clinical trials will enroll patients with similar degrees of disease involvement. New delivery devices for inhaled medications may deliver therapies to the location of disease with less toxicity.

In conclusion, the publication of this most recent inhaled antibiotic trial adds to our understanding of therapy (and trial design) for NCFB. Coupled with other inhaled antibiotic, oral macrolide, and inhaled mucolytic trials, this trial has helped us begin to understand the complexities of treating this disease. We need to remember that this is a very heterogeneous patient population, and ultimately, clinicians will need to target therapies and provide personalized care to patients with this disease. Combining different types of therapies and including airway clearance will also be important. Finally, we have barely scratched the surface on issues including early disease identification, the role of the microbiome (10), the impact and treatment of nontuberculous mycobacterial infection (11), and the interplay with chronic obstructive pulmonary disease (12). ■

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The Genomic Complexity Underlying Pulmonary Arterial Hypertension: From Mendel to Networks

Pulmonary arterial hypertension (PAH) is a life-threatening condition characterized by remodeling of pulmonary muscular arterioles and pulmonary vascular obstruction and vasoconstriction, leading to increased pulmonary vascular resistance (PVR), a mean pulmonary artery pressure of at least 25 mm Hg at rest, and right heart failure. PAH in the absence of an obvious cause is classified as heritable (HPAH) in the 6% of cases with a positive

family history and in an additional 10–40% of cases previously classified as idiopathic PAH who are found to have an underlying genetic mutation despite a negative family history. Mutations have been found most commonly in bone morphogenetic protein receptor 2 (*BMPR2*), a member of the transforming growth factor β (TGF β) superfamily of receptors; less commonly in genes encoding other members of the TGF β signaling pathway, such as *ALK-1*, *ENG*, *SMAD4*, *SMAD8*, and *CAVI*; and rarely in *KCNK3*, which encodes a member of the two-pore domain potassium channels expressed in pulmonary artery smooth muscle cells (1). However, there is considerable phenotype heterogeneity, including incomplete penetrance, so that only a fraction of individuals with the same mutation develop PAH. This phenotype heterogeneity is likely related to environmental factors and to

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variants in other genes (“modifiers”) that attenuate or exacerbate the pathophysiology of PAH. It has been proposed that the development of PAH requires multiple hits (2, 3). Genetic modifiers have proved challenging to investigate. In the May 1 issue of the *Journal*, Stearman and colleagues (4) report an elegant study that identifies functionally relevant noncoding variants in prostacyclin synthase (*PGIS*).

Stearman and colleagues hypothesized that *PGIS* might be one such modifier, wherein certain genetic variants in the promoter might confer protection to unaffected *BMPR2* carriers by increasing promoter activity. To validate their hypothesis, the authors divided the study cohort into three groups: (1) normal controls, (2) unaffected *BMPR2* carriers, and (3) PAH. All samples were analyzed for four single-nucleotide polymorphisms (SNPs) (T22G, G45A, C185T, and C325A; SNPs 1–4, respectively) and a variable number of tandem repeats (VNTR) with three to nine copies located in the 5′-flanking region of the *PGIS* promoter. VNTR length was found to be associated with transcript expression in normal heterologous epithelial and endothelial cell lines. Furthermore, the six-repeat VNTR allele combined with the SNP3 T mutant (M1) allele significantly increased *PGIS* protein expression as compared with shorter VNTR lengths combined with the SNP3 C wild-type (wt) allele. This finding is consistent with other studies that have shown that fewer repeats are associated with lower promoter activity in endothelial cells (5, 6). Similarly, the SNP4 A minor allele was associated with increased promoter activity in normal heterologous epithelial and endothelial cell lines. The genotypes associated with increased *PGIS* expression were overrepresented in unaffected *BMPR2* mutation carriers relative to patients with PAH, suggesting that this increased expression confers protection against the development of PAH and that these variants in *PGIS* are genetic modifiers of PAH.

Prostacyclin synthase, a member of the cytochrome P450 superfamily, is an enzyme localized in the endoplasmic reticulum that catalyzes the conversion of prostaglandin H₂ to prostacyclin in the arachidonic acid cascade. Prostacyclin is a major lipid metabolite of arachidonic acid in vascular endothelial and smooth muscle cells and in epithelial cells (7, 8), and plays a vital role in a large array of biological processes, including inhibition of platelet aggregation, dilation of vascular cells, and inhibition of smooth muscle cell proliferation (9), all of which are involved in the pathophysiology of PAH. Lung tissue from patients with PAH exhibits decreased expression of *PGIS* (10). Prostacyclin analogs have been successfully used in the treatment of PAH. Thus, there is a biologically plausible mechanism by which genotypes that increase *PGIS* expression may confer protection against the development of PAH, even in individuals with a “single-hit” mutation in *BMPR2*.

A limitation of Stearman and colleagues’ study is the lack of correlation between *PGIS* messenger RNA (mRNA) expression and clinical hemodynamic parameters such as mean pulmonary artery pressure and PVR. The authors attribute this result to the delay between acquisition of hemodynamic measurements and tissue specimens, to the end stage of disease in the collected specimens, and to the effects of medical therapy. However, epigenetic mechanisms, such as DNA methylation, histone modification, and silencing by noncoding RNA, may also be at play, particularly since the *PGIS* promoter is a CpG island. Epigenetic mechanisms may also underlie the lack of transcriptional effects of SNP 1 and SNP 3 *in vitro* in some human cell lines, and the

differences in transcriptional effects across different cell lines. Finally, although an association between *PGIS* genotype and expression has been convincingly demonstrated, the ultimate biological endpoint that should impact phenotype severity, prostacyclin synthase activity, was not assessed.

Determining the association between the functional effects of genetic modifiers and clinical outcomes will be critical to our understanding of the mechanisms of incomplete penetrance of PAH-associated mutations, and to optimizing individualized management strategies for patients. Other investigators have identified additional potential genetic modifiers of PAH. Analysis of the Asn453Ser (N/S) polymorphism in the gene encoding estrogen-metabolizing enzyme (*CYP1B1*) in *BMPR2* mutation carriers showed an increase in the wild-type N/N genotype with a corresponding increase in expression of urinary estrogen metabolites in affected carriers, consistent with the concept that estrogens increase the risk for PAH (11). SNPs in *TGFβ* that decrease *TGFβ*/BMP signaling are associated with later onset age and decreased penetrance in heterozygous *BMPR2* carriers (12). The serotonin transporter (*SERT*) LL promoter genotype has been associated with increased transcription and earlier onset of the disease in *BMPR2* carriers (13). Other genetic variants have been associated with an increased risk of PAH in individuals without *BMPR2* mutations. Variants in the gene cerebellin 2 precursor (*CBN2*) and in the promoter and translated regions of the gene encoding the Kv1.5 channel (*KCNA5*) are more prevalent in patients with PAH, and are associated with increased expression of these genes in vascular endothelial and/or smooth muscle cells isolated from the lungs of patients with PAH (14, 15). In forms of PAH associated with other diseases, variants in the gene encoding angiotensin I-converting enzyme (*ACE*) are associated with lower 6-minute-walk test distance and survival rate (16), and variants in the gene encoding interleukin 6 (*IL6*) with higher levels of interleukin 6 and increased susceptibility to PAH (17).

Collectively, these studies have allowed us to discern some of the complex genetic variables that underlie the development of PAH. It is now evident that it would be overly simplistic to describe HPAH as a Mendelian disorder. Rather, HPAH is likely the product of interplay between a familial germline genetic mutation, other germline genetic modifiers, epigenetic regulation, somatic mutations, and/or environmental exposures. The perturbed biological pathways are diverse and cannot always be predicted *a priori*. Furthermore, animal models do not fully replicate PAH at the histological or the hemodynamic levels. To capture this complexity, it will be necessary to use strategies complementary to the candidate gene approaches exemplified by the studies discussed above. One means of tackling these issues in a complex disease such as PAH is to use a systems biology approach. This method involves (1) performing genome-wide studies, including next-generation sequencing, to analyze DNA variation in whole genomes, expression of mRNAs, microRNAs, and long non-coding RNAs, proteomics, and metabolomics; and (2) the continued development of novel computational methods to integrate these “big data.” Systematic integration of these data will not only allow construction of a network of molecules associated with the pathophysiology of the disease, but also assist in the development of computational models of PAH. Such models have proved to be robust predictors of underlying cellular interactions. Recently, systems biology approaches have improved understanding of many

Figure 1. A strategy to integrate genetic modifier and other “big data” to elucidate the complex pathophysiology of pulmonary arterial hypertension.

complex diseases (18). In a rare disease such as PAH, such studies would benefit from worldwide collaborations both (1) to acquire data from thousands of patients with varying etiologies and disease severity and over multiple time points that will be necessary to obtain the statistical power to detect modifiers of small or moderate effect size, and (2) to develop novel computational methods to integrate these data into networks and models. At the same time, in-depth functional experiments, such as the one described by Stearman and colleagues, will still be required to validate predictions based on PAH models. These studies will lead to improved prognostication, optimization of individualized therapy for patients and at-risk family members, and the development of novel therapies (Figure 1). Finally, translation to the bedside will require the acquisition, interpretation, and communication of complex genetic data by a multidisciplinary team of geneticists, genetic counselors, and clinicians. Thus, patients will better understand their prognosis and their therapeutic options, presymptomatic mutation carriers will be identified, and prospective parents will be counseled about the risk of transmitting a heritable mutation and options such as preimplantation genetic diagnosis. ■

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