

REVIEW ARTICLE

Translating Asthma: Dissecting the Role of Metabolomics, Genomics and Personalized Medicine

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Abstract The management of asthma has largely stagnated over the last 25 years, but we are at the dawning of a new age wherein -omics technology can help us manage the disease objectively and rationally. Even in this new scientific age, getting the basics of asthma management right remains essential. The new technologies which can be applied to multiple biological samples include genomics (study of the genome), transcriptomics (gene transcription), lipidomics, proteomics and metabolomics (lipids, proteins and metabolites, respectively) and breathomics, using exhaled breath as a source of biomarkers, which is of particular interest in view of its non-invasive nature in pediatrics. Important applications will include the diagnosis of airways disease, including its components; the pathways driving airway pathology; monitoring the response to treatment; and measuring future risk (asthma attacks, poor lung growth trajectory). With the advent of a wide range of novel biologicals to treat asthma, -omics technology to personalize therapy will be especially important. The U-BIOPRED (Europe) and SARP (USA) groups have been most active in this field, especially using bronchoscopically obtained samples to perform cluster analyses to define new asthma endotypes. However, stability over time and consistency between investigators is imperfect.

This is perhaps unsurprising; results of biomarker studies in asthma will be a composite of the underlying disease, the (variable) effects of adverse drivers such as allergen exposure and pollution, the effects of treatment, and the effects of adherence or otherwise to treatment. Ultimately, the aim should be an exhaled breath based tool with a rapid result that can be used as a routine in the clinic. However, at the moment, there are as yet no clinical applications in children of -omics technology.

Keywords Biomarker · Transcriptomics · Bronchial biopsy · Bronchial brushings · Induced sputum · Airway inflammation · Asthma phenotype · Endotype

Glossary *Lipidomics* Large scale study of lipid composition of a biological system; *Machine learning* Technique used to discern patterns objectively in a given dataset; *Metabolomics* Analysis of the metabolome at a given time point; *Microarray* Microscopic DNA spots containing a specific sequence, on a solid surface, which with hybridisation to cDNA or cRNA targets allows the study of a large number of gene expression simultaneously; *Molecular network* Representation of interactions between molecules, generating potential pathways; *Proteomics* Study of the total protein components of an organism; *RNA sequencing* Deep sequencing of cDNA with high resolution using modern, high-throughput, next generation techniques; *Transcriptomics* The study of all synthesised RNA molecules, including non-coding RNAs; *VOCs* Volatile organic compounds, usually measured in exhaled breath, of lung or systemic origin

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Introduction

In perhaps no field anywhere in medicine has progress so stagnated over the last 25 y than in ‘asthma’, and indeed most but not all airway diseases; a review on the given topic which confined itself to present applicability of -omics tools would be very brief. Whereas asthma treatment has remained ‘the blue one, the brown one, measuring the urinary cotinine, and staring menacingly at the pet cat’ [1] (although likely in India the almost universal UK stupidity about cats does not apply). It is salutary to compare this dismal state of affairs with what has happened in less than 80 y in Cystic Fibrosis (CF). Until 1938, CF was indistinguishable from many other causes of childhood malnutrition, wasting and bronchiectasis; the current approach, with early specific diagnosis, novel molecular treatments with a solid evidence base and an *in vitro* approach to personalised medicine is summarised in Table 1. Although of course some CF management aspects are a work in progress, it is very clear that ‘asthma’ is not merely far behind, but also making little if any progress. This lack of progress has led to a *Lancet* Asthma commission to try to address this [2]; the present review will propose a way forward, try to modernise the approach to asthma, and specifically, discuss how the power of modern scientific tools can be harnessed to make asthma treatment truly personalised. This is particularly important as ever more novel biologicals are brought onto the market, targeting similar or different pathways (for *example*, TH2, IL17, TSLP [3]); either we find biomarkers to guide us, or we will be left embarking on a sterile series of N-of-1 therapeutic trials.

It cannot be stressed too often that low dose ICS (200 mcg/day fluticasone equivalent), if taken regularly and with appropriate technique, result in excellent

control of asthma in the vast majority of children. Clearly, the application of -omics technology to children who have not got the basics right in terms of treatment is a nonsense [4]. Failure to respond should lead to a focussed assessment, addressing co-morbidities and environmental and lifestyle factors, discussed in detail elsewhere, before deploying sophisticated biological and mathematical analyses. Adherence is crucial in asthma management, but often only lip-service is paid to this important topic. Methods of assessment (in ascending order of usefulness) include questionnaires, prescription uptake records, electronic monitoring of inhaler activation [5], and, most recently, assessment of inhalation as well as activation [6]. All are imperfect; but when assessing severe asthma manuscripts and -omics technology, it is important to assess in detail how carefully adherence has been addressed prior to study entry. A biological, -omics signal of differences between TH2 driven airway eosinophilia with and without ICS treatment would be a major step in the assessment of adherence. However it is clear that -omics technology is not needed in children whose disease is readily controlled by getting the basics right.

This article will review the potential of new techniques, and how they have been applied to date, which is largely in adults. For the purposes of this review, it is assumed that such reversible factors have been addressed and the issue is how to assess the airway disease in children of all ages who are not responding to conventional therapy. It is important to note that there are major differences between adults and children in the manifestation of severe asthma. Both ourselves [7, 8], ENFUMOSA [9] and the SARP group [10, 11] showed that children with severe asthma were markedly atopic with variable airflow obstruction, whereas in adults there is an inverse relationship between

Table 1 The contrast between the modern approach to cystic fibrosis and asthma

Clinical situation	Cystic fibrosis	‘Asthma’
Timing of diagnosis	Newborn screening increasingly available	Often after prolonged symptoms
Diagnosis	Highly specific molecular and electrophysiological tests universally applied	Mostly ‘clinical’ diagnosis which is often wrong; physiological and other testing often not deployed, no molecular testing at all
Evidence base for treatment	Huge and definitive randomised controlled trials	Very little hard pediatric evidence
Treatment	Moving from treating the downstream consequences of <i>CFTR</i> dysfunction to designer, gene class-specific molecules	‘One size fits all’, and if the child does not respond, give more of the same
Personalised medicine	<i>In vitro</i> systems (rectal biopsy spheroids) which appear to correlate with <i>in vivo</i> response being developed	Lip service only in clinical practice
Biomarker driven monitoring	Moving to gene expression signature driven identification of pulmonary exacerbations	Children dying because of failure to identify the seriousness of asthma attacks and treat them properly

CFTR Cystic fibrosis transmembrane regulator

atopy and severity. Other important differences will be highlighted in this review; it is essential that studies are not extrapolated uncritically across the age spectrum. A detailed review of the analytical tools, both in the laboratory and bioinformatics, is beyond the scope of this manuscript.

What Do We Want from –Omics?

Omics refers generically to a large dataset generated from a single sample with the aim of identifying pathways of disease and/or identifying novel pathways and mechanisms of disease. There are numerous different and powerful approaches to –omics [12]. Broadly, these involve either seeking specific and pre-determined patterns in the data, or using systems biology techniques to let the data speak for itself, although this may be less objective than its proponents believe (above). The mathematical and data-handling challenges of these approaches are formidable. Methods include:

- Genomics – the study of the genome
- Transcriptomics – the study of gene transcription
- Lipidomics, proteomics and metabolomics, studying lipids, proteins and metabolites respectively
- Breathomics – the study of exhaled breath which is of particular interest in view of its non-invasive nature in pediatrics

These techniques can be applied to multiple body fluids and tissues which can, in pediatric terms, be divided into biological material which may be obtained repeatedly (exhaled breath including condensate, induced or spontaneously expectorated sputum, urine, blood, nasal fluid, nasal cells) and invasive samples not suitable for longitudinal studies [bronchial biopsy and brushings, bronchoalveolar lavage (BAL), lung tissue]. Clearly, not all techniques are applicable to all samples; for *example*, transcriptomics requires DNA, and so cannot be applied to urine.

An important methodological issue is that there may be no direct relationship between the strength of a signal and its biological importance. Thus a small change in a low concentration metabolite may be hugely important biologically, but drowned out by larger signals of no biological importance. Dilutional effects are particularly important in some fluids, *e.g.*, BAL. A detailed description of the different analytical tools, including the increasing sophistication of different mass spectrometers, is beyond the scope of this article.

Currently there are many aspects of airways disease which should be put on an objective footing, and –omics technology is surely the way to do this. These are:

- The diagnosis of airways disease: does the child have airway pathology or is s/he just deconditioned
- What are the components of the airway pathology?
- What are the pathways driving airway pathology and how can we modulate them?
- Can we monitor the biological response to treatment?
- Can we identify the child at risk of future asthma attacks? [exacerbations]
- Can we identify the child on a low lung function trajectory [13, 14]?

The next section of this review focusses on the different –omics techniques and what role they may have.

Personalising Treatment: Approaching Airways Disease

The first and most important area in which a new, probably –omics based approach is needed is in asthma diagnosis. It is very clear that, in all age groups and most settings, ‘asthma’ is over-diagnosed, in part due to over-reliance on the history and the failure to perform objective tests. An objective, breath-based (ideally, below) test to diagnose eosinophilic airway inflammation and thus prevent children being wrongly treated with potent ICS is very urgently needed. One real barrier to achieving this is in the use of an umbrella term like ‘asthma’. Terms like ‘anemia’ and ‘arthritis’ have long been relegated from the status of diagnostic labels to merely descriptions of a clinical problem, looking pale and having red, swollen or painful joints, respectively. It is now time for ‘asthma’ to be relegated to the same status: breathlessness, cough and wheeze. It is abundantly clear that many different airway pathologies can lead to these non-specific symptoms, and that the right approach is to ask ‘does this child have an airway disease?’ and if the answer is affirmative, ‘what is contributing to this child’s airway disease, and especially, what is treatable?’ rather than turn the pages of guidelines. Just as the failing kidney can manifest only by a raised blood urea and creatinine, so the failing airway can only manifest by these symptoms.

Deconstructing Airway Disease

Adverse stimuli can affect any biological tube in relatively limited ways. These are:

- Fixed obstruction
- Obstruction varying spontaneously over time and with treatment
- Inflammation
- Infection

- Increased ‘twitchiness’ – this differs from variable obstruction, because an increased expulsive effort (cough) does not necessarily have to be accompanied by transient obstruction to flow
- Abnormal contents: either too wet, too many solids, or too dry.

Furthermore, there are domains of risk:

- Risk of acute asthma attacks
- Risk of impaired lung growth, either secondary to asthma attacks or as a separate entity
- (In pre-school children) risk of progressing from episodic wheeze to eosinophilic airway disease.

Clearly, not all are relevant to all pediatric airways diseases: CF is dominated by the effects of the airway being too dry (‘low volume hypothesis’) whereas some, at least of the asthmas are dominated by airway inflammation. What is also clear is that we need modern –omics or genetic tools to try to dissect out these components – and these are sadly lacking.

Omics –Who is Doing What?

Future Risks: Progression from Pre-School Episodic Wheeze to School Age Eosinophilic Airway Disease

This hugely complex subject can only be touched upon in this review. In brief, we do not know the molecular mechanisms causing babies to wheeze with viral infection, although antenatal and postnatal tobacco and pollution exposure are important; the reader is referred to a recent review [15]. A proportion of children with viral wheeze progress to school age eosinophilic airway disease, again *via* poorly understood pathways [16–19]. Important factors include multiple early atopic sensitization and severe attacks of wheeze [20]. Predictive indices [21, 22] have a good negative predictive value, but a positive predictive value little better than flipping a coin. Currently we know that in the pre-school years those who develop school age asthma lose lung function, which is never regained throughout life [23, 24]; they develop airway remodelling and eosinophilic inflammation; we do not know how to predict which children will go down this route, what the mechanisms are, and how to prevent this. There are some –omics data to help us, but much more is needed to be known.

A small study of gene expression profiles in peripheral CD4 + ve cells of transient and persistent wheezers, and normal controls [25] was observational and descriptive, but there were distinct differences in gene expression profiles between the two wheezing phenotypes. There were some commonalities in immunological pathways involving proliferation and apoptosis of T-cells. For example, interferon (IFN) related

genes were down-regulated in transient wheezers. Another study followed 202 preschool wheezers to school age, and attempted to use volatile organic compounds (VOCs) and exhaled breath condensate to enhance the prognostic value of conventional predictive indices [26]. VOCs and possibly inflammation related genes [Toll like receptor (TLR)-4, catalase, TNF- α] improved the predictive value. This sort of approach is admirable, but we are still a long way from truly understanding the complex and catastrophic series of events which lead to school age asthma.

Phenotyping Asthma

Perhaps the biggest groups using –omics and other technology to explore asthma phenotypes are U-BIOPRED [27, 28] and SARP [29], focussed largely on severe asthma. Increasingly, the aim is to use systems biology to allow clusters and phenotypes to emerge from the data [30], rather than try to fit the data to pre-set prejudices. This is of course a fallacy; there is inevitably investigator bias in selecting upon what data to perform the analysis. So for *example*, 10 years ago bacterial infection or mucosal colonization were thought not relevant to asthma, but a series of seminal papers (above) have over-turned this; however, until these papers were available, bacteria would not have been part of any clustering. There also needs to be more intellectual stringency in our use of clustering; as with ‘asthma gene’ studies, replication in another cohort should be insisted upon. Furthermore, longitudinal stability over time is another prerequisite; we have shown [31] that sputum cellular phenotypes are not stable in children, unlike in adults [32]. Furthermore, it is important to make the distinction between phenotyping which is of clinical value (changes treatment, prognostic value) from those determining mechanistic pathways, when assessing the value of these approaches.

The SARP group, using total of fifteen markers comprising clinical characteristics, peripheral blood markers and sputum analysis identified four clusters of adult asthma, with no replication or assessment of temporal stability [33]. These were (A) mild to moderate asthma, with early onset and no obesity and pauci-granulocytic or eosinophilic inflammation; (B) same inflammatory pattern, but older, more likely to be obese, with impaired spirometry and with a high proportion of African Americans; (C) and (D) both had predominant sputum neutrophilia, sometimes with eosinophilia; cluster (C) were older, likely to be obese and have severe asthma. They also had impaired spirometry and many were treated with oral corticosteroids. Cluster D was the oldest, male predominant, likely obese, with complex medication regimes and, surprisingly, atopy. The problems with this paper, other than lack of replication and knowledge of temporal stability, include an inability to untangle the effects of disease from that of treatment, that association and causation cannot be disentangled,

and the fact that this sort of cluster analysis does not (to this author at least) lead us anywhere. SARP themselves recognised the problems with the utility of clustering, in a paper reporting outcomes in their original five clusters [34]. This showed that in fact there were no important differences in outcomes in any of these clusters, calling the whole approach into question.

Overall, mathematical cluster analyses is an approach which has yet to prove its worth. Although the results of cluster studies are similar, they are by no means identical. I suggest the approach that is needed is clustering by disease pathways or endotypes, which will hopefully define treatment approaches, rather than on a loose aggregation of clinical characteristics.

Blood Transcriptomics

Blood transcriptomics is an approach which offers promise. A recent study in adults proposed that red cedarwood triggered asthma could be reliably diagnosed using a peripheral blood signature [35], and certainly this sort of approach, if confirmed, is a major advance on current diagnostic approaches. The U-BIOPRED group [36] identified 1693 genes differentially expressed in adult asthmatics as against controls, with a bigger effect size in severe asthmatics; however it should be noted that many (90%) but not all differences were related to differences in peripheral blood white cell count. Using a pathway analysis approach, the investigators found that genes involved related to chemotaxis, migration and myeloid cell trafficking, and decreased development of B-cells, hematopoietic progenitor cells and lymphoid organs. The findings held true in both training and validation sets. Similar changes, but of a lesser magnitude, were seen in mild-moderate asthmatics. There were differences in gene signatures in terms of molecular responses to corticosteroids. However, disturbingly the transcriptomic changes described did not align to any clinical cluster [37]; this finding reminds us that we have a long way to go before we understand the complexities of asthma.

A study relating activation status of CD4 and CD8 positive lymphocytes to non-coding RNA expression in adults with severe asthma showed significant changes in CD8 but not CD4 cells, with activation of multiple pathways involved in T-cell activation, with multiple changes in miRNA expression [38]. As with many studies, this is observational and poses more questions than it answers, and is an important starting point, rather than definitively driving the field forward. The whole field of the role of micro-RNAs is rapidly expanding, and has been reviewed in detail elsewhere [39].

Sputum Transcriptomics

Sputum cell transcriptomics has been used to define clusters in the adult U-BIOPRED cohort [40, 41]. One approach used by

U-BIOPRED [41] was to use clinical clustering to define phenotypes, with training and validation cohorts, and then assess differences in sputum proteomics and transcriptomics data. The clusters identified were (1) well-controlled, moderate-to-severe asthma; (2) late onset severe asthma in smokers, with chronic airflow obstruction; (3) is similar to (2) but in non-smokers; and (4) obese women with uncontrolled severe asthma characterized by asthma attacks but normal lung function. In terms of gene expression, in Cluster 1, IL-16 was differentially expressed compared with Clusters 2 and 3. Cluster 2 showed increased GM-CSF and CXCL7 expression. Cluster 3 showed decreased levels of Cathepsin G compared with phenotypes 1 and 4. LYN kinase was reduced in Phenotype 3 compared with Phenotype 2 (this kinase controls GATA-3 and thus TH2 differentiation. Phenotypes 2 and 3 showed differential expression of pathways related to fibronectin matrix and the actin cytoskeleton, whereas Phenotypes 3 and 4 showed pathways related to cytokine signalling, especially interferons, and fibroblast growth factor and its receptor.

The next approach was first to define eosinophil- and neutrophil-predominant sputum phenotypes, and generate Affymetrix arrays from sputum plugs. The data were analysed using hierarchical, unsupervised clustering. Three transcript associated clusters (TACs) were identified. TAC1 was characterised by eosinophilic sputum, immune receptors IL33R, CCR3 and TSLPR, with the highest enrichment for IL-13/TH2 and ILC2 gene signatures. These patients were oral corticosteroid dependent, with frequent asthma attacks, severe airflow obstruction, and the highest sputum eosinophil counts and FeNO levels. TAC2 was characterised by sputum neutrophilia, serum CRP and eczema, interferon-, tumour necrosis factor- α - and inflammasome related genes. TAC3 was characterised by genes of metabolic pathways, ubiquitination and mitochondrial, but with no TH2 signature, despite moderate elevation in sputum eosinophils, and had better preserved lung function. This important paper again highlights that eosinophilia is not synonymous with TH2 activation, confirming our own findings in severe asthma.

Bronchial Tissue Transcriptomics

Transcriptomics approaches can also be applied to bronchial brushings and biopsies. A pioneering study used this approach to determine TH2_{hi} and TH2_{lo} subgroups of mild to moderate asthmatics based on TH2 gene signatures [42]. The two groups could be differentiated by peripheral blood expression of periostin (which is also derived from growing bone, so cannot be used in pediatrics), CLCA1 and Serpin B2. Only the TH2_{hi} group had eosinophilic airway obstruction and were responsive to inhaled corticosteroids. The U-BIOPRED group took this approach further in severe asthmatics. They identified two steroid-resistant, eosinophilic subgroups [43]; one with high mucosal eosinophilia, raised FeNO, asthma attacks

and oral corticosteroid use. The other eosinophilic group had elevated sputum eosinophils and were more obese. This dissociation between sputum and biopsy eosinophils has been described in children [44], but the relative significance of each has not been determined. U-BIOPRED also described two non-eosinophilic groups, and developed a predictive model of the likelihood of steroid responsiveness. Certainly, there are subphenotypes of ongoing severe asthma which are not driven by airway eosinophilia [45].

Longitudinal phenotyping studies are much rarer than cross-sectional. The ADEPT study [46] derived adult clusters at baseline and validated them longitudinally as well as in a subset of the U-BIOPRED cohort [47]; they used a technique called fuzzy-partition-around-medoid clustering, including clinical and biomarker profiles, including classification into TH2_{hi} and TH2_{lo} using gene expression profiles on bronchial biopsies. These were: Phenotype 1, with mild, early onset disease and good lung function with a low, predominantly Type 2, inflammatory burden; Phenotype 2 consisted of moderately controlled asthmatics with mild airflow limitation, and moderate airway responsiveness and Type 2 inflammation; Phenotype 3 were a group with moderate asthma control, only low Type 2 inflammation, a non-eosinophilic, neutrophilic phenotype and predominantly fixed obstruction; and Phenotype 4 was a cohort of severe asthmatics with uncontrolled reversible airflow obstruction, a mixed inflammatory picture and type 2 inflammation. The strengths of this study include longitudinal stability and validation in another cohort, which are considerable. However, the problems of this approach are graphically illustrated. There is considerable overlap between the phenotypes for virtually every parameter measured. This does not invalidate the study, but leads to the conclusion that this approach may be excellent for determining phenotypes and endotypes in groups, but is unlikely to be a useful clinical tool. In this regard, neither in this or any other study has an attempt been made prospectively to allocate individuals to specific clusters, and determine if this approach has clinical utility.

BUT: What are the Problems?

A single snapshot measurement in asthma, be it –omics or anything else, measures a composite of the underlying disease, the (variable) effects of adverse drivers, the effects of treatment, and the effects of adherence or otherwise to treatment. Adverse drivers are unlikely stable over time – the most obvious examples being the thunderstorm and soya bean asthma epidemics [48, 49] which led to a surge in airway eosinophilia [50], but even varying allergen exposure at school may be an issue in children [51]. Adherence to treatment is notoriously variable and will impact on airway pathology. Generally, the longitudinal stability of phenotypes or supposed endotypes has

been little studied, and when this has been done, the evidence for stability is far from compelling [52]. Thus the challenge of the new –omics will be to sort out what biomarkers truly reflect disease pathophysiology (the disease causing pathway) and what represents intrinsically variable factors such as listed above. This area has not yet really been addressed or in many cases, even identified as a problem.

How Will We Make Omics Work in Practice?

Whereas it is completely valid to use invasive techniques to discern new pathways and mechanisms, in clinical practice, a non-invasive approach is essential, especially in children. Although blood, urine and induced sputum can and should be routine clinical tests, bronchoscopy never will be. Exhaled breath analysis has the attraction of being non-invasive, requiring only passive co-operation, and potentially with a rapid readout. There are proofs of concept studies in adults in which different airway diseases [Chronic obstructive pulmonary disease (COPD), asthma] can be distinguished by VOCs analysis [53, 54]. In children, VOCs of 945 compounds were initially analysed, but in fact only 8 were required to differentiate asthmatics from controls with a sensitivity of 89% and a specificity of 95% [55]. Of course what is needed is to show that this test can distinguish children brought with non-specific, non-asthma symptoms from true asthmatics, correlating with steroid responsiveness in the asthmatics. The ideal we should aim for is a pediatric ‘Breathalyzer’ which will give a readout of the important biomarkers to inform treatment.

Summary and Conclusions

There is no doubt we are on the brink of an era of exciting, –omics driven treatment of asthma. As ever more and diverse approaches to the use of biologicals come on the scene, we need to find rational ways of targeting the right treatment to the right child, making non-invasive measurements, a truly personalised approach. We need to understand steroid resistant eosinophilia, and the apparently non-inflammatory asthma pathways. But it is also salutary to reflect that these sophisticated and expensive approaches to monitoring and treatment will make clinical skills even more, not less, relevant. We better need to assess whether children really just need to get the basics right, rather than reach immediately for the latest test and expensive molecule. Unless we know how to be sure that the child is being given medication appropriately, that the environment is optimised, that psychosocial issues are tackled, and the family really understand the disease, no amount of –omics technology will reduce asthma morbidity and mortality.

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Compliance with Ethical Standards

Conflict of Interest None.

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