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How Variability in Clinical Phenotypes Should Guide Research into Disease Mechanisms in Asthma

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Abstract

Asthma is increasingly being considered as a collection of different phenotypes that present with intermittent wheezing. Unbiased approaches to classifying asthma have led to the identification of distinct phenotypes based on age of onset of disease, atopic state, disease severity or activity, degree of chronic airflow obstruction, and sputum eosinophilia. Linking phenotypes to known disease mechanism is likely to be more fruitful in determining the potential targets necessary for successful therapies of specific endotypes. A "Th2-high expression" signature from the epithelium of patients with asthma identifies a subset of patients with high eosinophilia and good therapeutic responsiveness to corticosteroids. Other characteristic traits of asthma include noneosinophilic asthma, corticosteroid insensitivity, obesity-associated, and exacerbation-prone. Further progress into asthma mechanisms will be driven by unbiased data integration of multiscale data sets from omics technologies with those phenotypic characteristics and by using mathematical modeling. This will lead to the discovery of new pathways and their integration into endotypes and also set up further hypothesis-driven research. Continued iteration through experimentation or modeling will be needed to refine the phenotypes that relate to outcomes and also delineate specific treatments for specific phenotypes.

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At the beginning of the last century, the concept that asthma represented a disease of intermittent airway narrowing that could explain the difficulty in breathing, wheeze, and chest tightness gained general acceptance. The discovery that adrenaline could relieve asthma symptoms, with the later development of β_2-adrenergic agonists as potent bronchodilators, further unified the concept of asthma as a bronchoconstrictor disease. With the description of the atopic state, asthma was divided into extrinsic or atopic asthma, which usually started in childhood and often improved through adolescence, and intrinsic or nonatopic asthma, which had an adult onset of symptoms that were often more persistent and severe (1). In the 1950s, the beneficial effects of corticosteroids (CSs) in the form of cortisone were recognized (2), and in the 1960s, with the introduction of inhaled CS (ICS) therapy, the importance of airway inflammation, later recognized as eosinophilic in nature, came to the fore, and asthma was defined as a disease of the airways characterized by intermittent bronchoconstrictor episodes associated with airway inflammation, with the airway inflammation considered to contribute to the bronchoconstrictor response. On the basis of this definition, the pharmacological approach to the treatment of asthma has since been based on a combination of bronchodilator and antiinflammatory treatments (3). The concept of treatment failure, particularly to CSs, was recognized with the description of CS-resistant asthma (4). More recently, the levels of asthma control and severity and the exacerbation state have been categorized, further dividing asthma into levels of severity (5). Patients with severe asthma were defined according to their lack of response to asthma therapies (6, 7).

Therefore, over the last 100 years, a disease that was considered to be one of intermittent airflow obstruction has now been subcategorized in terms of its clinical presentation, severity, and response to treatments. There is even the notion that asthma is an umbrella term that encompasses many different diseases (8). In severe asthma, many more disease characteristics are obvious (9), and the need to find new effective therapies in this condition (10) has focused efforts into phenotyping asthma. With the necessity for new therapies, it is clear that one of the objectives of phenotyping will be to link the clinical phenotype to mechanisms of disease, thus defining groups of patients

who may be responsive or unresponsive to specifically targeted treatments.

This review addresses the ways in which we can phenotype asthma, how we can link these phenotypes with pathophysiological mechanisms, and how the revolution in "-omics" technology can be harnessed to define more precise phenotypes.

Definition of Clinical Phenotypes

The definition of phenotype according to Wikipedia is as follows: "A phenotype (from Greek *phainein*, "to show" + *typos*, "type") is the composite of an organism's observable characteristics or traits, such as its morphology, development, biochemical or physiological properties, phenology, behavior, and products of behavior. Phenotypes result from the expression of an organism's genes as well as the influence of environmental factors and the interactions between the two." For the definition of asthma phenotypes, this term has been used in different ways. In asthma, many features, such as wheeze or measures of lung function, could be considered as traits. These characteristic traits may be used to determine which genes determine them, or a particular combined subset of these characteristics may be determined by specific genes. Phenotypes are artificial constructs, and in the case of asthma, it is useful for clinicians to characterize a particular subgroup of patients with asthma who define a particular risk factor, who respond to a particular treatment, or who have a particular good or bad prognosis. This would define phenotypes based mainly on clinical and physiological measures. Other ways of phenotyping asthma would be to include a disease pathogenic mechanism that may be distinct, such as virus-induced asthma or occupational asthma. Indeed, it has been suggested that the asthma syndrome be divided into distinct disease entities with specific mechanisms, which have been termed "endotypes" (11, 12). This is clearly very helpful in defining potential therapeutic targets, but not all mechanisms have been discovered yet.

One objective for phenotyping asthma could be to provide long-term prediction of outcomes and to find out which specific treatments may benefit selected phenotypes. Han and colleagues have argued for defining phenotypes of chronic obstructive pulmonary disease that have real predictive value, such that phenotypes could be "a single or combination of disease attributes that describe differences between individuals with chronic obstructive pulmonary disease as they relate to clinically meaningful outcomes" (13). Therefore, a phenotype should be prospectively validated and refined for each of the outcomes to which it relates.

A list of clinical phenotypes of asthma as would probably be agreed on by most asthma clinicians is provided in Table 1, but most still need "validation." Clinically, the use for these particular clinical phenotypes has been to describe particular types of patients with asthma that is recognized as difficult to treat. Phenotypes may be grouped together because of a common underlying mechanism. Table 2 lists some characteristic traits particularly associated with severe asthma in terms of the extremes of these traits. The pathophysiological basis for these characteristics remains to be determined, but these could be part of phenotypes of asthma. It will also transpire that a phenotype or endotype is not a fixed entity but may vary over time or with treatment, and that phenotypes of asthma are defined by the available characteristics of the disease documented and on the degree of understanding we have of the disease.

Defining Phenotypes of Asthma by Cluster Analysis

Recent use of unbiased approaches to phenotyping has been of great benefit in the analysis of phenotypes of asthma. Cluster analysis groups data objects based only on information found in the data that describes the objects and their relationships, with the objective of finding how objects within a group are similar or related to one another. The greater the similarity or homogeneity within a group, or the greater the difference between the groups, the better or more distinct the clustering. Using this specific analysis, the term "cluster" has been used interchangeably with the more commonly used term "phenotype," as defined above.

The Severe Asthma Research Program (SARP) adult and pediatric cohorts (14, 15) and the UK Leicester cohort (16) have used hierarchical cluster analysis, whereas for the European cohorts of European Community Respiratory Health Survey (ECHRS) Epidemiological Study on the Genetics and Environment of Asthma (EGEA) (17), a model-based clustering analysis was used to study a range of asthma severities. Despite differences in size and clinical variables chosen for analysis, these studies came up with similarities in the phenotypes identified (Table 3). The analyses identified patients with preserved lung function and little activity of disease, patients with early-onset disease with atopic background, and a more severe group associated with adult-onset disease and active disease. Thus, age of onset of disease, lung function, and atopic state featured highly in these clusters or phenotypes. Similarly, such clusters were also identified in patients from Korea and Japan (18, 19). A separate cluster analysis of a population of subjects with asthma in New York resulted in clusters that were qualitatively similar to those described for SARP, giving support to the robustness of the clinical phenotypes defined by the SARP cluster analysis (20). Further application of these analyses to other cohorts will be the best way forward to validate the robustness of these analyses.

Such analyses have also led to the definition of new clinical groups such as those associated with obesity, defined in the SARP and Leicester cohorts. This has now been confirmed in other analyses that have specifically examined the contribution of obesity (21, 22). Two clusters of obese individuals were described: obese uncontrolled and obese well-controlled, and these asthma clusters differed from one

Table 1. Potential "phenotypes" of asthma

1. **Clinically defined and responsiveness to therapy**
Defined by severity: mild, moderate, severe
Characterized by exacerbations
Early-onset extrinsic asthma
Late-onset intrinsic asthma
Corticosteroid-resistant asthma
2. **Defined by triggers and inducers and by association**
Exercise induced
Aspirin or nonsteroidal induced
Allergen induced
Occupational asthma
Obesity associated
Cigarette-smoking asthmatic
Viral induced
3. **Inflammatory phenotype**
Eosinophilic
Neutrophilic

Table 2. Characteristics of asthma listed as traits at extreme ends and associated biomarkers

Characteristics	Potential Biomarkers
1. Early onset/childhood vs. late onset/adult	Age of onset of asthma $<$ 40 yr vs. $>$ 40 yr
2. Obese vs. nonobese	Body mass index $>$ 95th percentile for age
3. Chronic airflow obstruction vs. no airflow obstruction	Post-bronchodilator FEV_1/FVC ratio of $<$ 0.7 vs. $>$ 0.7
4. Recurrent frequent exacerbations vs. occasional few exacerbations	Severe/moderate exacerbation frequency $\geqslant$2 vs. $<$ 1 per yr
5. Atopic/extrinsic vs. nonatopic/ intrinsic	Skin prick tests or specific IgE positive vs. negative for more than one aeroallergen
6. Eosinophilic vs. noneosinophilic	Sputum eosinophil count $>$ 3% vs. $<$ 2%
7. Th2 cytokine high vs. Th2 cytokine low	High vs. low expression of Th2 cytokines in epithelial cell brushings
8. Corticosteroid insensitive vs. corticosteroid sensitive	Failure to control vs. well-controlled asthma while on maximal asthma therapy including regular oral corticosteroids
9. β-adrenergic bronchodilator response vs. no β-adrenergic bronchodilator response	Post-β-adrenergic bronchodilator FEV_1 response of $>$ 15% vs. $<$ 15%

another regarding age of asthma onset, measures of asthma symptoms and control, exhaled nitric oxide concentration, and airway hyperresponsiveness but were similar with regard to measures of lung function, airway eosinophilia, and serum IgE (21).

Clusters of severe asthma were described as clusters 4 and 5 of the adult SARP cohort who were on high treatment and associated with severe airflow obstruction. In a small cluster analysis of refractory asthma in Korean patients (23), four clusters were described, with three of the four resembling closely clusters 4 and 5 of SARP. One cluster that was new was the Korean cluster 4, which consisted predominantly of men and cigarette smokers, which was a group that was recruited in SARP. In childhood asthma, this was exemplified by two clusters of severe asthma, one of asthma with severe exacerbations and multiple allergies and the other with severe asthma with bronchial obstruction (24).

Phenotyping and Endotyping of Asthma

In the SARP adult cohort analysis, the clusters fell in line with the treatment-progressive approach of the Global Initiative for Asthma (GINA) with the patients with severe asthma in clusters 4 and 5 having treatments at GINA steps 4 and 5. Approaches that include measurements of inflammatory markers are more likely to provide information about potential pathophysiological mechanisms and ultimately specificity of response to therapies. Our current understanding of the pathophysiology of asthma has increased to a large extent in the last couple of decades thanks to the ability to measure and localize mediators, cells, and cytokines in asthmatic tissues and to performing experimental studies in asthmatic cells and in asthmatic animal models. Eosinophils and immune and inflammatory pathways initiated through Th2 $CD4^+$ T-cell activation with the production of IL-4, IL-5, and IL-13 are considered to play an important role in the pathophysiology of asthma (10, 25, 26). The inflammatory process of asthma is likely to be more complex, such as the potential for innate immune responses to interact with the well-established acquired immune response mechanisms (25). Newly described cytokines, such as thymic stromal lymphopoietin (TSLP), IL-17, and IL-23, have now been implicated in asthma (25, 27, 28). The inclusion of pathophysiological mechanisms into phenotyping of asthma, referred to as endotyping, will be an important element of the process of understanding asthma and finding new treatments. Endotyping of asthma has been limited so far but will become an increasingly important feature of phenotyping. This section reviews the importance of phenotyping and endotyping in relation to the extreme characteristic traits listed in Table 2.

Eosinophilic and Neutrophilic Asthma

Sputum eosinophil counts were added to their cluster analyses in the Leicester cohort of patients with refractory asthma: one of the earliest findings was the discordance between symptoms and eosinophilic airway inflammation (16). One cluster was that of an early-onset, symptom-predominant group with minimal eosinophilic disease, with a high prevalence of obesity and female sex, whereas the other cluster consisted of an eosinophilic inflammation–predominant group with few symptoms, late-onset disease, and a greater proportion of men, with a high prevalence of rhinosinusitis, aspirin sensitivity, and exacerbations. The Leicester group had also previously shown that sputum eosinophils can be used as a guide to adjust asthma treatments with improved control of asthma with fewer exacerbations than the conventional use of symptoms or peak flow measurements (29, 30). The refractory patients with high sputum eosinophilia and recurrent exacerbations responded to specific anti-IL5 monoclonal antibody treatment with a reduced number of exacerbations (31). Thus, a subphenotype of asthma characterized by high-dose treatment with ICS and sometimes with oral CSs with recurrent exacerbations and increased sputum eosinophils would benefit from anti-IL5 therapies (30, 31).

The eosinophilic asthma subphenotype is likely to only constitute a minor proportion of asthma. A recent study of mild-to-moderately severe asthma indicated that sputum eosinophilia, defined as eosinophils present at 2% or more, was found in 36% of subjects with asthma not taking an ICS and in 17% of ICS–treated subjects with asthma (32). Antiinflammatory therapy caused significant improvements in airflow obstruction in eosinophilic asthma but not in persistently noneosinophilic asthma. Noneosinophilic asthma was more predominant in mild-to-moderate asthma, just as neutrophilic asthma is also predominant in severe refractory asthma (33, 34). One study of severe asthma indicated that there was no relationship between sputum eosinophilia and submucosal eosinophilia measured in bronchial biopsies and that sputum eosinophilia, but not submucosal eosinophilia, tracked with exacerbation rates (35). Periostin, which is stimulated

Table 3. Phenotypes or clusters of asthma as determined by cluster analysis

Leicester: primary care cohort (16)	
Cluster 1	Early-onset atopic asthma, with airway dysfunction and eosinophilic inflammation; increased number of hospitalizations
Cluster 2	Noneosinophilic inflammation; obese, female predominant
Cluster 3	Benign asthma with little evidence of active disease; no significant airway hyperresponsiveness in 58%
Leicester: secondary care (16)	
Cluster 1	Early-onset atopic asthma, with airway dysfunction and eosinophilic inflammation; increased number of hospitalizations
Cluster 2	Noneosinophilic inflammation; obese, female predominant
Cluster 3	Early onset, symptom predominant with minimal eosinophilic disease
Cluster 4	Eosinophilic inflammation predominant with few symptoms, late-onset disease
ECRHS II (17)	
Phenotype A	Active treated allergic childhood-onset asthma; atopic asthma, active disease, greater bronchial hyperresponsiveness
Phenotype B	Active treated adult-onset asthma; older subjects with adult-onset asthma; female, active disease; asthma attack in previous 12 mo
Phenotype C	Inactive/mild untreated allergic asthma
Phenotype D	Inactive/mild untreated nonallergic asthma
EGEA 2	
Phenotype E	Active treated allergic childhood-onset disease
Phenotype F	Active treated adult-onset asthma
Phenotype G	Inactive/mild untreated allergic childhood-onset asthma
Phenotype H	Inactive/mild untreated allergic adult-onset asthma
Trousseau Asthma Program childhood asthma (6–12 yr) (24)	
Cluster 1	Asthma with severe exacerbations and multiple allergies
Cluster 2	Severe asthma with bronchial obstruction
Cluster 3	Mild asthma
Trousseau Asthma Program childhood asthma (<3 yr) (83)	
Cluster 1	Mild episodic viral wheeze, mild disease, and normal chest X-ray results
Cluster 2	Nonatopic uncontrolled wheeze with moderate to severe disease, uncontrolled wheezing despite high doses of inhaled corticosteroids, parents with asthma
Cluster 3	Atopic multiple-trigger wheeze with eczema and a positive result from the Phadiatop Infant test, increased levels of IgE, IgA, and IgG, and abnormal chest X-ray
SARP pediatric cohort (15)	
Cluster 1	Late-onset symptomatic asthma
Cluster 2	Early-onset atopic asthma with normal lung function
Cluster 3	Early-onset atopic asthma with mild airflow obstruction and comorbidities
Cluster 4	Early-onset atopic asthma with advanced airflow limitation
Korean asthma cluster: COREA (18)	
Cluster 1	Smoking asthma
Cluster 2	Severe and obstructive asthma
Cluster 3	Early-onset atopic asthma
Cluster 4	Late-onset atopic asthma

by the Th2 cytokine IL-13, has been proposed as a serum biomarker of airway eosinophilia and high-Th2 asthma (36). Serum periostin was shown to be a good marker of the bronchodilator responses of patients with moderately severe asthma treated with an antibody to IL-13 (37). The phenotyping of eosinophilic asthma will certainly evolve further with the discovery of other associated biomarkers, improving the granularity of the phenotype.

Eosinophil and neutrophil sputum numbers show wide variability in severe asthma, with patients demonstrating none to very high levels of either cell (33, 34). Baines and colleagues investigated asthma phenotypes using gene expression profiling of induced sputum and unsupervised hierarchical clustering of these expression profiles (38). They described three phenotypes as (*1*) chronic airflow obstruction and less well–controlled asthma, increased exhaled nitric oxide, and sputum eosinophils; (*2*) airflow obstruction and higher sputum neutrophils; (*3*) higher sputum macrophages and lower eosinophils and neutrophils, and lung function in normal range (38). They also found that genes in the IL-1 and tumor necrosis factor-α/nuclear factor-κB pathways were overexpressed and correlated with clinical parameters and neutrophilic airway inflammation. Patients with severe asthma with mixed neutrophilia and eosinophilia have lower lung function and higher frequency of daily wheeze and health-care use (39). The mechanisms behind these diverse inflammatory profiles are likely to be complex, but a neutrophilic response may signify a non-Th2–driven mechanism and, most likely, non–steroid-responsive asthma. Bacterial colonization in the airways of patients with severe asthma could contribute to neutrophilic asthma (40, 41) and be associated with the defective phagocytosis of bacteria and apoptotic cells by macrophages (42, 43). The mechanisms for airway neutrophilia are less clear. Treatment with corticosteroids (CSs) themselves can contribute to the neutrophilia to some degree, and even Th1 factors may play a role. Th17 immunity has been implicated as a cause for neutrophilia, with some supporting data from severe asthma (44).

High Th2 Expression

Woodruff and colleagues divided a group of patients with mild-to-moderate asthma

into Th2-high and Th2-low groups, based on the mRNA expression in airway epithelial cell brushings of the IL-13–inducible genes, periostin, chloride channel regulator 1, and serpin peptidase inhibitor by examining the gene signature of airway epithelial brushings (45). The patients with Th2-high asthma had greater degree of bronchial hyperresponsiveness, serum IgE levels, blood and airway eosinophilia, subepithelial fibrosis, and airway mucin gene expression (46). Furthermore, the patients with high Th2 asthma responded well to inhaled CS therapy in terms of improvement in FEV_1, whereas those with a low Th2 signature did not respond at all to inhaled steroid therapy. Although this analysis of phenotypes in terms of high Th2 versus low Th2 has started to contribute to our understanding of asthma, further analysis is necessary to understand its relationship to eosinophilia, to innate immune responses, and to steroid resistance in the Th2-low group. Such an approach will necessitate mathematical modeling.

Chronic Airflow Obstruction

In the SARP cohort, airflow obstruction was partitioned into air trapping and airflow limitation (47). One characteristic of severe asthma was the prominence of air trapping, confirmed by the increase in residual volume to total lung capacity (TLC) ratio and by increases in TLC and FRC. Air trapping in severe asthma has been characterized by high-resolution computed tomography scanning and is highly suggestive of being secondary to an obstructive process in the small airways (48, 49). The degree of airflow obstruction has been linked to the degree of air trapping and also to the degree of airway wall remodelling and inflammation (50, 51). Using high-resolution computed tomographic assessment, increased airway wall thickness has been shown to be increased in severe asthma, and this thicker airway wall correlated with the pathologic measure of airway wall remodelling on biopsy and to the degree of airflow obstruction (52). However, another group, while finding that chronic persistent airflow obstruction was associated with longer disease duration, more inflammatory cells in sputum, and greater smooth muscle area, found no differences between groups for any cytokine biomarkers (53). In addition, no differences in airway wall thickness were observed between the chronic obstructed patients and the nonobstructed patients. These conflicting results probably reflect the small numbers of patients usually reported in these studies.

CS Insensitivity

CS insensitivity in patients with severe asthma is an issue, because severe asthma is usually defined as inadequate control of asthma despite the patient being established on high doses of ICS with or without the need for oral CSs (54). One defined group of CS-insensitive asthma could be represented by the oral CS–dependent asthma, with these patients deteriorating on reduction or cessation of oral CS therapy. There is no biomarker for CS insensitivity, and one of the ways of determining CS insensitivity is to observe the patient's response to an increased dose of CS above their maintenance dose. There is some evidence that an increase in neutrophils in sputum may indicate CS insensitivity. There are now several putative mechanisms for CS insensitivity. Activation of p38 mitogen-activated protein kinases (55, 56), inability to recruit HDAC2 to the glucocorticoid receptor (GR) transcriptional complex (57), and reduced effectiveness of the ligand for GR binding (58) have been proposed; in addition, certain groups of patients with asthma are more likely to develop CS insensitivity, such as smokers with asthma and obese patients with asthma (59, 60). To understand further the interactions of kinases in CS insensitivity, *in silico* mathematical modeling can be applied. In this case, the hypothesis is that a set of signaling kinases with specific cross-talk pathways in response to inflammatory and oxidative stresses results in a relative CS insensitivity in severe asthma through effects on GR and/or on GR-associated protein phosphorylation. In preliminary studies, we have used a Monte Carlo parameter estimation of the GR activation pathway and integrated this with the p38 mitogen-activated protein kinase pathway including feedback circuits. After several rounds of iteration using wet laboratory experiments and mathematical predictions to check the biological validity, we have delineated key nodal interaction points between these pathways (61). These were stimulus independent and may provide novel approaches to reversing CS insensitivity.

"-omics" Technologies and Approaches

Although we have an understanding of the role of eosinophils and Th2 cytokines in the pathogenesis of asthma, these may not be the sole drivers of the disease. Indeed, the primary causes of disease development in asthma remain largely unknown, and this will be a stumbling block toward endotyping. There have been advances in the understanding of biological regulatory networks made of proteins, RNA, and metabolites, thanks to the availability of high-throughput biological data. Asthma is likely to involve a large number of different types of molecular and cellular components interacting through complex networks in nonlinear dynamic modes. These impact in specific ways on biologic processes involved in processes as diverse as inflammation, immunity, cell cycle, apoptosis, or metabolism. These biologic networks are likely to be closely linked to the clinical and phenotypic expression of asthma. In addition, there is further complexity in that the developmental and disease processes occurring at various levels of the lungs and airways and the rapid subcellular molecular events can influence each other through upward and downward causation networks that operate across several levels of biologic organization. For example, molecular and signal transduction pathways can be activated or turned off according to the physical location within the cell or organ and the time point examined.

Applying unbiased -omics methods combined with disease-focused and hypothesis-driven approaches is one way to push forward our understanding of asthma phenotypes. The omics technologies make no assumption about what is important in a particular disease, and therefore are ideal tools for discovery of new disease pathways and processes. Use of transcriptomics alone or of proteomics alone may provide some information, but the combination of transcriptomics as a measure of gene regulation and of proteomics as a measure of post-translational modifications and biochemical activity will be powerful in understanding endotypes. Addition of metabolic readouts (metabolonomics) may also be important, because these may represent environmental influences or nutritional influences. Changes in the

airway microbiome of asthma, which have also been reported, may also influence certain phenotypic traits (62, 63).

Transcriptomic Approaches

Transcriptomic analysis of peripheral blood $CD4^+$ T cells in children with frequent and infrequent wheeze due to virus exacerbations have led to the discovery of different immunological pathways involving STAT-1 and inflammatory genes such as the PGE_2 receptor (64). In severe asthma, distinct differential microRNA (miRNA) profiles from blood $CD8^+$ T cells and not from $CD4^+$ T cells were seen as compared with nonsevere asthma, with reduced regulation of miRNA146a/b and miRNA28-5p (65). miRNA profiling of airway epithelial cells from patients with asthma revealed 66 miRNAs whose expression was significantly different from those without asthma (66). Using network analysis, the top-ranked predicted target of the extremely down-regulated miRNA-203 was aquaporin 4, the expression of which was up-regulated in asthmatic epithelial cells. In another study, a different subset of miRNA was differentially expressed in epithelial cells from mild asthma (67), whereas these differences were also observed in bronchoalveolar lavage fluid exosomes from subjects with mild asthma (68). Microarray analysis of sputum cells between patients with asthma with a neutrophilic inflammation with evidence of raised serum levels of C-reactive protein and IL-6 and those without showed alteration of 449 genes related to the innate immune and neutrophilic responses, indicating the need to target systemic inflammatory factors (69).

Proteomic Approach

Proteomics has been performed on sputum samples. A combination of two-dimensional gel analysis and GeLC-MS/MS allowed assignment of 191 proteins, representing the proteome of induced sputum from a normal female smoker (70). Using a shotgun proteomic approach, Gharib and colleagues found 17 proteins to be different in sputum samples from subjects with asthma compared with healthy individuals, among them calcium-binding proteins, S100A9, S100A8, α_1-antitrypsin, SMR3B, and Clara cell 10kd protein (71). The proteomes of bronchial biopsy samples from healthy subjects and subjects with asthma have been compared using the iTRAQ methodology coupled with nano-LC-LTQ-Orbitrap mass spectrometer (72). By contrast to the results obtained from a proteomic analysis of sputum samples, they found up to 1,800 proteins that were differentially regulated in the asthmatic samples, with pathway analysis revealing acute phase response signaling, cell-to-cell signaling, and tissue development proteins. Similar transcriptomic and proteomic analysis of nasal fluids, nasal fluid cells, and nasal mucosa demonstrated both known and unknown genes, proteins, and pathways modulated by CS therapy (73).

Systems Biology Approach to Asthma

Systems biology is recognized as a strategy to obtain information from complex quantitative biological data (74). It is the quantitative analysis of dynamic interactions among many components of a biochemical system, leading to an understanding of the behavior of the whole system. A major approach is to collect and analyze clinical, physiologic, and high-throughput data from genomic, transcriptomic, lipidomic, and proteomic data using complex statistical and computational methods (75). Although often regarded as being hypothesis-free, this is not the case. This approach has been used to demonstrate that different combinations of genomic and proteomic signatures can phenotype breast cancer and chronic lymphocytic leukemia and can link these phenotypes to the development or progression of disease or indicate responsiveness to a particular specific intervention (76, 77), which are the same objectives set out for phenotyping asthma.

Systems biology approaches will therefore depend on the types of questions that are being asked (75). These questions are: (*1*) Can integrated -omics markers define phenotypes of asthma? (*2*) Does a disease biomarker identified from a cell or biopsy from the disease site of a single patient or a small number of clearly defined and clinically phenotyped patients track out to peripheral tissues or blood? And (*3*) Can this map to bigger populations? It is predicted that the integration of -omics data will provide the answer to the causes of specific diseases or disease subphenotypes and also, possibly more importantly, provide estimations of risk in currently healthy subjects or patients with subclinical manifestations of disease. As such, a set of different -omics data lists will not solve the problem, but analysis of networks or pathways associated with each -omics platform, which alone are not indicative of disease drivers, may point to distinct pathways or networks that *in toto* may provide this information. The ultimate aim is to obtain multilayer modules that include information about all layers and regulatory elements.

Link to Personalized Medicine

A recognition that the -omics parameters measured are dynamic rather than static is critical in understanding the potential for systems biology in disease. Lipidomic, transcriptomic, proteomic, and epigenetic readouts are variable and will change with health and disease and with environmental exposures. However, changes in DNA sequence can occur over time with some diseases (77). This has led to the concept that a systems approach will lead to truly personalized medicine. In the first example of its kind, a complete integrative personal omics profile was measured 20 times over a 14-month period by merging the genomic sequence with RNA, protein, metabolic, and autoantibody profiles in a single "healthy" individual. This analysis of more than 3 billion molecular signals led to the discovery of the changes in -omics readouts resulting from the effects of two bouts of viral infection in that individual (75). After the second viral infection, the subject developed raised glucose levels and was successfully treated for type 2 diabetes, which has been subsequently controlled by lifestyle changes. Importantly, the combined -omics approach revealed changes in distinct inflammatory pathways linked to infection. In addition, the combination of transcriptomic and proteomic biomarkers together, but neither alone, indicated novel risk pathways for the development of type 2 diabetes in a subject with no other risk factors and family history of disease. These networks controlling the response to infection and the onset of diabetes would have been detected by the use of transcriptome or proteome analysis alone. This integrated approach on an n = 1 basis shows the potential for systems biology in defining patient risk, which led to the focusing of clinical outputs toward measurement of biomarkers for specific disease risks. The

challenge is to apply these types of approaches to larger numbers of patients, where the question being addressed is distinct from that of the n = 1 study: Will this provide a set of biomarkers to clearly define a disease phenotype or endotype that can be used to subsequently screen larger cohorts for potential drug responders or nonresponders?

Benson and colleagues as part of the MultiMod EU consortium (http://www.multimod-project.eu/project.html) have identified modules of highly interconnected genes in disease-specific networks derived from integrating gene expression, DNA sequence, and protein interaction data from 13 different complex diseases (78). These modules tended to overlap in a hub with disease-specific components protruding like petals from a flower and were enriched for pathways related to oncological, metabolic, and inflammatory diseases. This suggested that this network hub would be associated with a general increase in susceptibility for complex diseases. The authors went on to demonstrate that these pathways were enriched in 145 other complex diseases by using genome-wide association studies.

Challenges of Systems Biology

In the implementation of systems biology approaches, there are a number of challenges to bear in mind: (*1*) the very complex biological regulatory networks; (*2*) the multiscale nature of the various systems of biological organization at molecular, protein, cellular, organ, and whole organism levels; (*3*) the large number of data points generated by -omics technology; (*4*) the need to centralize and relate to heterogeneous knowledge. Therefore, the approach has been to combine mathematical and computational methods (79) for modeling the pathophysiological and biochemical processes underlying a disease to dissect out the function and regulation of biological networks (80). Mathematical modeling offers the opportunity to relate the genetic or epigenetic causes of a disease such as asthma to their phenotypic effects at the organ/whole organism level. An example of a multiscale mathematical model is the one set up to dissect out airway hyperresponsiveness (81), which integrates experimental data related to the bronchoconstriction of asthma at the intracellular (actin–myosin interaction), at the cellular (calcium signaling), at the tissue (mechanical forces), and at the whole organ (airway smooth muscle contraction) level.

An Example: UBIOPRED

In the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcome (UBIOPRED) project funded by the Innovative Medicines initiative (82), a systems biology approach has been taken to phenotype severe asthma. A large part of the effort has been to set up the tools needed for application of systems biology to asthma. Data processing and preliminary analysis of -omics data sets has been organized with specific bioinformatics tools. Analysis of biological networks to identify key proteins or genes and application of principal component analysis, clustering, classification, or probabilistic causal networks using Bayesian networks will be the techniques used. The transMART platform first developed by Janssen pharmaceutical company has been adopted by UBIOPRED to enable large research teams to use this as a platform for translational research collaboration, with the ability of each team to analyze results of -omics and patient parameters. This will allow for testing hypotheses and formulating experimental design. Continued iteration through experimentation or modeling will be needed to refine the phenotypes that relates to outcomes and also delineating specific treatments for specific phenotypes.

Conclusions

Phenotyping asthma using mechanisms is the way forward to finding specific treatments for specific phenotypes of asthma. Taking advantage of the -omics technologies and application of mathematical modeling are essential steps in delineating the known and unknown biological networks involved in asthma that may underline the characteristic traits of asthma. The outcome will be very much dependent on perfecting the tools of systems biology that will lead to phenotyping into pathophysiological mechanisms. ■

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