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Promises and Pitfalls of Multiomics Approaches to Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is an incurable illness of the pulmonary vasculature resulting from an interplay of dysregulated biological pathways. Despite great recent gains in scientific knowledge and therapy, the molecular determinants of PAH remain incompletely understood, and current PAH therapies target three signaling pathways: prostacyclin, endothelin, or nitric oxide pathways (1). A deeper understanding of disease pathogenesis is needed to identify novel therapeutic targets and disease-specific biomarkers.

Refinements of high-throughput molecular techniques have led to multiple omics approaches toward unraveling PAH pathobiology. These designs pivot away from traditional reductionist approaches to biological questions that use a hypothesis-driven framework and instead take an unbiased approach to discovering molecular differences between biological conditions (e.g., disease vs. health). In recent years, these pages have featured omics studies that have described the genetic underpinnings of vasodilator responsiveness in idiopathic PAH (2), a whole-blood RNA signature of PAH susceptibility and outcomes (3), and dysregulated gene expression in rodent PAH models at single-cell resolution (4).

These omics reports illustrate advances made possible by these powerful study designs. This prompted the NHLBI's Division of Lung Diseases to initiate the PVDOMICS (Pulmonary Vascular Disease Phenomics Program) study aimed at defining novel subphenotypes of pulmonary vascular disease using multiomics methods (5, 6). Integrated omics strategies typically merge data from two or more omics

Supported by funding from the NIH/NHLBI grants K23HL153781 (C.E.S.) and R01HL114910 (P.M.H.) and the Scleroderma Foundation New Investigator Award (C.E.S.).

Originally Published in Press as DOI: 10.1164/rccm.202203-0537ED on April 22, 2022

modalities (genomics, proteomics, or metabolomics) to better understand the flow of molecular information and gain insight into mechanisms underlying biological processes. They have the potential to reveal druggable targets and novel molecular signatures suitable for further study; however, great care must be taken in harmonizing and analyzing the wealth of high-dimensional data generated.

In this issue of the *Journal*, Harbaum and colleagues (pp. 1449–1460) report a systematic analysis of the plasma proteome in a multicenter cohort of patients with PAH followed longitudinally for ascertainment of outcomes (7). This group has already made several seminal contributions to omics in PAH (8–10). Here, they aimed to identify genetically influenced proteins that distinguish PAH from healthy controls and inform prognosis, thus reflecting fundamental PAH pathobiology. By coupling protein quantitative trait loci (pQTL) analysis to Mendelian randomization (MR) analysis, the authors were able to infer causal relationships between protein concentrations and PAH. Their analyses implicated, in particular, two secreted matrix-binding proteins, NET4 (netrin-4) and TSP2 (thrombospondin-2), in PAH pathobiology.

The authors should be commended for their rigorous approach to the study, with inclusion of patients from multiple centers, discovery and replication subgroups, robust false discovery rate correction for multiple testing, and performance of sensitivity analyses to examine effects of important confounders. The stability of pQTL associations over time was confirmed in a subset of subjects with longitudinal samples available, and pQTL results were cross-checked in three publicly available databases. MR analysis limits the potential for erroneously concluding reverse causation; thus, use of this method supports (without proving) that these pQTLs are causally linked to PAH. The authors also analyzed serial plasma samples from initially healthy related volunteers who were followed over time. Concentrations of the two implicated proteins, NET4 and TSP2, rose in subjects who went on to develop PAH.

There are a few reasons to remain circumspect about the study's findings, however. The rigor of the approach, although admirable, may have excluded some proteins of clinical or biological significance from the results. The authors required that proteins of interest be *1*) genetically influenced; *2*) quantitatively different in PAH from those in healthy controls; and *3*) prognostically informative in patients with PAH. Limiting results to genetically influenced proteins may not capture the effects of environmental second hits, polygenic contributions to disease, and the complex interactions of integrated biological networks, all thought to play a role in PAH. MR is most powerful as a technique when it links a single intermediate phenotype to a disease outcome and is less useful in the face of pleiotropy and multifunction of genes. Furthermore, some proteins may be mechanistically important in disease initiation, but not in disease maintenance or progression (or vice versa). Such proteins could be carved out by the requirement that results be predictive of both disease and outcomes.

Effect sizes for the proteins that did result from this approach are rather small, particularly for TSP2, and although associations with PAH were statistically significant in meta-analysis, associations were not consistently significant across individual genome-wide association sub-studies. Would these proteins have adequate discriminatory utility if applied as biomarkers in individual centers? Are they biologically relevant enough such that targeting their molecular pathways would produce meaningful therapeutic effects? TSP2 is elevated in left heart failure and associated with other cardiovascular diseases, raising the question of whether its association with PAH is disease specific (11, 12). The discordant observations that TSP2 rises as individuals develop disease and is associated with poor outcomes, although higher TSP2 is associated with reduced odds of PAH by MR, raises the question of whether TSP2 actually serves a compensatory function in PAH.

Nonetheless, this report demonstrates the promise of integrated omics approaches for driving the field forward. The requirement for genetic associations with protein concentrations limits the potential for reporting out proteins that reflect late-stage manifestations of disease. Notably, NET4 and TSP2 were both *cis*-pQTL results of MR analysis. These results likely reflect upstream events in PAH pathobiology. By revealing novel associations with PAH (i.e., deleterious effects for NET4 and protective effects for TSP2), these findings pave a path for future hypothesis-driven cellular and molecular studies examining effects of inhibiting NET4 and augmenting TSP2 signaling. Studies such as this one can help to prioritize and focus downstream efforts, allowing a concentration of energies on the molecular targets with greatest potential for future drug development.

In order for the promise of multiomics approaches to be fully realized in PAH, careful attention must be dedicated to mitigating inevitable pitfalls, and follow-through is needed to capitalize on results. Without the kinds of rigorous statistical methods applied by the authors here, there is potential for type I error from multiple comparisons and erroneous conclusion of reverse causation. To facilitate translation to clinical applicability, omics studies require rigorous follow-up with well-designed *in vitro* and *in vivo* studies. Integrated omics studies may implicate potentially druggable targets but cannot, on their own, elucidate underlying mechanisms of action. Novel molecular associations yielded by multiomics approaches translate to useful biomarkers only if additional studies demonstrate reproducibility of measurements, adequate discrimination of clinically relevant outcomes, and disease specificity. With appropriate design and follow-up, however, a strategy of coupling multiomics studies to a pipeline for examining the most promising findings has immense potential for broadening scientific discovery in PAH. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Drug Therapy for Obstructive Sleep Apnea: Are We There Yet?

Obstructive sleep apnea (OSA) is highly prevalent and can lead to major neurocognitive and cardiovascular impairments (1). Once believed to be a purely anatomical problem, it is now clear that nonanatomical factors (endotypes) are necessary for OSA to develop in up to 70% of patients (1–3). This paradigm shift has set the stage for increasingly promising research into endotype-targeted pharmacotherapies, which are urgently needed to help the many patients with OSA who are unable or unwilling to use current treatment options such as continuous positive airway pressure (CPAP) (4).

One strategy is to augment insufficient upper airway dilator muscle tone during sleep via drugs with combined adrenergic and antimuscarinic effects. In a landmark study by Taranto-Montemurro and colleagues, one dose of atomoxetine-oxybutynin at bedtime improved OSA severity as measured by the apnea–hypopnea index (AHI) by 63% (−16 events/h) (5). But it remains to be seen whether longer-term use of these or similar drugs maintains efficacy and, importantly, whether these reductions of the AHI (a surrogate outcome) translate into improved clinical outcomes. At least in theory, adrenergic medications may disrupt sleep quality and could have net adverse effects on cardiovascular health by increasing sympathetic tone despite improving OSA.

Another line of investigation aims to improve unstable ventilatory control (high loop gain), which is not only a driver of central sleep apnea but also a major contributor to OSA pathogenesis in 30–40% of patients (2). An individual with high loop gain tends to have periodic drops in respiratory drive that result in reduced activation of upper airway dilator muscles and thus can directly lead to repetitive respiratory events (i.e., OSA). One important regulator of ventilation is CA (carbonic anhydrase), which has 15 different isoforms that are ubiquitous in human tissues including kidneys, erythrocytes, endothelium, and central/peripheral chemoreceptors (6–8). By inducing a renal metabolic acidosis and other complex effects (7), CA inhibitors (CAIs) such as acetazolamide augment ventilation, which dampens fluctuations in CO_2 and thus respiratory drive (i.e., lower loop gain) (9). Consequently, acetazolamide has been found to reduce the AHI in patients with sleep apnea on average by ∼38% (−14 events/h) based on a meta-analysis of 26 small studies (10). Of note, effects were similar in obstructive and central sleep apnea, and a subsequent meta-analysis using a different methodology reported a nonsignificant but similar effect size for OSA (AHI −10 events/h), supporting the findings of the former analysis (10–12).

However, acetazolamide is just one among dozens of CAIs, which vary somewhat in their affinity for the different CA isoforms and tissues, likely explaining a portion of the variability of clinical

C.N.S. is supported by the ATS Foundation (ASPIRE Fellowship). A.M. is supported by the National Institutes of Health.

Originally Published in Press as DOI: 10.1164/rccm.202202-0301ED on March 23, 2022