

Advancing Understanding of Mechanisms of Severe Asthma and Drug Effects Using Transcriptomics

In this issue of the *Journal*, Weathington and colleagues (pp. 837–856) report on an in-depth analysis of gene expression in BAL cells from patients with a range of asthma severities who were enrolled in SARP (Severe Asthma Research Program) (1). Transcriptomics has been successfully used to date in this program and its sister program in the European Union, U-BIOPRED (Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes), resulting in a step-change in our understanding of the mechanisms of severe asthma through analysis of blood, sputum, and epithelial brushing and biopsies (2–8). The study by Weathington and colleagues is the first to analyze BAL cells, and because the compositions of sputum and BAL are quite different, reflecting processes in the proximal and peripheral airways, respectively, this study is a welcome addition to asthma research. Another value of this study is the use of cell culture with THP-1, a human acute monocytic leukemia cell line, and BAL macrophages to validate some of the *in vivo* observations made in controlled conditions *ex vivo*.

Gene expression in samples composed of a mix of cells is likely to reflect their relative proportions. In this study, the authors found that gene expression was most strongly correlated with neutrophil and lymphocyte numbers, even after correction for age, sex, race, and body mass index reduced the strength of the correlation. Among the top 20 genes that correlate with asthma severity in this paper, we find the *ADRB2* (adrenoceptor β_2), *ZBTB16* (zinc finger and BTB domain-containing protein 16), and *KFL9* (Krueppel-like factor 9) genes. The *ADRB2* gene, localized to the 5q31–q32 region, encodes the *ADRB2* β_2 -adrenergic receptor, a member of the G protein-coupled receptor superfamily. It has been studied in multiple populations and found to be highly polymorphic, and meta-analysis has shown it to be associated with asthma severity (9). It is localized to many cell types, including smooth muscle cells and inflammatory cells. Expression of both the *ZBTB16* gene, a member of the Krueppel C2H2-type zinc-finger protein family, and the *KFL9* gene has been associated with corticosteroid response in studies of asthma (10). Additionally, oxidative stress increases expression of Klf9 via activation of NRF2 (NF-E2-related transcription factor 2), and overexpression of the Klf9 gene sensitizes cells to oxidative stress (11). In the severity-related gene set, many were within or close to three asthma susceptibility loci (5q12–32, 17q11–23, and 1p13–36), adding strength to the observations. Applying weighted gene coexpression network analysis, a data-mining method for studying biological networks,

the authors identified 49 gene coexpression networks, and a pathway enrichment analysis showed that “regulation of cAMP-dependent protein kinase activity” was the top pathway that was negatively correlated with β -agonist use and asthma severity. Importantly, the culture model reinforced these *in vivo* findings.

Unbiased clustering is now commonly used to study multiple biomarkers because it allows the identification of as yet unknown associations and mechanistic networks. This follows our appreciation that stratification by clinical criteria is inadequate because a comparison of biomarker levels within groups defined by clinical severity shows very large variability within individual clinical severity strata. Using unbiased clustering of combined BAL and epithelial brushing data, Weathington and colleagues found five clusters, four of which were either highly enriched or composed entirely of subjects with asthma. Three of the clusters had typical features of type 2 (T2) asthma, and one of them was enriched for subjects with severe asthma with the longest duration and a profile that was consistent with T2-low asthma.

As the authors acknowledge, adherence was not formally assessed, which is a shared weakness of SARP and U-BIOPRED because the fractional exhaled nitric oxide suppression test, now validated for clinical application (12), was not available at the time of recruitment in both consortia. The finding of upregulated genes that have been shown by other investigators to be induced by corticosteroids does indeed suggest, as the authors state in the paper, that prescribed medication was taken, but because nothing is known about the time course of steroid-induced induction of these genes, this association cannot be taken as a guarantee of regular treatment at the time samples were taken.

This study further reinforces the view that severe asthma is now widely considered a disease composed of a number of clinically defined phenotypes and mechanism-defined endotypes (13). As the authors propose, it offers a fertile ground for hypothesis testing. Because we know so little about non-T2 asthma, the single cluster that was characterized by low expression of T2 genes could be studied in more depth, which we should be able to do by using as metadata other biomarkers not reported in this paper. The authors caution that their finding that β -agonist exposure had the strongest impact on gene expression may not relate to disease mechanisms; however, one way or another, treatment with this class of drugs is likely to modulate a multitude of mechanisms. How and to what extent this may occur remains incompletely understood, even though we have been using these drugs for decades. The data from this study could be linked to the genetics data that the SARP consortium has acquired to see how the *ADRB2* polymorphisms fit into the observed endotypes. Furthermore, links with obesity, which is also genetically associated with *ADRB2* polymorphism (14), could be explored, not least because obesity is a known risk factor for asthma and is independently correlated with oxidative

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stress. The same goes for the observations of corticosteroid-dysregulated genes. Of note, *ADRB2* gene activity is positively regulated by glucocorticoids. It is difficult to study how these two drug classes impact mechanisms, because both are central to asthma management. Therefore, other ways, including the use of mouse and cell culture models and clinical studies applying different doses of corticosteroids, need to be considered to determine how different doses influence gene expression. The challenge is to decide how best to take these findings forward. Now in its third funding period, SARP continues to show the enormous value and cost-effectiveness of large collaborative research studies to address questions in ways that could never be done by individual academic institutions or pharmaceutical companies. U-BIOPRED has been similarly productive. The findings from both consortia need to be validated, and cross-interrogation of the respective datasets would be of enormous value. We are now well into the era of asthma biologics, and a set of approved monoclonal antibodies that target different pathways is available. To understand how these drugs work on mechanisms, we need to use omics methods like the ones applied in this study. ■

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Ratko Djukanović, M.D.
Faculty of Medicine
University of Southampton
Southampton, United Kingdom
and
NIHR Biomedical Research Centre
Southampton, United Kingdom

ORCID ID: 0000-0001-6039-5612 (R.D.).

References

1. Weathington N, O'Brien ME, Radder J, Whisenant TC, Bleecker ER, Busse WW, *et al.* BAL cell gene expression in severe asthma reveals mechanisms of severe disease and influences of medications. *Am J Respir Crit Care Med* 2019;200:837–856.
2. Rossios C, Pavlidis S, Hoda U, Kuo CH, Wiegman C, Russell K, *et al.*; Unbiased Biomarkers for the Prediction of Respiratory Diseases Outcomes (U-BIOPRED) Consortia Project Team. Sputum transcriptomics reveal upregulation of IL-1 receptor family members in patients with severe asthma. *J Allergy Clin Immunol* 2018;141:560–570.
3. Kuo CS, Pavlidis S, Loza M, Baribaud F, Rowe A, Pandis I, *et al.*; U-BIOPRED Study Group. T-helper cell type 2 (Th2) and non-Th2 molecular phenotypes of asthma using sputum transcriptomics in U-BIOPRED. *Eur Respir J* 2017;49:1602135.
4. Modena BD, Bleecker ER, Busse WW, Erzurum SC, Gaston BM, Jarjour NN, *et al.* Gene expression correlated with severe asthma characteristics reveals heterogeneous mechanisms of severe disease. *Am J Respir Crit Care Med* 2017;195:1449–1463.
5. Modena BD, Tedrow JR, Milosevic J, Bleecker ER, Meyers DA, Wu W, *et al.* Gene expression in relation to exhaled nitric oxide identifies novel asthma phenotypes with unique biomolecular pathways. *Am J Respir Crit Care Med* 2014;190:1363–1372.
6. Östling J, van Geest M, Schofield JPR, Jevnikar Z, Wilson S, Ward J, *et al.*; U-BIOPRED Study Group. IL-17-high asthma with features of a psoriasis immunophenotype. *J Allergy Clin Immunol* [online ahead of print] 15 Apr 2019. DOI: 10.1016/j.jaci.2019.03.027.
7. Schofield JPR, Burg D, Nicholas B, Strazzeri F, Brandsma J, Staykova D, *et al.*; U-BIOPRED Study Group. Stratification of asthma phenotypes by airway proteomic signatures. *J Allergy Clin Immunol* 2019;144:70–82.
8. Bigler J, Boedigheimer M, Schofield JPR, Skipp PJ, Corfield J, Rowe A, *et al.*; U-BIOPRED Study Group II. A severe asthma disease signature from gene expression profiling of peripheral blood from U-BIOPRED cohorts. *Am J Respir Crit Care Med* 2017;195:1311–1320.
9. Contopoulos-Ioannidis DG, Manoli EN, Ioannidis JP. Meta-analysis of the association of beta2-adrenergic receptor polymorphisms with asthma phenotypes. *J Allergy Clin Immunol* 2005;115:963–972.
10. Leigh R, Mostafa MM, King EM, Rider CF, Shah S, Dumonceaux C, *et al.* An inhaled dose of budesonide induces genes involved in transcription and signaling in the human airways: enhancement of anti- and proinflammatory effector genes. *Pharmacol Res Perspect* 2016;4:e00243.
11. Zucker SN, Fink EE, Bagati A, Mannava S, Bianchi-Smiraglia A, Bogner PN, *et al.* Nrf2 amplifies oxidative stress via induction of Klf9. *Mol Cell* 2014;53:916–928.
12. Heaney LG, Busby J, Bradding P, Chaudhuri R, Mansur AH, Niven R, *et al.*; Medical Research Council UK Refractory Asthma Stratification Programme (RASP-UK). Remotely monitored therapy and nitric oxide suppression identifies nonadherence in severe asthma. *Am J Respir Crit Care Med* 2019;199:454–464.
13. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. *Lancet* 2008;372:1107–1119.
14. Arner P, Hoffstedt J. Adrenoceptor genes in human obesity. *J Intern Med* 1999;245:667–672.

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Can Macrolide Antibiotics Prevent Hospital Readmissions?

Exacerbations of chronic obstructive pulmonary disease (COPD) are one of the most common causes of impaired health status and hospital admissions, as well as readmissions that are costly to healthcare

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services (1). The European COPD audit has shown that after an exacerbation, 35% of patients will be readmitted to the hospital within 90 days (2). Therapy for an acute exacerbation is currently dependent on courses of oral corticosteroids and/or antibiotics, and there is no evidence that this impacts exacerbation readmissions. Other interventions are urgently required, and although some studies have investigated the use of antiinflammatory agents for acute exacerbations, including TNF- α (tumor necrosis factor- α) inhibitors (3) and phosphodiesterase E4 inhibitors (4), no effect has been shown either on exacerbation recovery or on time to the next