TBX4 variants and pulmonary diseases

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REVIEW

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CURRENT OPINION

TBX4 variants and pulmonary diseases: getting out of the 'Box'

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Purpose of review

In 2013, the association between T-Box factor 4 (*TBX4*) variants and pulmonary arterial hypertension (PAH) has first been described. Now – in 2020 – growing evidence is emerging indicating that *TBX4* variants associate with a wide spectrum of lung disorders.

Recent findings

TBX4 variants are enriched in both children and adults with PAH. The clinical phenotype associated with a *TBX4* variant seems to be milder than that in other PAH-associated gene mutations. Further, *TBX4* variants have increasingly been associated with a variety of clinical and histopathological phenotypes, including lethal developmental parenchymal lung diseases such as not only acinar dysplasia in neonates, but also less outspoken parenchymal lung diseases in children and adults.

Summary

The clinical phenotype of a *TBX4* variant has recently been recognised to expand from bone disorders to different types of lung diseases. Recent data suggest that variants of *TBX4,* a transcription factor known to be an important regulator in embryonic development, are not rare in both children and adults with PAH and/or developmental parenchymal lung diseases.

Keywords

lung disease, pulmonary arterial hypertension, T-Box factor 4

INTRODUCTION

In 1927, Nadezhda Alexandrovna Dobrovolskaya-Zavadskaya described a heterozygous mutation in mice that affected both tail length and the sacral vertebrae [1]. This mutation was called *brachyury* (short tail, *Greek*) mutation. Later, because of the T(tail)-locus of the gene product, the mutation was renamed into T-Box mutation [2,3]. In 1996, T-Box transcription factor 4 (*TBX4*) was identified, in a series of other T-Box genes (*TBX1–5*), as a gene highly expressed during organogenesis [4,5].

This review deepens into the *TBX4* gene and its variants in pulmonary diseases, since in the recent years, the spectrum of clinical phenotypes associated with variants of the *TBX4* gene [including both mutations and copy number variations (CNVs)] is expanding from bone abnormalities to conditions affecting other organs, especially the lungs. We aim to discuss the current knowledge on the role of *TBX4* variants in human lung diseases.

EMBRYOGENESIS

T-Box genes are DNA-binding transcription factors that play a crucial role during embryogenesis [6]. The *TBX4* gene is located on chromosome 17q23.2 and has shown to be important for hindlimb

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KEY POINTS

- *TBX4* variants are associated with idiopathic and hereditary pulmonary arterial hypertension (IPAH/HPAH) and seem to be enriched in children compared with adults.
- *TBX4* variants are associated with a variety of parenchymal lung diseases, ranging from lethal neonatal developmental lung diseases, including acinar dysplasia and congenital alveolar dysplasia, to milder forms of growth abnormalities.
- Both infants, children and adults can present with *TBX4*-associated parenchymal lung diseases.
- The presence of parenchymal lung disease in a patient with *TBX4*-associated pulmonary hypertension (PH) may complicate clinical classification of the patient in HPAH or group 3 PH associated with developmental lung disease.

and pelvic development in both animals and humans [5,7,8]. Animal studies have also suggested an important role for *TBX4* in the formation of the body wall, development of external genitalia and anorectal development [5,9,10].

In addition, *TBX4* is expressed in the lung mesenchyme, already at an early stage during embryogenesis [10]. Together with *TBX5*, *TBX4* regulates the process of lung branching by controlling the expression of the secreted fibroblast growth factor (*FGF*)*10* and activation of *FGF10* signalling [11,12]. *FGF10* is expressed in the mesenchyme surrounding the distal epithelial tips that mark the site of future bud formation and acts by mainly binding to the FGF receptor 2b [13–15]. *FGF10* is involved in the initial production of paired buds of endoderm from the ventral foregut and their eventual outgrowth forming the precursors of the main bronchi [14]. Second, *FGF10* induces new bud formation and therefore plays a key role in branching morphogenesis [14,16].

Another important regulatory gene that is affected by *TBX4* and *TBX5* signalling is canonical wingless-2 (*WNT2*), a mesenchymal protein that is essential for lung specification [11,17].

Reduction of *TBX4* or *TBX5* leads to a severe decrease in branching by a loss of expression of both *FGF10* and *WNT2*. This results in a defect of formation of lung lobes and failure of lung lobe separation, eventually leading to a smaller overall lung size [11,18]. Absence of both *TBX4* and *TBX5* leads to complete branching arrest [19].

In addition, *TBX4* and *TBX5*, via *SOX9* and independently of the *FGF10* signalling pathway, regulate the condensation of cells to form the cartilage rings of the trachea and bronchi, and also the development of the smooth muscle cells in the trachea [11,20]. Reduction of both *TBX4* and *TBX5* causes a decreased number of tracheal cartilage rings and can therefore result in tracheomalacia or tracheal stenosis [11]. *TBX4* has also been shown to be present in pulmonary veins and arteries, suggesting that pulmonary vessels arise from the surrounding lung mesenchyme [10]. These experimental data, derived from cell and animal studies, indicate rather convincingly that *TBX4* plays an important role in foetal development, including both parenchymal and vascular lung development.

CLINICAL PHENOTYPES

Clinical consequences of *TBX4* variants in humans were first revealed in 2004, when the association between a spectrum of limb and skeletal abnormalities, known as small patella syndrome (SPS) or ischiocoxopodopatellar syndrome, was identified (Fig. 1) [22]. This syndrome causes aplasia or hypoplasia of the patella and developmental anomalies of the pelvis and feet.

In 2013, Kerstjens-Frederikse *et al.* [23] discovered that mutations in the *TBX4* gene or deletions encompassing the *TBX4* gene were associated with pulmonary arterial hypertension (PAH) in childhood. Pulmonary hypertension, an increased pulmonary arterial pressure, is a haemodynamic symptom, and its underlying diseases are characterised by a clinical classification (Table 1) [24,25]. PAH is a specific form of PH and is a rare, progressive and often lethal disease of the pulmonary vasculature, characterised by an increased pulmonary vascular resistance, leading to right ventricular failure and eventually death [26,27]. It may be associated with a variety of underlying diseases, including connective tissue diseases, congenital heart diseases, portal hypertension, HIV infection and drugs. In the absence of associated conditions, it is classified as idiopathic PAH (IPAH) or it can be caused by a genetic defect (hereditary PAH; HPAH) (Table 1) [24,25]. In recent years, a progressive number of gene mutations is being been identified as cause for PAH, including mutations in bone morphogenetic protein receptor type II (*BMPR2*) being the most frequently found gene mutation in both children and adults with PAH [28–30]. Further, mutations in activin receptor-like kinase 1 (*ACVRL1*), endoglin (*ENG*), potassium channel subfamily K member 3 (*KCNK3*), eukaryotic translation initiation factor 2-alpha kinase 4 (*EIF2AK4*), Caveolin-1 (*CAV1*) and *SMAD9* have currently been identified as associated with the occurrence of PAH, and this list is growing [31–36].

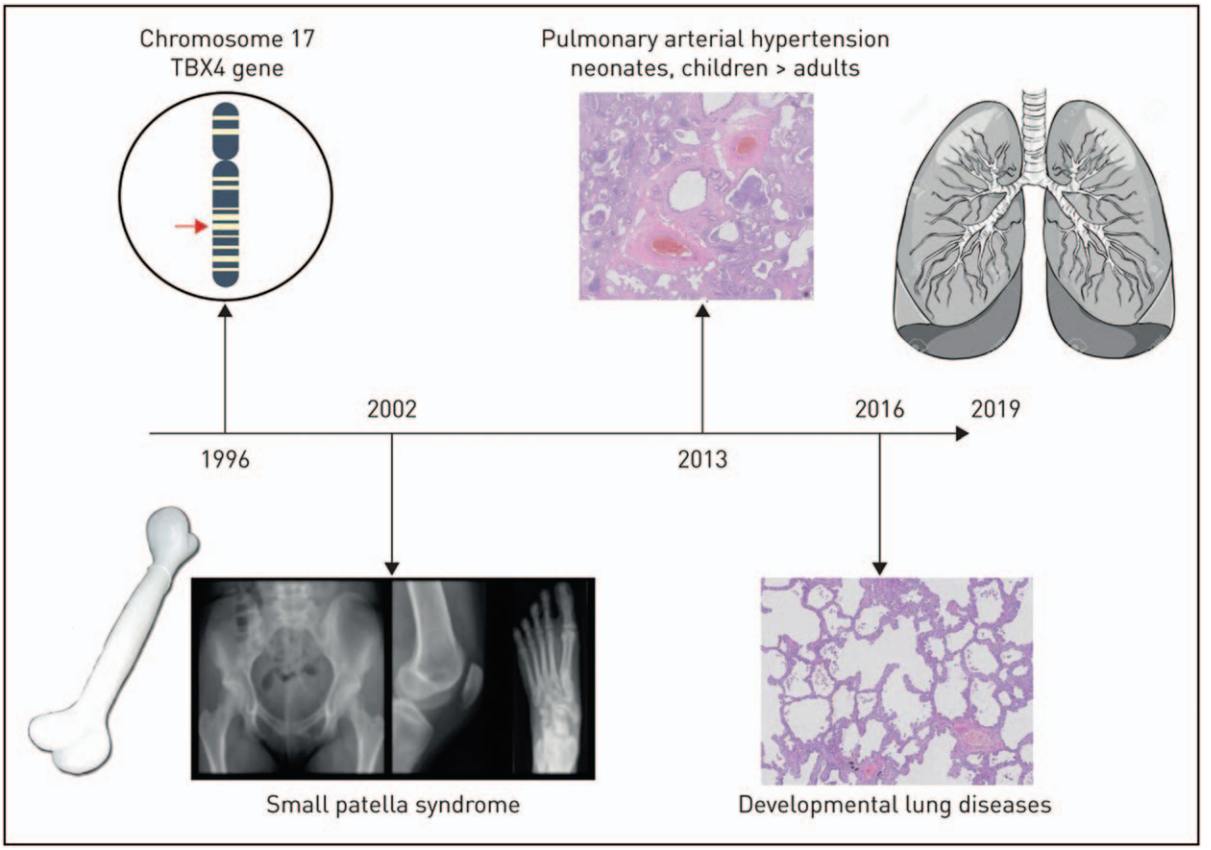

FIGURE 1. Timeline of the expanding clinical phenotypes of *TBX4* variants since the discovery of this gene in 1996. Reproduced with permission from [21].

In the study of Kerstjens-Frederikse *et al.* [23], genetic analyses in a cohort of 20 children with IPAH revealed deletions at 17q23.2 with an overlapping region harbouring the *TBX4* gene in three children with PAH and mental retardation, and an intragenic *TBX4* mutation in three children with PAH with normal intelligence. All appeared to have skeletal malformations characteristic for SPS.

Since the discovery of the association between *TBX4* variants and paediatric PAH, different cohorts confirmed an enrichment of *TBX4* variants in childhood P(A)H. Levy *et al.* [33] reported three *TBX4* truncating mutations in a subgroup of 40 paediatric IPAH/HPAH patients (8%). Zhu *et al.* [37▪] found in a cohort of 155 patients with paediatric-onset IPAH/HPAH that *TBX4* variants were present in a substantial proportion of children (8%). Of these, 5% were pathogenic and likely pathogenic (LP) variants in *TBX4*, while another 3% were variants of unknown significance (VUS).

In summary, the prevalence of *TBX4* variants in children with IPAH/HPAH is estimated 7–8%, which makes *TBX4*, after *BMPR2*, the second most frequently affected gene in these children [33,37▪,38].

A lower number of *TBX4* variants seemed to be present in the adult population with IPAH/HPAH. Kerstjens-Frederikse *et al.* [23] in the same study, as previously mentioned, identified two *TBX4* variants (one LP, one VUS) in a cohort of 49 adults with IPAH. In a larger cohort of 257 adults with IPAH/HPAH, Zhu *et al.* [37▪] identified three adult patients with a *TBX4* variant (one LP, two VUS) (1.2%). Navas *et al.* [39] reported a prevalence of 2.4% of *TBX4* variants in patients with IPAH/HPAH. In a cohort of 966 adult patients with IPAH/HPAH, Gräf *et al.* [40] reported that 1.4% of the patients had a *TBX4* variant. Finally, in a French cohort, Eyries *et al.* [38] reported that 2.6% of the patients with IPAH/HPAH had a *TBX4* variant, concluding that also in adulthood PAH, *TBX4* may be the most frequently mutated gene after *BMPR2*. In summary, the overall prevalence of *TBX4* variants in adults with IPAH/HPAH is estimated around 1.7%.

The reason why *TBX4* variants seem to be more frequent in childhood-onset compared with adulthood-onset IPAH/HPAH is insufficiently clear. A detrimental disease course of *TBX4*-associated HPAH with early decease before reaching adulthood would have explained such phenomenon, however

Table 1. Clinical classification of pulmonary hypertension (PH) [24,25]

1 PAH
1.1 Idiopathic PAH
1.2 Heritable PAH
1.3 Drug- and toxin-induced PAH
1.4 PAH associated with:
1.4.1 Connective tissue disease
1.4.2 HIV infection
1.4.3 Portal hypertension
1.4.4 Congenital heart disease
1.4.5 Schistosomiasis
1.5 PAH longterm responders to calcium channel blockers
1.6 PAH with overt features of venous/capillaries (PVOD/PCH) involvement
1.7 Persistent PH of the newborn syndrome
2 PH due to left heart disease
2.1 PH due to heart failure with preserved LVEF
2.2 PH due to heart failure with reduced LVEF
2.3 Valvular heart disease
2.4 Congenital/acquired cardiovascular conditions leading to postcapillary PH
3 PH due to lung diseases and/or hypoxia
3.1 Obstructive lung disease
3.2 Restrictive lung disease
3.3 Other lung disease with mixed restrictive/obstructive pattern
3.4 Hypoxia without lung disease
3.5 Developmental lung disorders
4 PH due to pulmonary artery obstructions
4.1 Chronic thromboembolic PH
4.2 Other pulmonary artery obstructions
5 PH with unclear and/or multifactorial mechanisms
5.1 Haematological disorders
5.2 Systemic and metabolic disorders
5.3 Others
5.4 Complex congenital heart disease

LVEF, left ventricular ejection fraction; PAH, pulmonary arterial hypertension; PCH, pulmonary capillary haemangiomatosis; PVOD, pulmonary veno-occlusive disease.

currently available, preliminary outcome data invalidate such speculation. The clinical implications of the presence of *TBX4* variants in children with PAH have been scarcely studied. Where *BMPR2* mutation carriers have shown to have a worse survival in both children and adults with HPAH when compared with noncarriers, children with *TBX4* variants in a Dutch national cohort of 70 children with PAH showed less severe clinical presentation and more favourable survival compared with *BMPR2* mutation carriers, similar to nonmutation carriers (own data). Similar findings have been reported by Levy *et al.* [33] in a French cohort of 66 children with PAH.

Navas *et al.* [39] confirmed these findings in adults with IPAH/HPAH and showed that patients with a *TBX4* variant tended to have a milder clinical presentation compared with patients with IPAH, *BMPR2* mutation carriers, *KCNK3* mutation carriers and patients with familial PAH without a mutation. Also, survival in patients with a *TBX4* variant was better compared with patients with a *BMPR2* mutation or a *KCNK3* mutation.

Zhu *et al.* [37▪] reported that HPAH patients carrying a *TBX4* variant had a 20-year younger age-of-onset than *BMPR2* mutation carriers (mean ± SD: 7.9 ± 9.0 compared with 28.2 ± 15.4, respectively, $P < 0.0001$). Although the clinical phenotype of PAH in *TBX4* variant carriers in general seems to be milder than in *BMPR2* mutation carriers, it is not necessarily favourable, and varies from mild/moderate PAH to also more severe forms of PAH, eventually fatal or requiring lung transplantation [37▪].

Until today, available clinical data do not justify patients with HPAH harbouring a *TBX4* variant to be treated differently than patients with other forms of PAH-associated conditions.

TBX4 AND PARENCHYMAL LUNG DISEASES

After the first discovery of a role for *TBX4* variants in the development of PAH, Szafranski *et al.* [41] described in 2016 a neonate with signs of acinar dysplasia (AcDys), a severe diffuse developmental lung disease, who showed to have a de novo *TBX4* mutation. This was the first report suggesting a relation between a *TBX4* variant and developmental parenchymal lung disease. Subsequently, in 2019, German *et al.* [42] described another case of a preterm born neonate with respiratory failure due to a diagnosis of AcDys, harbouring a deletion from chromosome 17q23.2 to 17q23.2 involving 14 genes including *TBX4* (Fig. 2). In the same year, Karolak *et al.* [43▪▪] described a cohort of 26 children with AcDys, congenital alveolar dysplasia (CAD) or other rare pulmonary hypoplasias and reported in 16 children, a *TBX4* variant or *FGF10* variant. In almost 50% of these cases, the developmental lung disease was accompanied by PH. Suhrie *et al.* [44] identified pathogenic *TBX4* mutations in two cases of infants presenting with respiratory failure. One child had abnormal lung lobulation with marked deficiency in acinar/alveolar development, and eventually deceased. The other child had a lung biopsy that demonstrated areas of the lung with near normal alveolarisation adjacent to regions with deficient alveolar growth, characterised by simplified, cystically dilated alveolar spaces [45].

Galambos *et al.* [46▪▪] subsequently further extended the spectrum of paediatric diffuse parenchymal lung disease associated with *TBX4* variants. The authors described a cohort of 19 children with *TBX4* variants, including six children with loss of the *TBX4* gene due to 17q23 deletions and thirteen

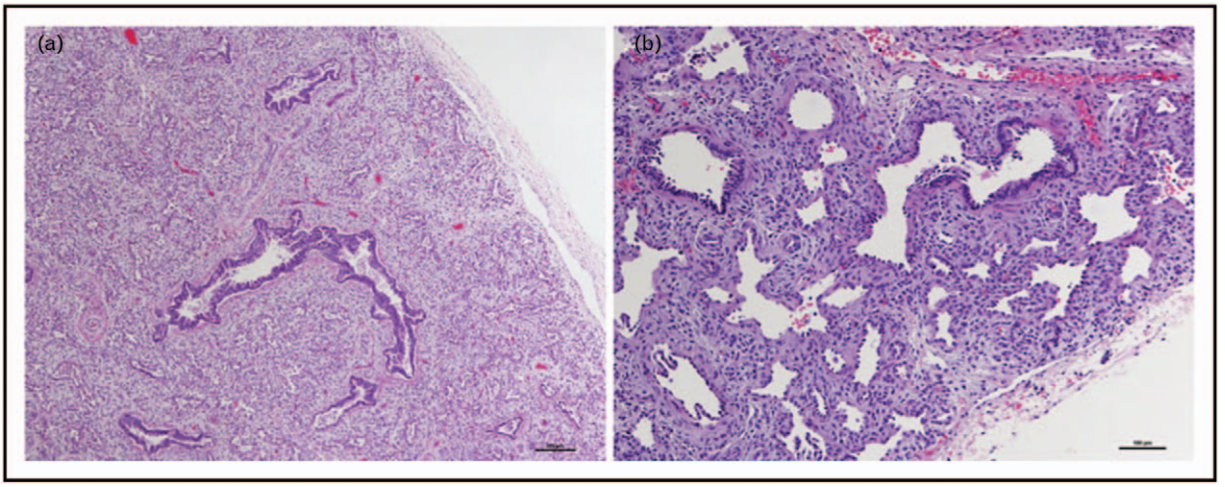

FIGURE 2. Lung histopathology of a patient with acinar dysplasia. (a) Lung from the infant's autopsy, showing an immature appearance for gestational age with no saccular structures or alveoli. (b) Higher power demonstrating abnormal acinar structures with abundant intervening mesenchyme (hematoxylin and eosin). Reproduced with permission from [42].

children with intragenic mutations. The majority of the children (63%) presented with a biphasic clinical course of neonatal hypoxic respiratory failure and persistent pulmonary hypertension of the neonate (PPHN), which seemed to resolve around the age of 1 month, but followed by chronic PH later in infancy or early childhood. Histopathological assessment was available in seven children and showed mainly signs of severe disruption of all compartments of distal lung development similar to CAD or AcDys, or signs of pulmonary interstitial glycogenosis (PIG). The children with a severe presentation of PPHN in the beginning of their life also showed more severe disruption of the distal lung development compared with children who were diagnosed later in childhood. Some children had prominent interstitial lung disease. This series unequivocally shows that *TBX4* variants are not only associated with lethal developmental lung diseases such as AcDys or CAD, but may also present with milder growth abnormalities or nonspecific interstitial lung diseases. Interestingly, a recent case report of Maurac *et al.* [47▪] suggests that such developmental growth anomalies are not necessarily associated with neonatal presentation. The authors describe a 34-year-old woman presenting with unexplained PAH and right heart failure [47▪]. On x-rays of the feet and the pelvis, the patient showed signs of SPS. Chest computed tomography (CT) revealed peripheral reticular lines, centrilobular nodules, thin-walled cysts in the upper lobes and an appearance resembling diverticula in the trachea and large bronchi. Lung biopsy from the left upper lobe showed densification foci of the lung parenchyma with cholesterol crystals. Although the histopathological abnormalities were not typical for developmental distal lung abnormalities, the tracheal abnormalities in this patient highlight a potential role of *TBX4* in a disturbed development of the larger airways [11].

The studies described here strongly suggest that *TBX4* variants can be associated with a broad spectrum of lung diseases with a wide age range of clinical presentations. Neonates and infants with *TBX4* variants are clearly overrepresented in the reports on parenchymal lung diseases and authors have frequently used the terminology 'developmental lung disease'. Different classifications for children's interstitial and diffuse lung disease (chILD) have been proposed. The American Thoracic Society published a classification of chILD in 2013 where AcDys, CAD, alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV) are classified as diffuse developmental disorders, whereas primary pulmonary hypoplasia is classified under growth abnormalities (Table 2) [48,49▪]. The structural changes seen in these diseases are associated with the different stages during lung embryogenesis in which growth arrest occurs; the pseudoglandular (AcDys), canalicular (CAD) and saccular/alveolar (both ACDMPV and primary pulmonary hypoplasia) phase [50▪▪]. Also, PIG, characterised by interstitial expansion due to increased mesenchymal cellularity, is regarded a disease of altered lung development [49▪,51]. Also, other diffuse lung diseases such as chronic neonatal lung disease (i.e. bronchopulmonary dysplasia), or surfactant dysfunction disorders (i.e. nonspecific interstitial pneumonia) are associated with disturbed or disrupted vascular and parenchymal lung development [46▪▪,47▪,48].

ChILD is a complex spectrum of disorders with a high variety in clinical phenotypes for which the

Table 2. Classification scheme for paediatric diffuse lung disease [48]

I. Disorders more prevalent in infancy
A. Diffuse developmental disorders
1. Acinar dysplasia
2. Congenital alveolar dysplasia
3. Alveolar-capillary dysplasia with pulmonary vein misalignment
B. Growth abnormalities
1. Pulmonary hypoplasia
2. Chronic neonatal lung disease
A. Prematurity-related chronic lung disease (bronchopulmonary dysplasia)
B. Acquired chronic lung disease in term infants
3. Structural pulmonary changes with chromosomal abnormalities
A. Trisomy 21
B. Others
4. Associated with congenital heart disease in chromosomally normal children
C. Specific conditions of undefined aetiology
1. Pulmonary interstitial glycogenosis
2. Neuroendocrine cell hyperplasia of infancy
D. Surfactant dysfunction mutations and related disorders
1. *SPFTB* genetic mutations – PAP and variant dominant histologic pattern
2. *SPFTC* genetic mutations – CPI dominant histologic pattern; also DIP and NSIP
3. *ABCA3* genetic mutations – PAP variant dominant pattern; also CPI, DIP, NSIP
4. Others with histology consistent with surfactant dysfunction disorder without a yet recognised genetic disorder
II. Disorders not specific to infancy
A. Disorders of the normal host
1. Infectious and postinfectious processes
2. Disorders related to environmental agents: hypersensitivity pneumonia, toxic inhalation
3. Aspiration syndromes
4. Eosinophilic syndromes
B. Disorders related to systemic disease processes
1. Immune-related disorders
2. Storage disease
3. Sarcoidosis
4. Langerhans cell histiocytosis
5. Malignant infiltrates
C. Disorders of the immunocompromised host
1. Opportunistic infection
2. Disorders related to therapeutic intervention
3. Disorders related to transplantation and rejection syndromes
4. Diffuse alveolar damage of unknown aetiology
D. Disorders masquerading as interstitial disease
1. Arterial hypertensive vasculopathy
2. Congestive vasculopathy, including veno-occlusive disease
3. Lymphatic disorders
4. Congestive changes related to cardiac dysfunction
III. Unclassified – includes end-stage disease, nondiagnostic biopsies and those with inadequate material

CPI, chronic pneumonitis of infancy; DIP, desquamative cell interstitial pneumonia; NISP, nonspecific interstitial pneumonia; PAP, pulmonary alveolar proteinosis.

associated pathophysiology is insufficiently understood. Therefore, although the notable role of *TBX4* in embryonic development may be tempting, the term 'developmental lung disease' as a common denominator in the phenotypical spectrum of *TBX4*-associated lung diseases should be used with caution.

The reason for the variety in clinical presentation in patients carrying a *TBX4* variant remains unclear. It has been hypothesised that the type of mutation may determine both phenotype and its severity, but currently, there does not seem to be a pattern when comparing all described different mutations [41,46▪▪,50▪▪]. Genetic modifiers – coding or noncoding – may play a role in the variability of the phenotype. Karolak *et al.* demonstrated that all infants with developmental lung disease with either a *TBX4* variant or *FGF10* variant also harboured at least one noncoding mutation in *BCAS3* (microtubule associated cell migration factor), which has overlap with the predicted regulatory elements that have been identified in the human foetal lung fibroblast cell line [43▪▪,52]. However, children with deletions of chromosome 17q23.2, harbouring both *TBX4* and *BCAS3*, do not necessarily present with lung disease or PAH [45].

Karolak *et al.* [53] recently published a case report on an infant with PIG, harbouring both a *TBX4* variant and a *CTNNB1* mutation (associated with intellectual disability and neurodevelopmental abnormalities). The authors state that a heterozygous *TBX4* variant alone may not be sufficient to cause lung disease and suggest that mixed phenotypes may be due to complex compound inheritance [54]. In addition, environmental and maternal/pregnancy-related factors may also influence the severity of the clinical phenotype [55].

DIAGNOSTICS

Characterising chILD is difficult especially in neonates [48]. Symptoms are nonspecific and neonatal presentation will complicate the diagnostic process. For a long time, lung biopsy for histopathological analyses has been the ultimate diagnostic, limited by high procedural risks and often low diagnostic yield. Genetic testing is gaining ground due to the identification of an increasing number of genetic causes for several interstitial lung diseases [48]. The presence of a certain mutation can have profound effects for clinical decision making: the dismal prognosis of a *FOXF1* mutation causing ACDMPV will affect treatment decisions in a neonate dependent on intensive ventilatory support and will avoid the otherwise required lung biopsy [50▪▪]. Standard testing for *TBX4* variants in neonates with respiratory insufficiency has been proposed: Vincent *et al.* [50▪▪] proposed to screen infants with AcDys, CAD or other lung hypoplasias on *TBX4* variants when there is a family history of PAH. However, as long as the prevalence and incidence of *TBX4* variants in neonates and the consequences in terms of treatment strategies are not defined, the role of standard testing on *TBX4* variants is unclear.

CLASSIFICATION OF PULMONARY ARTERIAL HYPERTENSION ASSOCIATED WITH *TBX4*

During the most recent World Symposium on Pulmonary Hypertension in 2018, the clinical classification of PH was updated [25]. In this classification, unexplained PAH-associated *TBX4* mutations are assigned to group 1 PAH (HPAH). The Pediatric Task Force introduced in Group 3: PH due to lung diseases and/or hypoxia, a subclass 'PH associated with developmental lung disease', in order to accommodate neonatal PH (other than PPHN) in the classification. Now, as *TBX4* variants are frequently associated with developmental lung diseases, the appropriate classification of an infant or child with a *TBX4* variant and PH becomes more complicated and may be hazardous. This forces the diagnosing physician to a meticulous assessment of any lung disease and, if present, its causal role in the presence of PH. This is obviously a challenging task in small infants, but crucial in defining optimal treatment strategy.

CONCLUSION

Animal studies have demonstrated an important role of the *TBX4* gene in the embryonic development of limbs and tail mesoderm, the lung mesenchyme and the genital papilla [5,9]. The clinical phenotype of *TBX4* variants in humans has expanded from bone disorders to vascular and parenchymal lung diseases and is likely to expand further. The enrichment of *TBX4* variants in PAH justifies inclusion of this gene in genetic analyses in patients with PAH. Further, *TBX4* variants have shown to contribute to diffuse lung diseases, especially but not exclusively in neonates, and seems to be multifaced. Importantly, the exact genotype-phenotype correlations have to be further 'unboxed'.

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Conflicts of interest

There are no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

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