles as central regulators of the asthmatic response.

3.2. Asthma Burden and Prevalence

300 million individuals are estimated to suffer from asthma worldwide and by 2025; there might be an addition of 100 million more to this number. (Bijanzadeh et al, Indian J Med Res., 2011) In India the prevalence of asthma in adults is 1.69-3.47. (Aggarwal et al, Indian J Chest Dis Allied Sci., 2006)

According to the Global Burden of Asthma Report, asthma is now one of the world’s most common long-term conditions. Based on standardized epidemiology study data performed in more than 80 countries, this report provides a comprehensive survey of the prevalence and impact of asthma around the world. The Report was commissioned by the Global Initiative for Asthma (GINA), in 1993, to reduce the burden of asthma by working with healthcare professionals and public health officials around the world. Data on prevalence of asthma is now available from several countries. Prevalence varies from region to region depending upon the definition used for diagnosis of asthma. Asthma is currently reported to affect 1.2 to 6.3% adults in most countries. The clinically diagnosed asthma in adults is generally reported as 2.7 to 4.0% in most European countries, 12.0% in England and 7.1% in the United States (Los et al, Twin Res., 2001). In Australia, the prevalence is quite high (9.5 to 17.9%). In India, the overall burden of asthma is estimated at more than 15 million patients (Pal et al, Indian J Community Med., 2009). An increasing prevalence, mortality, incidence and severity of asthma have been reported worldwide. Recent reports suggest wide variation (4-19%) in the prevalence of asthma from different geographic areas of India (Aggarwal et al, Indian J Chest Dis Allied Sci., 2006), and further increase in mortality is expected in the future (Lancet, 1998).
The prevalence of asthma varies internationally, but there is narrowing of these differences between various countries owing to increased prevalence in low and middle income countries and plateauing in high income countries (World Allergy Organization (WAO) White Book on Allergy, 2011). It is estimated 300 million people worldwide suffer from asthma, with 250,000 annual deaths attributed to the disease (World Health Organization. Global surveillance, prevention and control of chronic respiratory diseases: a comprehensive approach, 2007). Almost all of these deaths are avoidable. Workplace conditions, such as exposure to fumes, gases or dust, are responsible for 11% of asthma cases worldwide. About 70% of asthmatics also have allergies. A significant contribution to the global burden of asthma is occupational asthma, since the condition accounts for approximately 15% of asthma amongst adults (World Health Organization. Global surveillance, prevention and control of chronic respiratory diseases: a comprehensive approach, 2007).

According to the National Commission on Macroeconomics and Health (Govt. of India, 2005), there were approximately 25 million cases of asthma in India in 2001, which may increase by 50% in 2016.

**Figure 3: Different triggers of asthma** (http://mymedicalassistant.org/types-of-asthma)

### 3.3. Types of Asthma

Depending upon whether the symptoms are precipitated by allergens or not, asthma may be classified as atopic or non-atopic. Atopic and non-atopic asthma are also sometimes referred to as extrinsic and intrinsic asthma, respectively. On the basis of severity, however it could be classified into major two types:
3.3.1. Mild Asthma

A reduction of 20-40% in FEV1 (Forced expiratory volume) with a 20-30% variability is a characteristic of clinically mild to moderate asthma. Its pathological features involve acute or chronic airway inflammation. In the most common allergic subtype this consists of activated Th2 lymphocytes and eosinophil infiltration in association with IgE production. Asthma also involves remodeling of the airway wall and AHR, hyperplasia of mucus secreting cells and goblet cell metaplasia. Up-regulation of Th2 cytokines including IL-13, IL-4 and Thymic Stromal Lymphopoietin (TSLP) contribute to various pathological features of the disease (Shikotra, Choy et al. 2012).

3.3.2. Severe Asthma

Clinically characterized by reduction of more than 60% in FEV1 (forced expiratory volume) with >30% variability. Since severe asthmatics are usually resistant to glucocorticoid treatment, it is particularly difficult to treat. The pathological features of the severe type differ from mild asthma, involving a mixed Th2/Th1 phenotype with a possible emerging role of Th17 cells. Lymphokines such as tumor necrosis factor (TNF)-α, IFN-γ, IL-17 and IL-27 are also found to be elevated in the lungs of severe asthmatics that induce the influx of neutrophils (vs. eosinophil in mild asthmatics) or a mixed granulocyte airway infiltrate.

Figure 4: Schematic diagram representing differences in airways between a healthy individual and an asthmatic patient. (http://thehealthanddiseaseblog.blogspot.in/2013/03/Asthma-Epidemiology-Classification-Causes-Pathogenesis-Morphological-changes-of-airway-Signs-and-Symptoms-Investigations-Treatment-Management-Asthma-in-pregnancy-and-Prognosis-of-Asthma.html)
Normal individuals have a well-defined open airway lumen through which ventilation can take place; on the other hand, asthmatic individuals have severely constricted bronchi which hamper the flow of air, hence leading to difficulty in breathing.

### 3.3.3. State of the Art Asthma Medication

For each individual the medication varies depending upon the age, symptoms, and asthmatic trigger, but invariably most can be classified under two categories: (https://www.mayoclinic.com/health/asthma/DS00021/DSECTION=treatments-and-drugs)

#### 3.3.3.1. Long-term Asthma Control Medications

These are taken on a daily basis and reduce the chances of having an asthma attack. Types of long-term control medications include:

1. **Inhaled corticosteroids**: These include fluticasone, budesonide, mometasone and ciclesonide etc. These are generally safe for long-term usage in comparison to oral corticosteroids, having relatively lesser side effects.
2. **Leukotriene modifiers**: These are oral drugs including montelukast, zafirlukast and zileuton. They help relieve asthma symptoms for up to 24 hours.
3. **Long-acting beta agonists**: These inhaled drugs include salmeterol and formoterol, which prevents airway closure and reduce inflammation. They usually are taken in combination with an inhaled corticosteroid.
4. **Combination inhalers**: It is a combination of long-acting beta agonist with a corticosteroid, such as fluticasone-salmeterol etc.
5. **Theophylline**: It is a routine bronchodilator that helps in keeping the airways open.

#### 3.3.3.2. Quick-Relief (Rescue) Medications

These are helpful for quick relief during an asthma attack and used as SOS medication. Various quick-relief medications include:

1. **Short-acting beta agonists**: These are quick relief bronchodilators acting within minutes of administration, which includes albuterol, levalbuterol and pirbuterol.
ii) **Ipratropium:** It immediately helps in relaxation airways for easier breathing. Mostly used for emphysema and chronic bronchitis, but sometimes is used for asthma treatment also.

iii) **Oral and intravenous corticosteroids:** These include prednisone and methyl prednisolone which help relieve airway inflammation. However, owing to their severe side effects during long term use, they are mostly used for short term therapies to treat severe asthma cases only.

iv) **Allergy medications:** These medications help during complications of asthma involving allergy. They include allergy shots, omalizumab, oral and nasal sprays.

---

![Figure 5: The peri-operative management of asthma](Applegate et al, Journal of Allergy and therapy, 2013).

### 3.4. “-Omics”: What is it?

-Omics can be defined as a broad term encompassing different biological and engineering science discipline techniques for analyzing biological interactions ([http://www.nature.com/omics/about/index.html](http://www.nature.com/omics/about/index.html), [http://omics.org/index.php/Omes_and_Omics](http://omics.org/index.php/Omes_and_Omics)).
The suffix – ‘om’ dates back to “genome” as there is a common belief that there exists some root “-ome” in Greek referring to wholeness or completion, implying complete information.

It is a recent term that has become popular owing to massive experiments being performed in various “-omes”. The main focus of which are on:

1. Mapping information to objects such as genes, proteins, and ligands;
2. Finding interaction relationships among the objects;
3. Engineering the networks and objects to understand and manipulate the regulatory mechanisms; and
4. Integrating various omes and omics subfields.

It mainly includes disciplines such as genomics, transcriptomics, metabolomics, proteomics, phenomics etc. to look into the complex biological phenomenon from a holistic perspective.

The field is relatively new, with the early papers coming around a decade back.

The initial breakthrough paper came out in Nature Biotechnology in 2002 wherein data from different technologies – genomic, transcriptomic, proteomic, and metabolomic were analysed in silico to make some sense out of it (Palsson B, Nature Biotechnol., 2002). There are other reports as well wherein omics approach has been used to look into a problem from a holistic perspective (Nibbe et al, Plos Comput Biol., 2010; Fukushima, A.Curr Opin Chem Biol., 2009).

3.5. ‘–Oomics’ Perspective to Biology

Of the different disciplines that constitute –omics, metabolomics is the comprehensive analysis in which all the metabolites of a biological system are identified and quantified. It is the study of unique chemical signatures left by cellular metabolic processes, their changes over time as a consequence of multi-parametric pathophysiologial stimuli.

To address such metabolite discovery, Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry have been the techniques of choice. There are reports of NMR
spectroscopy being used in the discovery of small molecules (Atzori et al, Front Biosci (Elite Ed)., 2010; Tiziani et al, Nat Commun., 2011).

The reasons for using NMR in metabolite hunt from biological fluids include:

- It is powerful in providing overall biochemical profiles of low-molecular weight endogenous metabolites in biological fluids, without requiring any pre-selection of measurable analytes.
- The biochemical compounds detected are represented in a spectrum, the intensity of each of which correlates with the metabolite’s concentration.
- Helps differentiate between disease and health.
- Provide clues as to the metabolic derangements associated with asthma and such findings may help us sub-phenotype asthma into its component disease processes.

NMR has been previously used to detect the metabolite fingerprints from exhaled breath although without much reproducible success.

This is a relatively new area which requires introduction and discussion.

**Exhaled Breath Condensate (EBC) as a Matrix**

EBC is a relatively unexplored biological medium that could provide an assessment of lung pathobiology. This biological fluid is a convenient and relevant matrix in which biomarkers may be identified (Horvath et al, Eur Respir J, 2005; Corradi et al, Acta Biomed, 2005). Recently, there has been an increasing interest in using exhaled breath as a simple non-invasive means to detect non-volatile macromolecules (protein, lipids, oxidants, and nucleotide) and volatile molecules (acetic acid, formic acid, ammonia etc), which may represent biomarkers of various pathological processes in the lung (Holz O, Eur Respir J., 2005; Deykin A, J Allergy Clin Immunol., 2006; Zhang et al, J Biomol Tech, 2004).

Exhaled breath condensate (EBC) is condensed from water but also contains droplets that carry solute from the lower respiratory tract. The collection of EBC by cooling exhaled air has several advantages compared to many other methods of sampling the airspaces. The main advantage of working with exhaled breath is that it is non-invasive,
relatively inexpensive and could be collected multiple times from subjects without discomfort. (Kharitonov et al, Am J Respir Crit Care Med., 2001). So far, it has no reported role on airway inflammation or any significant influence on airway function (Baraldi et al, Arch Dis Child, 2003), making repeated sampling possible. Because it is effort-independent, children and even mechanically ventilated patients can participate in the collection. The non-invasive nature of sampling is highly encouraged as it causes least discomfort in patients with exacerbating symptoms. The sampling becomes easier and exhaustive from all age groups and conditions.

Very little is known actually regarding the utility and importance of EBC and Nasal scrape as matrices for studying asthma. Small molecule and protein signatures obtained from these matrices in patients and controls would help us gain insights into the understanding of the disease.

Evidences suggest that the abnormalities in the severity of asthma and COPD is better reflected in the constituents present in EBC than that could be ascertained by either spirometry or clinical symptoms alone (Kharitonov et al, Am J Respir Crit Care Med, 2001; Montuschi et al, Am J Respir Crit Care Med., 1999). The major drawbacks of EBC, in spite of reflecting the composition of the extracellular lining fluid (ELF) are contamination of saliva, anomalies due to dilution, and variability. The plethora of potential markers that cannot be consistently or easily analyzed in these highly diluted samples pose major limitation in the use of EBC (Louhelainen et al, Int J Chron Obstruct Pulmon Dis., 2008).

A recent report on NMR study of exhaled breath in asthmatics and healthy volunteers revealed models on the basis of spectral regions, which could differentiate the classes with high confidence in both training and validation sets. Secondary analysis was performed to determine the models for sub-group identification based on clinical features and medication (Ibrahim et al, Allergy 2013).

Despite the drawbacks mentioned here, exhaled breath based tests have important practical advantages that makes such research important.
There have been a few reports on the metabolomic profiling in the context of asthma which revealed novel metabolites and moreover ways to detect small molecules as prognostic markers (Ibrahim et al, Allergy, 2013; Serkova et al, Am J Physiol Lung Cell Mol Physiol, 2008).

Reports of NMR profiling of other bio fluids include a study on urine in children with asthma and healthy controls. They used the NMR data (70 metabolites) to create a model of separation between cases and controls and further validated the model efficiency on a separate group of subjects. The model could predict the controls from stable asthmatics and exacerbating emergency patients with 94% accuracy (Saude EJ et al, J Allergy Clin Immunol., 2011).

Another interesting study includes metabolomics of Broncho Alveolar Lavage Fluid to look for direct pulmonary changes in small molecule fingerprints in mice model of asthma. BALB/c mice were sensitized and challenged with ovalbumin to develop experimental asthma. Dexamethasone was administered to study the effects of corticosteroids on lung metabolism. Metabolites in BALF measured using liquid chromatography–MS and gas chromatography–MS, and multivariate statistical analysis was performed by orthogonal projections to latent structures discriminant analysis. Novel changes revealed in ovalbumin challenged mice as compared to controls. These metabolite changes suggest alterations of energy metabolism in asthmatic lungs, with increases of lactate, malate, and creatinine and reductions in carbohydrates, such as mannose, galactose, and arabinose. Lipid and sterol metabolism were also affected with significant reduction in phosphatidylcholines, diglycerides, triglycerides, cholesterol, cortol, and cholic acid. Dexamethasone treatment effectively reversed many key metabolite changes, but was ineffective in repressing lactate, malate, and creatinine, and induced additional metabolite changes. Metabolomic analysis of BALF offers a promising approach to investigating allergic asthma. Hence such a metabolomic study revealed important metabolites (small molecules and lipids) leading to insights into asthma (Ho et al, Am J Respir Cell Mol Biol., 2012).

There have been reports of lipid profiling of asthma (a different form of metabolomics, larger sized molecules). Oxylipins (e.g. eicosanoids) are derived from fatty acids by
Chapter 3

mono- or dioxygenase-catalyzed oxygenation. These act as endogenous signaling molecules playing important role in pathophysiological processes in the lung, especially in disease such as Asthma, COPD, etc. (Lundstrom et al, Curr Pharm Biotechnol., 2011).

Apart from general metabolite profiling, targeted profiling of breath and other lung related matrices seem to attract more attention as they minimize the systemic noise and directly sample pulmonary evidence. As a result exhaled breath condensate, which samples air and solutes lining respiratory tract, has received a lot of scientific attention due to its potential informational wealth.

Early reports of exhaled breath profiling include metabolic profiling of breath condensate in childhood asthma.

One of the earliest reports of exhaled breath metabolic profiling by Carraro et al includes asthmatic children (persistent with corticosteroid and intermittent steroid naïve) and normal subjects. The combination of exhaled nitric oxide and Forced Expiratory Volume (FEV1) (maximal amount of air expired in one second expressed to a percentage of normal) discriminates children with asthma and healthy children with a success rate of approximately 81%, whereas selected signals from NMR spectra offer a slightly better discrimination (approximately 86%). The selected NMR variables derive from the region of 3.2 to 3.4 ppm, indicative of oxidized compounds, and from the region of 1.7 to 2.2 ppm, indicative of acetylated compounds (Carraro et al, Am J RespirCrit Care Med., 2007).

Carraro et al built a model Bidirectional-Orthogonal Projections to Latent Structures-Discriminant Analysis (O2PLS-DA) to discriminate between healthy, moderate and severe asthmatics based on NMR profiling of breath. The model was robust and could discriminate the groups based on retinoic acid, adenosine and vitamin D (Human Metabolome Database). Hence they show that breathomics could be potential in differentiating different asthma metabolic phenotypes.

Studies of profiling lipid mediators of breath have also been performed. They provide a distinct pattern in aspirin intolerant asthma. EBC from 115 adult asthmatic subjects (62 with aspirin intolerance) and 38 healthy control subjects were assessed quantitatively.
for 19 eicosanoids by using complementary HPLC, gas chromatography-mass spectrometry, or both. Palmitic acid concentrations were used as a marker for dilution of condensate samples. Levels of arachidonate lipoxygenase products and cysteinyl leukotrienes were found to be upregulated in asthma along with COX pathway. A sharp increase in the levels of prostaglandin D(2) and E(2) metabolites distinguished subjects having aspirin intolerant asthma along with higher levels of 5- and 15-hydroxyeicosatetraenoic acid than in aspirin-tolerant subjects. A classical discriminant analysis correctly classified 99% of asthmatic subjects (specificity 97 %.) The eicosanoid profiling allowed for 92% correct classification of aspirin-intolerant subjects (Sanak et al, J Allergy Clin Immunol., 2011).

These were some of the major metabolomic reports in asthma.

Transcriptome is an extremely important front characterized with the involvement of RNA species (mRNAs, miRNAs, siRNAs, piRNAs etc.). Early reports include species that play crucial roles in regulation of health and disease (Gower et al, Proc Am Thorac Soc., 2011; de Fougerolles et al, Nat Rev Drug Discov., 2007; Osman, A, Clin Lab., 2012 ; Soifer et al, Mol Ther., 2007).

Reports on transcriptome analysis in the context of asthma include the following:

Transcriptome sequencing (RNA-Seq) of human endobronchial biopsies revealed forty-six genes to be differentially expressed between asthma and controls. The important ones include pendrin, periostin, BCL2 etc. Ten gene networks were also found involved in cellular morphology, movement, and development.

Novel genes were found to be linked to asthma with high confidence. These indicate the pathophysiology between asthma and health differs considerably and that the transcriptome seems to reflect these differences well (Yick CY et al, Eur Respir J., 2013).

Another report states activation of circulating CD8+ T cells in subjects with severe asthma in comparison with controls. Previously, it was known that CD4+ T cells regulate inflammation in asthmatics without attributing much function to CD8+ T cells
for the same. MicroRNA and noncoding RNA expression in circulating T cells was measured by microarray, quantitative real-time PCR, etc.

Changes in the circulating CD8(+) T cells from patients with severe asthma were observed on comparison of mRNA expression. On the other hand, patients with non-severe asthma versus healthy controls showed no change in CD4(+) and CD8(+) T cells. Changes in expression of non-coding RNA species was observed, including natural antisense, pseudo genes, intronic long non-coding RNAs (IncRNAs), and intergenic IncRNAs in CD8(+) T cells from patients with severe asthma, which were not known yet. MicroRNA expression analysis revealed down-regulation of miR-28-5p in CD8(+) T cells and reduction of miR-146a and miR-146b in both CD4(+) and CD8(+) T cells. Thus a thorough transcriptome study revealed novel miRNA mediated regulation of asthma pathogenesis along with attributing important functions to hitherto unknown non-coding RNAs (Tsitsiou et al, J Allergy Clin Immunol., 2012).

Although there have been quite a few reports of differential mRNA expression attributed to inflammation and allergy, miRNA expression changes have only been recently documented in asthma and health.

miRNAs are small RNA species of 22-25 nucleotide length and have been reported in almost all biological fluids (Cortez et al, Nat Rev Clin Oncol., 2011). They have been known to be stable, involved in multiple pathways and influencing plethora of genes in almost all conceivable pathways (Mendell et al, Cell, 2012).

miRNAs are extremely useful in diagnosis as robust biomarkers in complex diseases (Cortez et al, Nat Rev Clin Oncol., 2011). Profiling of miRNAs in pediatric asthma and murine models has revealed upregulation of miRNA-221 and miRNA-485-3p. Initially miRNA microarray was performed, the results of which were further validated in mouse models using Real Time PCR.

There are reports of Let-7 micro RNA mediated regulation of IL-13 and allergic airway inflammation by (Kumar et al, J Allergy Clin Immunol., 2011). Induced levels of IL-13 inversely correlate with Let-7 levels in T cell cultures. Inflammation was associated with a reduction in most of the members of the let-7 family in IL-13 dependent murine
models of allergic airway inflammation. Exogenously administered let-7 mimics to allergically inflamed mice tend to decrease IL-13 levels, leading to resolution of airway inflammation, reduction in airway hyperresponsiveness, and attenuation of mucus metaplasia and subepithelial fibrosis typically seen in asthma.

There are reports of miRNA-21 being up-regulated in allergic inflammation and thereby regulating IL-12 p35 expression which helps in T-cell polarization (Lu et al, J Immunol., 2009).

Apart from general transcriptomic studies of blood or lung tissue, specific transcriptome profiling of other lung related matrices are rare. Exhaled Breath is not known to contain RNAs and hence there are no reports of EBC transcriptome profiles. The transcriptome of nasal scrape is similarly poorly defined, despite it being respiratory epithelial tissue that could reflect lung related processes to a great degree. Although there are scanty reports of nasal epithelia profiling, but these do not compare states of health and disease, limiting its utility as a potential matrix to pick up diseases (Simoes et al, J Proteomics., 2011).

Proteomic studies of asthma could also provide valuable understanding of the different mechanisms operating in the pathogenesis of the disease. Proteomics deals with the study of different protein signatures encoded by the genome that are associated with different biological phenomena. It is exceedingly popular and relevant as these protein signatures ultimately gives rise to phenotypic expression of individuals.

Reports of proteomic studies of asthma are few. The complexity of asthma, combined with the inaccessibility and invasiveness of disease relevant samples has been the biggest deterrent to respiratory proteomics. One study suggests possible utility of plasma proteomics in discriminating early from dual responses in asthmatic individuals subjected to allergen inhalation. Although pre and post challenge samples could not be differentiated on the basis of this, but discriminant analysis indicated that certain proteins responded differentially to allergen challenge with respect to responder type (dual and early). At pre-challenge, fibronectin was significantly elevated in Dual Responders, when compared to Early Responders, and remained significant in the validation set. This is thus a proof of concept indicating that proteomic approaches
could be used to delineate respiratory subtypes (Singh et al, Proteomics Clin Appl., 2012). In another study, Plasma based proteomic biomarkers such as EGF, MMP-9, IL-8, PAI-1 etc. could distinguish Non Small Cell Lung Carcinoma patients from Asthmatics and healthy controls which shows the utility of the approach towards disease diagnosis (Izbicka et al, Cancer Genomics Proteomics., 2012).

Genomic aspects of asthma are understood much better than other “omics”. This is attributable to the known strong hereditary risk of asthma as well as greater maturity of genotyping technology. Large scale genome wide association studies have been performed to probe genetic associations of complex diseases. There have been many reports of genomic studies in case of Asthma and allergy.(Cookson W, Immunol Rev., 2002; Ober et al, Immunol Rev., 2011; March et al, Int J Gen Med., 2013). More than a hundred genes have been identified already but most of the hereditary risk remains unexplained.

These were various accounts of different ‘omics’ approaches to delineate asthma pathophysiology. However, information from different disciplines still needs to be stitched together to solve the biological riddle of complex diseases. There has been little progress in this direction so far.

3.6. Use of Systems Approach to Unravel Complex Biology

There are a handful of Systems Approach papers, which have tried to integrate multiplatform data to find novel mechanisms and pathways predomination in different subtypes of complex diseases. It is expected that such an approach will be an essential step to establish personalized or stratified therapy.

Personalized medicine aims to assess medical risk factors, monitor, diagnose and treat patients according to their specific genetic composition and molecular phenotype. A review by Dahlin A et al attempted a Systems Biology approach towards pharmacogenomics to tackle asthma. Pharmacogenomic studies have explained a portion of the variability in drug response and provided an increasing list of candidate genes and SNPs involved in a disease. However, there is phenotypic variation in a network of complex interactions among genetic and environmental factors, and hence a
A multidisciplinary, systems-level approach is required in order to understand the interrelationships among these factors. Systems biology aims to capture these interactions between genetic factors and other variables, offering promise to better understanding and management for asthma.

Another review published in the Chest Journal in 2010 talks about an integrative Systems Biology approach in pulmonary disease biology. It clearly states the importance of these varied data sets and integrating them to make actual sense of disease biology.

A report which warrants a special mention was published in 2012 (Chen et al, Cell, 2012). Here an integrative account of personal omics profile (iPOP), has been done - an analysis which combines genomic, transcriptomic, proteomic, metabolomic, and auto-antibody profiles from an individual over a 14 month period. It revealed various medical risks, including type 2 Diabetes and also uncovered extensive, dynamic changes in diverse molecular components and biological pathways across health and disease. The iPOP analysis revealed extensive hetero-allelic changes during healthy and diseased states and an unexpected RNA editing mechanism. Genomic information with additional – omics studies lends a dynamicity and could be used to interpret physiological states.

Use of state of the art machine learning algorithms to analyze and integrate large scale multi-parametric data has been attempted. Support Vector Machine (SVM) has been used to analyze NMR data with fairly appreciable results, but it does not work well with skewed data sets (Lienemann et al, Pattern Recognition., 2008).

Apart from SVM, other machine learning techniques such as Linear Discriminant Analysis (LDA), Iterative Dichotomiser 3 (ID3), C4.5, 5 Decision tree etc. have been used for classification of objects in biological settings. Random Forest, a recent and robust technique involving an ensemble of decision trees has not been used till date in the analysis of exhaled breath metabolome data.

Although there are reports of looking into disease physiology separately (at metabolomic, proteomic levels) but joining the different parts of the jigsaw puzzle to make one complete sense has been really lacking.