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Understanding Genotype–Phenotype Correlations in Patients with *TBX4* Mutations: New Views Inside and Outside the Box

Gene mutations associated with pulmonary arterial hypertension (PAH) have greatly advanced our understanding of the underlying etiologies of pulmonary vascular disease in adults and children (1, 2). In contemporary series, nearly 20% of sporadic and 70% of familial cases of PAH are found to have mutations in one of several identified PAH-associated genes (3). Mutations of *TBX4* (T-Box transcription factor 4 gene) on chromosome17q23.2 are associated with idiopathic and heritable PAH (4, 5) and small patellar syndrome (SPS) (6), underscoring the important role of developmental pathways in the pathogenesis of pulmonary vascular and lung parenchymal diseases. TBX4 is a DNA-binding protein that, with transcription factor TBX5, is critical for lung growth and branching during embryogenesis (7). Phenotypes associated with *TBX4* mutations include not only infantile, pediatric, and adult pulmonary hypertension but also acinar dysplasia and lethal neonatal lung disorders, as well as bronchial and parenchymal lung abnormalities (8–11).

In this issue of the *Journal*, Prapa and colleagues (pp. 1522–1533) provide important genotype–phenotype correlations for patients with *TBX4* mutations (12). Notably, their study combines detailed phenotypic clinical investigation with *in vitro* functional analysis of genetic variants to understand how loss or gain of protein function might affect the clinical manifestations of *TBX4* mutations. They identified 137 subjects with TBX4 variants from 22 published studies and analyzed phenotypic and demographic data. Twenty-one subjects had a primary diagnosis of SPS, and 116 subjects had lung disease, with 45% presenting in adulthood, 36% in childhood, and 19% in the perinatal period, a complete range of ages that adds value to this analysis.

Mutations were localized to protein domains, including the highly conserved T-Box domain containing the first nuclear localization segment (NLS1) as well as the predicted second nuclear localization segment (NLS2) at the C-terminus and the transactivating region. Remarkably, 108 distinct *TBX4* variants were identified among the 116 subjects, including 43 missense, 54 truncation (39 frameshift and 15 nonsense), 3 splice site mutations, 6 indels, and 1 *TBX4* promotor variant. A key feature of this study was the determination of functionality for many of the TBX4 mutations, using site-directed mutagenesis to create defined variants of TBX4, and gauging protein function by measuring binding to T-Box–binding motifs with a readout on the basis of a downstream luciferase reporter assay. All indels, and 23 of 42 missense variants, caused TBX4 loss of function (LoF), whereas 11 missense variants were found to be benign. Intriguingly, eight missense variants resulted in TBX4 protein gain of function (GoF). Of three splice site variants, two resulted in exon skipping expected to alter protein structure.

Analysis of mutation localization and functional assessment identified novel genotype–phenotype correlations. Mutations within the T-Box and nuclear localization domains were associated with younger age at diagnosis and a higher incidence of interstitial and developmental lung disease. GoF mutations were associated with later-age presentation compared with LoF. Secondary skeletal manifestations of small patellar syndrome were found with higher frequency in variants outside the T-Box and NLS2 domains and in those with protein-truncating versus missense variants. Among 89 subjects with follow-up, variants localized to the T-Box domain were associated with shorter event-free survival, though younger age at diagnosis also remained a significant adverse factor. The authors compared patients with TBX4-related PAH against those harboring mutations in another common genetic contributor to PAH, *BMPR2,* and those without known variants in PAH-associated genes. Compared with PAH patients with *BMPR2* or no identified variants, patients with *TBX4* variants had a younger age at presentation, better performance on the 6-minute-walk test, worse pulmonary function testing, higher frequency of airway abnormalities, and longer event-free survival.

The data show that not all *TBX4* mutations are the same. Mutations in specific domains are associated with different ages at presentation, the presence of interstitial lung disease, and survival. In particular, mutations in the T-Box domain are associated with earlier presentation, greater interstitial lung disease, and shorter event-free survival. Although most genetic mutations are linked to loss of function, the investigators found eight variants with GoF

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using *in vitro* assays. As we move into the next phase of genomic understanding, it seems no longer sufficient to merely acknowledge *TBX4* mutation status. Instead, it is essential to know the biological consequences of a specific mutation and how that translates into PAH outcomes.

Such work will be challenging. Fundamentally, PAH, especially genetically linked PAH, is a rare disorder and even large centers see relatively few cases. For that reason, studies such as this rely on pooled data from multiple published reports. However, in pooling outcomes from published papers, researchers may not have a complete picture of the clinical manifestations of the TBX4 variants across ages. For instance, assessment for clinical features of SPS was lacking in most of the cases primarily presenting with lung disease, meaning that the full clinical phenotype was not captured. Furthermore, neonates and children are unlikely to have undergone pulmonary function testing because of their young age, so diffuse lung disease phenotypes may not have been fully appreciated. Patients with *TBX4* mutations causing severe disease may be underrepresented in published series because of selection biases related to recognition of the syndrome and genetic testing, whereas patients with mild/asymptomatic *TBX4* mutations are likely absent because of a lack of referral for genetic testing. Some cases of PAH are linked to multiple genetic abnormalities, and such are not explored in this report or may not have been identified.

Defining the functions of specific variants in PAH-associated genes in the context of other determinants of PAH such as gene penetrance, comorbid conditions, epigenetic modifications, and environmental factors is a vital task. For example, why do some LoF mutations become apparent in neonates, whereas others remain occult until adulthood? Several strategies can help fill in gaps in our understanding. One essential clinical task is genetic testing for all patients with PAH. A second task is more complete phenotyping of PAH syndromes, including both pulmonary vascular findings, intrinsic lung abnormalities, and other nonvascular, nonpulmonary assessments, including musculoskeletal, neurologic, and cardiac features. These genetic syndromes may have consequences beyond the lungs. Finally, we need larger sets of highly annotated clinical and genetic data to capture the full impact of genetic etiologies in PAH and to share these data sets in a uniform fashion between large treatment centers. Such initiatives are emerging and are offering a more detailed, vivid picture of genetically based PAH. In the meanwhile, we now know the importance of specific mutations in *TBX4*, greatly advancing our appreciation for the variety and complexity of genotype–phenotype relationships in PAH. ■

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