TBX4 syndrome: a systemic disease highlighted by pulmonary arterial hypertension in its most severe form

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Thoré and co-workers add to our understanding of TBX4-associated pulmonary vascular disease as a precapillary form of pulmonary hypertension (PH), showing that TBX4 mutations may also cause multisystem anomalies concurrent with, or independent of, PH https://bit.ly/2yIqb9v

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Sometimes we miss the "ah-ha" moment, or, at least require multiple reminders to pay attention. This may have been the case for many of us in the pulmonary vascular field regarding rare variants (mutations) in the gene T-box transcription factor 4 (*TBX4*) and pulmonary hypertension (PH). In 2004, investigators in the Netherlands published the first description of humans with mutations in the T-box transcription factor 4 (*TBX4*) gene. While an interesting manuscript, it undoubtedly created no ripple in the pulmonary vascular disease field, as it described a cohort of individuals with small patella syndrome (SPS) without known cardiopulmonary abnormalities, consistent with prior animal model studies [1, 2]. At that time, SPS was known as a rare autosomal-dominant condition characterised by skeletal dysplasia, including irregular development of the patella and additional anomalies involving the limbs and pelvis. Subsequently, in 2010, geneticists expanded our understanding of the phenotype of subjects with mutations in *TBX4* (and perhaps other loci nearby, including *TBX2*), by describing paediatric cases with a more broadly syndromic condition characterised by variable expression of anomalies, such as skeletal dysplasias (including hand and foot irregularities), developmental delay, hearing loss, congenital heart defects (*e.g.* patent ductus arteriosus, atrial septal defect, aortic valve defects), and PH (no specific PH-related data was provided) [3, 4]. Again, the pulmonary vascular field, current authors included, failed to take notice. Then, in 2013, Kerstjens-Frederikse *et al.* [5], from a paediatric PH specialty centre in the Netherlands, described variations in *TBX4* and surrounding loci in six out of 20 paediatric pulmonary arterial hypertension (PAH) cases associated with syndromic anomalies similar to those previously described in *TBX4* mutants; overall, their data suggested that *TBX4* mutations contribute to the PAH condition in paediatrics. Yet, many in our field retained the impression that *TBX4* mutations were more prominent among children with highly "syndromic" features, and thus associated with a niche subgroup of PH in children.

In hindsight, these important work products implicating *TBX4* in the pathogenesis of PH set the stage for the subsequent emergence of impactful findings elucidating a notable prevalence of *TBX4* mutations

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among individuals previously felt to have a very "PH-specific phenotype" with familial (FPAH) or idiopathic PAH (IPAH). The gaze of the pulmonary vascular field has shifted dramatically to *TBX4* over the past 4 years, starting with a Spanish report of heterozygous *TBX4* mutations in three out of 136 (2.2%) adults diagnosed with IPAH [6]. Shortly thereafter, Zhu *et al.* [7] reported a US cohort of 412 individuals diagnosed with FPAH or IPAH in which deleterious heterozygous mutations in *TBX4* were discovered, with both paediatric-onset (12/155; 7.7%) and adult-onset (1/257; 0.4%) cases. Subsequently, *TBX4* was the second most common (23/2572; 0.89%) mutation identified in PAH cases reported from the US PAH Biobank, with 12/266 (4.5%) paediatric-onset and 11/2345 (0.47%) adult-onset cases. The PAH Biobank cohort included all subtypes of PAH (*i.e.* it was not restricted to FPAH and IPAH cases) [8]. Of the 23 cases with *TBX4* mutations in the PAH Biobank, two (8.7%) had an associated connective tissue disease (one paediatric and one adult-onset case) while three (13.0%) had congenital heart disease-associated PAH (two paediatric and one adult-onset) [8]. Deeper phenotypic information regarding skeletal or other features was not readily available from the PAH Biobank publication.

These studies and several others suggest that heterozygous *TBX4* mutations are rare but detectable among patients with what we typically consider as group 1 PH (PAH), according to the most recent Nice Classification System [9–11]. Yet, not all of the subjects with PH and heterozygous *TBX4* mutations fit neatly in the group 1 classification. In addition to the reports described above, a growing body of literature describes profound defects of lung development associated with heterozygous *TBX4* mutations (or large gene deletions encompassing *TBX4*), including death during infancy due to acinar dysplasia, congenital alveolar dysplasia, and pulmonary hypoplasia [12–15]. This is not surprising, given the important role in development, including but not limited to the lung and skeletal system, of TBX4 and related molecular pathway members [16]. Consistent with this recognition, children with severe persistent precapillary PH diagnosed during infancy with detectable anomalies such as congenital heart disease, skeletal defects, and developmental disabilities are now recognised to populate many of our paediatric PH programmes [17].

In the current issue of the *European Respiratory Journal*, Thoré *et al.* [18], from the French National Registry, report their experience in the care of 20 patients from 17 families known to transmit a variety of heterozygous mutations in *TBX4*. The authors provide comprehensive phenotypic and long-term outcome data on these PH subjects with a precapillary form of PH consistent with severe PAH. Of the 448 family probands with PAH studied, 6% of paediatric-onset, *versus* 3% of adult-onset, had *TBX4* mutations. *TBX4*-associated PAH in France demonstrated a female predominance and a bimodal age distribution, with presentation typically either during childhood or after age 40 years [8]. The majority of subjects had skeletal abnormalities including a high SPS penetrance, while congenital heart disease was less prevalent (15% of cases). The degree of detectable lung irregularities among these patients otherwise diagnosed with PAH was striking, including obstructive or restrictive abnormalities of pulmonary function, significant reductions in diffusing capacity of the lung for carbon monoxide corrected for haemoglobin and structural changes in the lungs. The high degree of bronchial and/or tracheal diverticula of the large airways, as well as parenchymal lung lesions, were consistent with concerns that disruptions of TBX4 signalling may result in profound or more subtle developmental lung lesions.

The non-vascular lung disease among adult heterozygous *TBX4* mutation carriers highlighted by the French National Registry is consistent with recent smaller reports of adult PAH patients [19], including in this issue of the *European Respiratory Journal*. Jansen *et al.* [20] in the Netherlands described one paediatric-onset and four adult-onset PAH cases with *TBX4* mutations, all among females, as well as three male *TBX4* mutation carriers without PAH. Every subject had skeletal anomalies as well as tracheal and/or bronchial diverticulosis, regardless of PAH status. Among those with PAH, additional functional and imaging-based irregularities were detected, similar to *TBX4* mutation carriers in the French National Registry.

Taken together, there is now ample data from both paediatric- and adult-onset cases to demonstrate that *TBX4*-associated pulmonary vascular disease is haemodynamically a precapillary PH consistent with PAH. However, the story is more complicated. We propose that those subjects with deleterious heterozygous *TBX4* mutations or *TBX4*-containing deletions have "*TBX4* syndrome", which, while heterogenous, is characterised by precapillary PH in its most severe form. The reason that "*TBX4* syndrome" presents with profound perinatal cardiopulmonary disease for some, SPS alone in others, or a combination of features that may include PAH remains unclear. The variability in presentation likely has to do with undetermined genomic, transcriptomic, epigenomic, or other factors that shape lung development, as well as susceptibility to injury and environmental influences. As with *BMPR2*-associated and other forms of PAH, the female predominance suggests sex-related factors contribute to PAH pathogenesis in *TBX4* mutants, but further work is needed. What is clear is that the prevalence of *TBX4* syndrome in our PH clinics is not trivial; and, even prior to genetic testing, careful examination for skeletal abnormalities, airway diverticulosis, abnormal pulmonary function test results, or the presence of atrial septal and other congenital heart defects, should heighten suspicion of heritable *TBX4* syndrome.

Thoré *et al.* [18], and other recent studies, have contributed important advances to our understanding of *TBX4* syndrome. Future studies are needed to explore the molecular and other features of *TBX4* signalling, especially as it relates to the pulmonary vasculature and phenotypic variations. Given its relatively rarity, international collaborations will be important to build sufficient numbers of patients to support evaluation of *TBX4* syndrome in more depth.

Finally, the placement of *TBX4* syndrome within the PH diagnostic classification demands careful consideration. This issue also highlights a broader challenge for PH specialists: as we learn more about phenotypic diversity in PH conditions, allocating our patients into discrete PH classification groups becomes more difficult. Is PH in *TBX4* syndrome a developmental lung disease (Nice group 3), or a heritable form of PH most consistent with group I (PAH), or both? Regardless, the emergence of *TBX4* syndrome as a recognised cause of PH is further evidence that PH may occur as a component of many, at times heritable, different conditions.

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