EDITORIAL
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Lessons from tracheal tube development for understanding congenital tracheal malformations

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Novel functions of the extracellular protein fibrillin-2 were discovered in tracheal tube development: fibrillin-2 has important roles in tracheal cartilage development and smooth muscle cell orientation and polarity, all required for tracheal contraction http://ow.ly/rn3Z30nId3N

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Fibrillins constitute a family of extracellular proteins critical for the biogenesis of elastic fibres and for the activity regulation of growth factors of the transforming growth factor (TGF)-β superfamily. All three fibrillins are present during development of tissues and organs, including lung, aorta, bones and skin [1–3]. Typically, fibrillin-2 and -3 expression is limited to prenatal and early postnatal development in humans, whereas fibrillin-1 expression persists throughout adulthood. In mouse, the situation is simplified by the fact that fibrillin-3 is not expressed due to chromosomal rearrangement events [4]. In the developing mouse embryo, fibrillin-1 and -2 co-distribute in elastic and non-elastic tissues, with fibrillin-2 accumulating preferentially in elastic fibre-rich matrices [1]. Unlike fibrillin-1, the specific roles of fibrillin-2 (and fibrillin-3 in humans) in tissue development and homeostasis as well as in disease is still relatively little explored.

Fibrillins assemble into beaded microfibrils through homotypic or heterotypic head-to-tail and lateral self-assembly mechanisms [5–8]. Microfibrils appear to incorporate the fibrillin isoforms that are present at the time of assembly [9]. Fibrillin-2 can also persist as an inner core within postnatal microfibrils [10]. Microfibrils serve as a scaffold for the assembly of elastic fibres, which provide elasticity to various soft tissues such as lung, blood vessels and skin [11]. Mutations in the fibrillin-2 gene (*FBN2*) cause an autosomal dominant connective tissue disorder, congenital contractural arachnodactyly, characterised by arachnodactyly, congenital contractures, kyphoscoliosis and camptodactyly [12, 13]. A loss-of-function mutation in *Fbn2* results in syndactyly in mice [14, 15]. This correlates with the radiation-induced mouse mutants "shaker with syndactylism", which is a multigene deletion that includes the *Fbn2* locus [14, 16, 17].

In the current issue of the *European Respiratory Journal*, Yin *et al.* [18] have employed forward genetic screening and analysis in mice to identify novel functions of fibrillin-2 in tracheal tubulogenesis (figure 1). In this study, loss of fibrillin-2 resulted in compromised tracheal tubulogenesis, characterised by a shortened tracheal tube as well as fewer and fragmented cartilage rings. This phenotype was caused by defective chondrocyte differentiation, whereas chondrocyte proliferation and apoptosis were unaffected. Fibrillin-2 is expressed by tracheal chondroblasts early during embryogenesis, and expression decreases over time. These findings correlate with a previous cell culture study using the chondrogenic cell line

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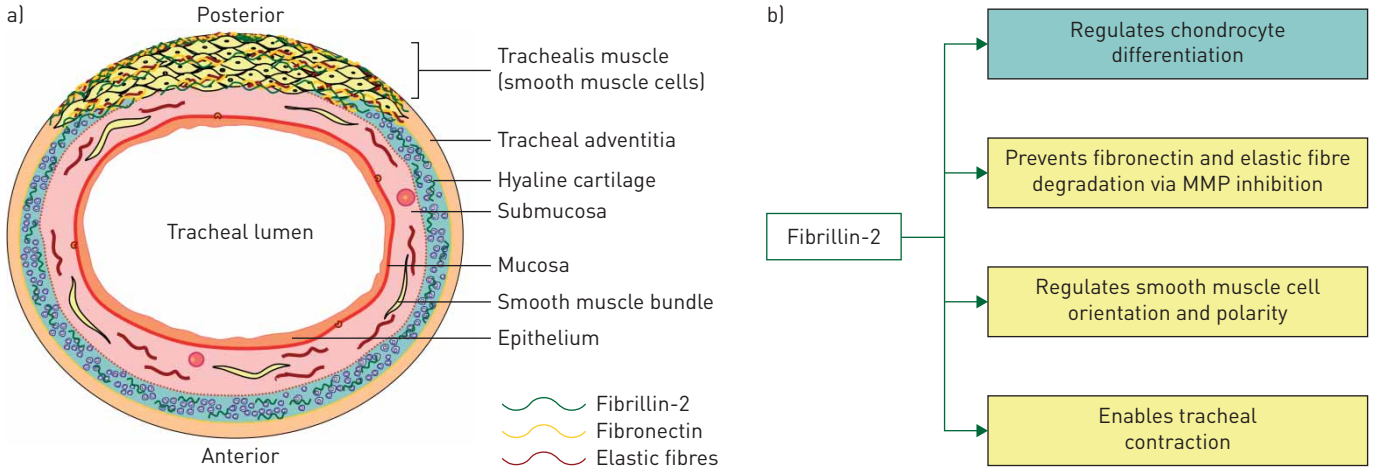

FIGURE 1 Schematic representation of a trachea in cross section, and fibrillin-2 functions in the trachea. a) The tracheal wall comprises an outermost adventitial layer, followed by a C-shaped layer of hyaline cartilage including chondroblasts/chondrocytes. Underneath the cartilage is a submucosal layer including smooth muscle bundles and elastic fibres. A thin mucosa layer separates the submucosa from an epithelium lining the tracheal lumen. The trachealis muscle bridges the gap in the cartilage, and consists of compactly packed smooth muscle cells, and extracellular matrix components. Fibrillin-2 is expressed by smooth muscle cells in the trachealis muscle, and by chondroblasts in the cartilage tissue during chondrogenic differentiation. b) YIN *et al.* [18] demonstrated the following functions for fibrillin-2. Fibrillin-2 aids in tracheal development by regulating chondrocyte differentiation. It also facilitates elastic fibre and fibronectin accumulation around smooth muscle cells by matrix metalloproteinase (MMP) inhibition. This leads to proper smooth muscle cell orientation and polarity. All these functional aspects of fibrillin-2 are required to support tracheal contraction. Colours of the boxes correlate with the respective colours of the tissues and proteins in a).

ATDC5, demonstrating that fibrillin-2 was highly expressed early during chondrogenesis and declined over the course of differentiation [19]. Together, these data show the importance of fibrillin-2 in early tracheal chondrogenesis. How fibrillin-2 mechanistically regulates chondrogenesis remains to be determined.

The study by YIN *et al.* [18] also deciphers a function of fibrillin-2 in the regulation of smooth muscle cell orientation and polarity in the trachea. The authors demonstrate that p38 mitogen-activated protein kinase (MAPK)-mediated downregulation of matrix metalloproteinases (MMPs) is instrumental for this function. More specifically, reduction of fibrillin-2 in the trachea resulted in elevated p38 MAPK phosphorylation, which in turn led to an upregulation of MMP-2 and -9, key elastin and fibronectin cleaving proteases [20]. The authors describe enhanced degradation of extracellular matrix proteins (elastin and fibronectin) around smooth muscle cells as the leading cause for the improper positioning of smooth muscle cells in the trachea, correlating well with one of the fundamental functions of the extracellular matrix being to provide environmental cues to the embedded cells [21]. The improper orientation of smooth muscle cells, and possibly the defective chondrogenesis, also led to reduced contractility of the trachea, represented by a decreased amplitude of spontaneous contractions and weakened contractile forces.

It is interesting that the role of fibrillin-2 is specific for tracheal tube development and is not observed in other organ tube development. YIN *et al.* [18] have shown the absence of a phenotype in the oesophagus of the *Fbn2* mutant mice, which also expresses fibrillin-1. Previous studies have demonstrated the absence of a phenotype in the vasculature of *Fbn2* null mice in the presence of fibrillin-1 [22]. Presumably, fibrillin-1 in these tissues can rescue the functions of fibrillin-2. In the vasculature, loss of fibrillin-1 in *Fbn1*$^{-/-}$ mice results in similar phenotypes to those of the trachea in *Fbn2* mutant mice, with smooth muscle cell disorientation, upregulation of p38 MAPK and MMP levels, and elastin degradation due to a loss of contact between the cells and the matrix [18, 22, 23]. Altogether, these data illustrate that fibrillin-1 can fully compensate for the loss of fibrillin-2 in the vasculature and the oesophagus, whereas cell and tissue deficiencies can develop in the trachea, presumably due to the absence of fibrillin-1.

YIN *et al.* [18] also connect the findings obtained with the *Fbn2* mutant mice with individuals suffering from tracheomalacia. Fibrillin-2 levels are reduced in the ventral parts of the trachea obtained from patients. It is important to note that administration of a p38 MAPK inhibitor or MMP inhibitors in the *Fbn2* mutant mice partially rescued some defects in tracheal tube development. Therefore, administration of p38 MAPK inhibitors such as ralimetinib [24] or pyridinyl-imidazol compounds [25], or MMP inhibitors such as doxycycline [26], marimastat [27] or batimastat [28], may be a useful avenue to explore in the future to improve the severity of tracheomalacia in patients.

Fibrillin-containing microfibrils serve as important regulators of TGF-β bioavailability. Elevated active TGF-β has been correlated with disease progression in disorders caused by fibrillin-1 deficiency [29].

Fibrillins also adopt a crucial role in bone morphogenetic protein (BMP) signalling. The N-terminal region of fibrillin-1 serves as a docking site for the prodomains of BMP-2, -4, -7 and -10 and also imparts latency to BMP-7 [30–32]. Additionally, fibrillin-2 and BMP-7 were identified in the same genetic pathway regulating limb and muscle development, as demonstrated in mouse studies [15, 33]. Given that some BMPs have been implicated in tracheal development, exploring how BMP growth factor bioavailability and activity are affected after the loss of fibrillin-2, and how this influences tracheal tube development, might provide additional important details of the mechanism in the future.

Overall, this study illuminated a novel function of fibrillin-2 in tracheal tube development and identified mechanisms by which cartilage development and smooth muscle cell orientation influence tracheal tube formation, elongation and contraction. This basic research also provided important leads that can be potentially further explored in future clinical studies with patients suffering from tracheomalacia and congenital tracheal stenosis.

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