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# Clarifying the Pulmonary Arterial Hypertension Molecular Landscape Using Functional Genetics

Pulmonary arterial hypertension (PAH) is a complex cardiopulmonary disease that is associated with numerous pathogenetic molecular mechanisms and results in mixed hypertrophic, plexigenic, and fibrotic vascular remodeling of distal pulmonary arterioles. Enhanced clinician awareness and early implementation of multiple PAH-specific therapies have improved the 3-year survival rate to 84% from 52% in the prior era (1). Nonetheless, PAH remains highly morbid, including impaired health-related quality of life that is akin to that of chronic obstructive pulmonary disease, particularly regarding physical inactivity and mental health burden (2). Despite widely heterogenous pathobiology, approved PAH medical therapies (still) target only nitric oxide, prostacyclin, or endothelin receptor biology. Furthermore, treatment responsiveness to PAH pharmacotherapies is highly variable even under tightly controlled circumstances customary among randomized clinical trials, leaving no doubt that as-yet undiscovered therapeutic targets exist by which to subgroup patients and modify their clinical course.

Precision-based methods for diagnosing and prognosticating PAH have focused largely on single genetic variants. In 2001, Newman and colleagues leveraged the wider availability of gene sequencing to complete an observational cohort study spanning 20 years and reported that a thymine-to-guanine transversion at position 354 in exon 3 of the *BMPR2* gene was present in 18 families with PAH (3). This finding gave rise to the era of hereditary PAH and, ultimately, the description of 17 disease-causing variants (4) and important advances using genetics for PAH diagnosis, prognosis, and family screening (5). However, <30% of patients have single variants in causative genes, and posttranscriptional mechanisms in numerous cell types have been reported in PAH (4). Together, these findings suggest that, akin to other complex disorders, it is unlikely a single sentinel genetic event underlies the entire PAH phenotypic spectrum.

In 1995, findings from the first *bona fide* microarray technology were published by Schena and colleagues using a high-speed robotic printing of complementary DNAs on glass (6). Transcriptomic platforms have expanded greatly since then in both sophistication and availability. Greater reliance on multiplex big data platforms, however, has not necessarily been coupled with definitive progress in understanding the mechanistic basis of disease (7). Indeed, data on differentially expressed genes from array probes have been published widely in PAH, although these outputs do not in and of themselves inform the pathobiological function of specific transcripts, and numerous examples showing an uncoupling between transcript quantity and disease relevance exist.

These shortcomings in PAH science establish the following major objective for our field in the modern era: integrating genetic



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context with transcriptomic data to identify causative pathways underlying the clinical phenotype. In this issue of the *Journal*, Rhodes and colleagues (pp. 586–594) (8) use a comprehensive, clever, and sound approach to address this challenge head-on. The investigators studied the transcriptomic profile of 359 patients with PAH who were randomized to one of the following three data analysis groups: RNA discovery, RNA validation, and analytical model validation. Each of the three groups was compared with transcriptomic data from 24 distinct age- and sex-matched volunteer control subjects. They identified 507 transcripts that were differentially expressed relative to control subjects in both the discovery and validation cohorts. A LASSO (Least Absolute Shrinkage and Selection Operator) analysis, which is a statistical model reported originally in the geophysics literature to optimize linear regression fitting for variable selection (9), was then used to identify a combination of 25 RNAs that optimally discriminated patients with PAH from control subjects. This approach successfully stratified patients into low- and high-risk groups using survival as an endpoint. Additional outcome analyses yielded internally consistent findings; the RNA signature also associated with World Health Organization functional class, 6-minute-walk distance, and biochemical evidence of heart failure.

Pathway analyses affirmed that many differentially expressed RNAs share annotated function with established PAH pathobiological mechanisms, including HIF-1α signaling, DNA repair, and zinc finger–containing transcription factors (10). However, Rhodes and colleagues recognized that despite this impressive synthesis of data, additional steps were needed to decipher a molecular cue with causative bearing on PAH, and to accomplish this end, they turned to Mendelian randomization (MR). This approach focuses on the effect of genotypic variance on variance in mRNA quantity. The resulting quantitative trait locus (eQTL) map is one basis of functional genetics, which aims to filter out signals in genetic variance that may be associated with a phenotype but are less likely to be pathogenic (thus, more likely associative) (11).

The authors accessed two publicly available eQTL databases and their own previously published PAH genome-wide association study (12) to perform a two-sample MR analysis. From 293 eQTLs available for the 507 RNAs, *SMAD5* was one of two genes to reach significance, and investigators focused on a specific SNP (rs4146187). They observed that in PAH, the C/C genotype was associated with decreased SMAD5 mRNA quantity and was present in ∼50% of patients with PAH, whereas the A/A genotype was linked to increased transcript quantity and greater risk reduction for developing PAH. By focusing their method on functional analyses (e.g., eQTL), the results provide a measure of specificity and boost confidence that modifying SMAD5, in this case, indeed modulates the clinical phenotype.

Identifying the relevance of SMAD5 to PAH is an important step forward, but clarifying the mechanistic implications of this finding nonetheless requires additional experimental data. As the authors assert, analyzing transcript quantity does not account for protein posttranslational modifications that are important in PAH (13) and also known to regulate SMAD5 bioactivity (14). Future avenues of research should consider transcriptomic biomarkers to predict PAH pharmacotherapy selection, escalation, or discontinuation. This, in turn, has further implications for PAH clinical trial design and patient enrollment.

Overall, Rhodes and colleagues transform the scientific landscape in PAH by expanding the continuum of biological data used to inform clinical risk. Through a multilayered and comprehensive approach culminating in MR methodology that emphasizes functional genetics via eQTL analysis, transcriptomic array data narrow toward causative molecular pathways. This work, therefore, advances knowledge on the genomic–transcriptomic axis in PAH while identifying *SMAD5* and its transcript *per se* as novel potential therapeutic targets. Further evidence to support these data and repurpose this approach to clarify other PAH subgroups, including differences across the temporal spectrum of the disease, are just a sampling of exciting future opportunity suggested by this important work. ■

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# Ventilator Sharing during Shortages
## A Siren's Song?

*La seule façon de lutter contre la peste, c'est l'honnêteté.*
—Albert Camus, La Peste, 1947

Coronavirus disease (COVID-19) is an extremely dynamic condition, and as of this writing, a total of more than 8 million cases and more than 450,000 global deaths have been confirmed (1). About half of these cases have occurred in Europe and North America. On March 26, an article in *The New York Times* entitled "'The Other Option Is Death': New York Starts Sharing of Ventilators," echoed that New York Presbyterian Hospital began ventilator sharing during the pandemic. It also mentioned that Governor Andrew M. Cuomo of New York said, "We need 30,000 ventilators. We have 11,000" (2). This critical shortage of life-saving devices and the colossal pressure and uncertainty created by social media and COVID-19 drive an urgent search for solutions. With few companies having the expertise to build ventilators, boosting supplies is no easy task in the midst of a pandemic.

In this issue of the *Journal*, and in the context of COVID-19–associated acute respiratory distress syndrome in New York Presbyterian Hospital, Beitler and colleagues (pp. 600–604) (3) discuss the feasibility of ventilator sharing, using a single ICU ventilator to support two patients. This approach has been addressed in recent bench studies (4, 5). The original and novel aspect of this research letter, however, is that the authors tested the short-term feasibility of ventilator sharing in patients with COVID-19 with acute lung injury. They provide an accurate description of its technical implementation as well as the potential risks and the way to limit these. Beitler's comprehensive strategy requires not only careful independent monitoring of each patient but also cautious selection of pairs of patients so as to minimize the risks of major mismatching when patients share a machine with the same settings. How to assess patient compatibility is not straightforward. As the authors underlined, numerous criteria must be met. For example, ventilator settings, respiratory system mechanics, and hemodynamic status must be similar. There can be no contraindication for neuromuscular blockade, and respiratory pathogens should be the same. The authors used the pressure control mode of standard ICU ventilators—a key safety feature. They also used a freestanding respiratory monitor so as to have a continuous display of patient-specific airflow, $V_T$, airway pressure, and capnography during ventilator sharing. In addition, the indications for this approach were cautiously limited to a 48-hour time span. The authors precluded the use of anesthesia machines for ventilator splitting owing to technical reasons.

The practical limitations of ventilator sharing, however, cannot be overlooked. First, the need for an unoccupied rescue ventilator within each cluster of ventilator-sharing patients, for example, may perhaps defeat the purpose of putting two patients on the same machine. Furthermore, this strategy impedes weaning from mechanical ventilation, a fundamental step to get rid of a ventilator. Ventilator sharing also demands additional patient care, adding even further strain to health systems with already limited resources. And besides the shortage of ventilators, there is also a shortage of personnel with sufficient physiological background and skills to manage severely hypoxemic individuals under mechanical ventilation (6, 7). The clinical approach itself is daunting. Beitler and his colleagues explained that whenever patients were on ventilator-sharing mode, patient care was assured by the usual clinical team and a consult of either of two intensivists familiar with the system who alternated around the clock. They also pointed out that "In acute ventilator shortages, after exhausting alternatives, ventilator sharing is a reasonable stopgap…." What they meant by "exhausting alternatives" is somewhat vague. One may wonder if, as a temporizing measure, manually bagging patients—this time with the only innovation being the addition of a positive end-expiratory pressure valve—would also be a reasonable alternative. After all, manually bagging patients by volunteers was successful during the polio epidemics in August 1952 in Copenhagen (8): mortality dropped from near 90% to <30%. It was a dramatic moment, and its consequences constituted a turning point in the history of medicine (9). Delivering "gentle" and noninjurious ventilation, however, may be more challenging in conditions of acute lung injury than in pure ventilatory failure. An additional strategy might be to have a repository of old ventilators that could be used to provide life-saving treatment in an acute shortage (10). Appropriate upkeep of these machines is possibly more practical than trying to manufacture new ventilators from scratch in a moment of crisis. One may remember that old pneumatic systems, in spite of their limitations, had much longer half-lives than their more modern "electronic" counterparts.

To summarize, what the study by Beitler and coworkers shows is that if patients can be matched in pairs and paralyzed, they can be kept alive under mechanical ventilation using a single



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