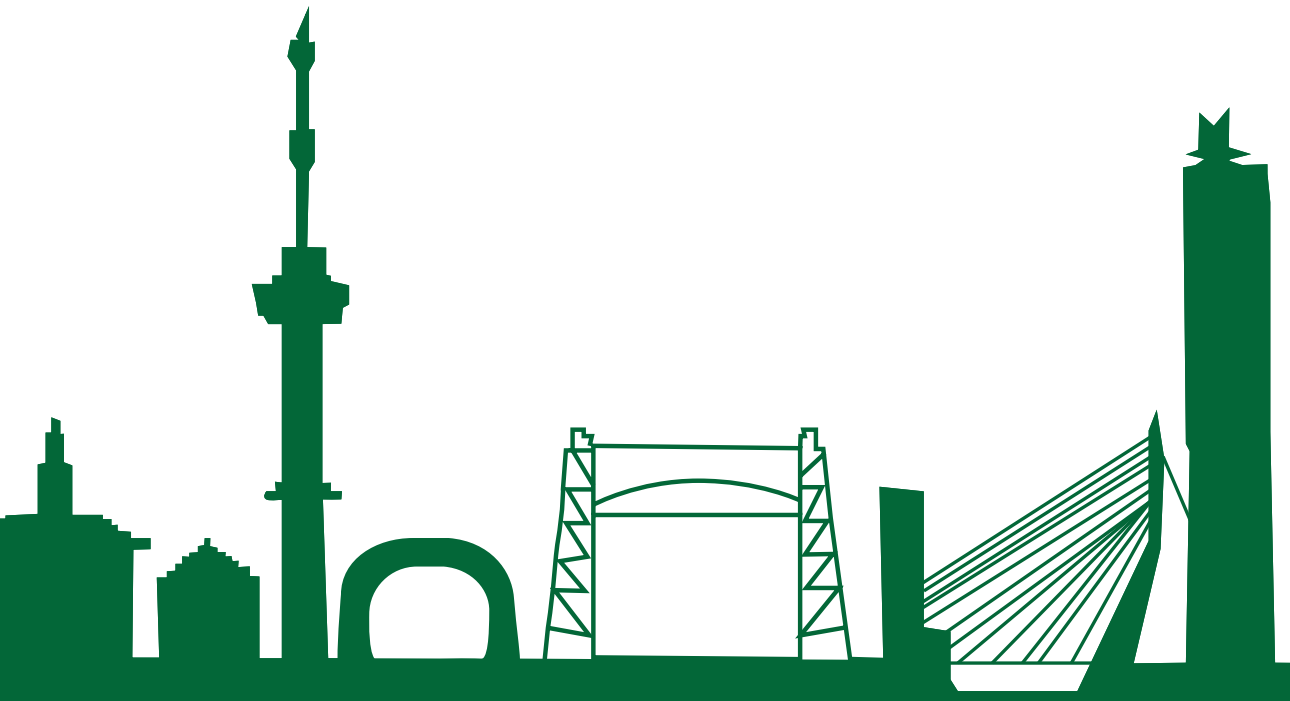

5b

The ever-expanding phenotypical spectrum of human *TBX4* mutations: from toe to lung

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Transcription factors of the T-Box family are known to be involved in the regulation of embryonic developmental processes. T-Box factor 4 (*TBX4*), one of its members first discovered in 1996, is expressed in a wide variety of tissues during organogenesis.[1,2] The *TBX4* gene is located on chromosome 17, region q23.2.[3] Most information on *TBX4* defects have been obtained from animal models that have revealed that *TBX4* plays a critical role, governing multiple processes during early limb and respiratory tract development. Loss of *TBX4* has been shown to block hindlimb and pelvic development, disrupts the development of the respiratory system and affects early embryonic vascularization.[4] In mice, *TBX4* (in concert with *TBX5*) regulates the process of lung branching by controlling the expression of the secreted fibroblast growth factor (*FGF) 10* and activation of *FGF10* signalling. Also, in the trachea, *TBX4* and *TBX5* are important for the formation of cartilage rings, although a distinct pathway that does not involve *FGF10* regulates this.[5] The clinical phenotype of *TBX4* defects in humans however, has begun only very recently to reveal itself (**Figure 1**).

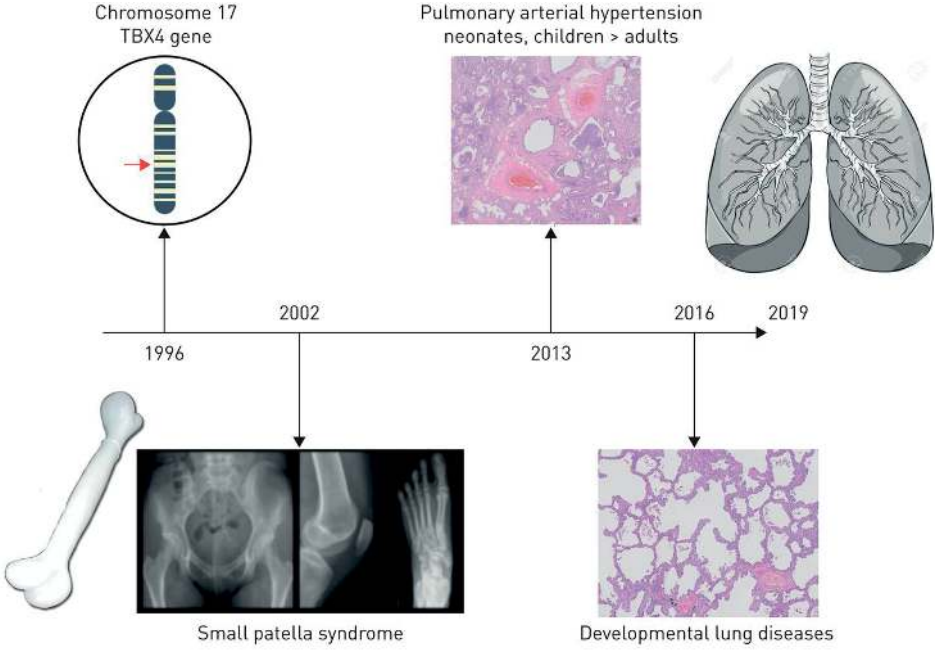

Figure 1. The ever-expanding phenotypical spectrum of *TBX4* mutations since the discovery of the gene in 1996.

First, in 2002, mutations of the *TBX4* gene were found to be associated with a spectrum of limb and skeletal abnormalities referred to as small patella syndrome (SPS), also known as ischiocoxopodopatellar syndrome. This autosomal-dominant skeletal dysplasia is characterised by aplasia or hypoplasia of the patella and developmental anomalies of

the pelvis and feet.[6] The pathogenic mechanism by which *TBX4* mutations cause SPS remains to be elucidated.

It was not before 2013 that *TBX4* gene mutations were discovered to be associated with pulmonary arterial hypertension (PAH) in childhood.[7] In contrast to the identification of most other PAH-related genes, this discovery was primarily based on clinical observations, recognising phenotypical similarities in six children diagnosed with PAH considered idiopathic at diagnosis. In these children PAH was associated with unexplained dysmorphic features and mental retardation. Genetic analyses revealed overlapping 17q23.2 deletions as a common denominator in three of them, where the minimal overlapping region of 1Mb contained several candidate genes including *TBX4*. Subsequent gene sequencing in an additional cohort of 14 paediatric patients diagnosed with idiopathic PAH but without associated dysmorphic features, identified *TBX4* mutations in about 20% of these cases. Since then, the association of *TBX4* mutations/deletion and PAH has been confirmed in different cohorts of paediatric PAH patients (idiopathic or hereditary) with a prevalence of approximately 7–10%.[8,9] Although available data suggest a lower occurrence in adults with PAH (2–3%), evidence is emerging that *TBX4* gene mutations might form the second-most frequently mutated gene, after *BMPR2,* in both children and adults.[10,11]

Recently, incidental cases have been reported of neonates presenting with pulmonary hypertension and respiratory failure, due to various lung diseases, who were found to carry pathogenic *TBX4* mutations. These included severe diffuse developmental lung disorders, such as acinar dysplasia and congenital alveolar dysplasia, conditions that are thought to represent a spectrum of growth arrest at different stages in lung development. The *TBX4–FGF10–FGFR2* epithelial–mesenchymal signalling pathway has been recently suggested to play an important but complex role in neonatal disease conditions associated with lethal lung maldevelopment.[12–14]

In the current issue of the European Respiratory Journal, Galambos et al.[15] describe a selected series of 19 patients, collected from different institutions over the world, presenting with pulmonary hypertension and carrying different *TBX4* variations, including mutations and deletions, and a variety of developmental lung disorders. With that the authors further define the ever-expanding spectrum of clinical manifestations and pulmonary histopathology associated with human *TBX4* variations.

The authors report six children with 17q23 deletions and loss of *TBX4* and 13 children with intragenic mutations. Interestingly, the majority of the children in this series (63%) showed a biphasic clinical course presenting with persistent pulmonary hypertension

of the newborn (PPHN) and respiratory failure with delusive resolution around the age of one month, followed by the emergence of chronic, progressive pulmonary hypertension later in infancy or early childhood. In seven patients, lung histopathology showed diffuse alveolar growth abnormalities and a variable degree of pulmonary arterial wall remodelling, with or without neointimal fibrosis. The severity of presentation of PPHN in early life seemed to correlate with more severe disruption of the distal lung development and contrasted with those who presented later in childhood.

Also in the current issue of the European Respiratory Journal, Maurac et al.[16] report on a very interesting case of a 34-year-old woman, presenting with pulmonary hypertension, right heart failure and SPS, in who a *TBX4* mutation was demonstrated. Although pulmonary function tests were unremarkable, except for a decreased carbon monoxide diffusion capacity, computed tomography imaging suggested bronchial and pulmonary parenchymal abnormalities. Lung biopsy tissue became available in this patient, showing pulmonary vascular remodelling corresponding with pulmonary hypertension, and in addition various airway and parenchymal abnormalities suggesting disturbed lung development.

A very interesting aspect of both these manuscripts was the availability of clinical course and lung histology in infants, children and adults with pulmonary hypertension associated with *TBX4* mutations. All available tissue specimens showed, in addition to various degrees of pulmonary vascular hypertensive remodelling, histological features of abnormal distal lung development affecting alveolar, interstitial and vascular structures. These developmental lung abnormalities seem to form a spectrum with on one hand severe and diffuse features of growth arrest, including acinar dysplasia and congenital alveolar dysplasia, which are associated with severe and early neonatal clinical presentation, while on the other hand milder features of bronchial abnormalities, simplified alveolar development and interstitial remodelling that seem to be associated with presentation at post neonatal age, childhood or even adulthood.[15, 16]

Accordingly, these reports show that the clinical course of *TBX4* mutation-associated pulmonary hypertension is highly variable and seem to correspond with the degree of lung developmental disorder. Patients with *TBX4* mutations and heritable PAH, thus without clinical signs of pulmonary bronchial or interstitial disorders, have been suggested to have a more favourable outcome compared to, for instance, those with *BMPR2* mutations. However, genotype–phenotype relations within *TBX4* mutations, as well as in comparison with other gene mutations, are still insufficiently studied.

The relationship between *TBX4* gene mutations and developmental lung and bone disorders in humans is becoming increasingly clear and is not that surprising, given current knowledge derived predominantly from genetically manipulated animal models on the important roles of both *TBX4* and *TBX5* in the developing lung and trachea. However, the exact role of *TBX4* in the development of the pulmonary structures, both the vasculature and the airways, is complex and currently insufficiently understood. This is illustrated by the unexplained variety in penetrance between *TBX4* mutation-associated bone anomalies (SPS), diseased pulmonary vasculature (PAH) or developmental lung disorders, as well the unexplained variety in expression between mutation carriers in the same family.[6,7] *TBX4* is known to affect the activity of members of the *FGF*-, *Wnt*- and *BMP*-pathways linking *TBX4* mutations to the TGF-β signalling pathway. Recent experimental observations indicate that *TBX4* mutations may be associated with developmental disorders also in other organs than bones and lung, including anorectal malformations.[17]

The emerging awareness that *TBX4* variations are associated with both pulmonary hypertension and developmental parenchymal lung disease, potentially leading to respiratory compromise, challenges the diagnostic classification of pulmonary hypertension in a patient with *TBX4* mutation. According to the clinical classification of pulmonary hypertension, proposed at the 2018 World Symposium of Pulmonary Hypertension, pulmonary hypertension associated with *TBX4* mutations has been classified as group 1 PAH, heritable PAH.[18] At the same time, the paediatric task force at this meeting suggested to include patients with pulmonary hypertension and a *TBX4* mutation in group 3 pulmonary hypertension, associated with developmental lung disease.[19] Moreover, parenchymal lung disease may induce alveolar hypoxia inducing hypoxic vasoconstriction. In these particular patients, it seems even more obvious that a meticulous diagnostic work-up has to be performed in each individual patient in an attempt to distinguish PAH from pulmonary hypertension associated with hypoxia and/or disrupted development. In both cases, the presence of pulmonary hypertension will seriously affect clinical course and treatment most appropriate to the associated condition is mandatory.

In summary, Galambos et al.[15] and Maurac et al.[16] must be commended for their contribution to the further comprehension of the still-emerging wide phenotypic spectrum associated with human TBX4 mutations. From neonate to adult, from bone to lung, mutations in the *TBX4* gene are associated with developmental organ disorders, presenting with pulmonary hypertension that cannot be easily placed under one single denominator. Further studies on the mechanisms through which disturbed function of the *TBX4* leads to pulmonary (arterial) hypertension and disrupted pulmonary development, are required in order to better understand phenotypic expression, inheritance and, ultimately, the optimal treatment approach of *TBX4*-associated pulmonary hypertension.

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