# The Impact of Postnatal TBX4 Insufficiency on Lung Development and Pulmonary Hypertension in Infant Mice

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## Introduction

Pulmonary hypertension (PH) is characterized by elevated pulmonary arterial pressure and contributes to high morbidity and mortality across the lifespan. There are gaps in the literature regarding underlying genetic variants that are associated with pediatric PH, including T-box transcription factor 4 (TBX4).

TBX4 is ubiquitously expressed in lung mesenchyme during development and encodes transcription factors critical in limb and lung development [1]. In transgenic mouse models, prenatal homozygous TBX4 insufficiency is embryonic lethal, and prenatal heterozygous mutations in TBX4 interfere with lung development and is likely related to abnormal distal lung development [2].

In clinical TBX4 related PH, there are key differences between early onset and late onset disease. Early onset disease presents in neonates with a more severe presentation than late onset disease which presents in early adulthood [3]. Importantly, the mechanisms contributing to the timing of early and late onset phenotypes associated with TBX4 related disease are poorly understood. We seek to investigate the postnatal homozygous disruption of TBX4 and hypothesize that the postnatal disruption of TBX4 is sufficient to impair the distal lung airspace and vascular growth.

## Methods

A TBX4 conditional knockout mouse on a global ERT2 Cre was used to temporally but not spatially disrupt TBX4. TBX4 deletion was activated by tamoxifen injection on day of life (DOL) 1. There were four experimental groups: Het (TBX4/Cre-), TBX4 (TBX4-KO/Cre-), HetCre (TBX4/Cre+), and TBX4Cre (TBX4-KO/Cre+). The TBX4Cre represents the primary experimental group (TBX4 Deficient), and the Het mouse represents the primary control group (Control).

On DOL21, lung function parameters total respiratory system resistance and compliance were measured using Scireq Flexivent. Then, lung and cardiac tissue was collected, and lungs were inflated with 4% PFA and held at 20 cm H2O inflation pressure for one hour. Alveolarization was measured by radial alveolar counts (RAC). Vessel density was quantified after immunostaining with von Willebrand's factor and counted as vessels per high powered field. The presence of right ventricular hypertrophy was then assessed by weighing the RV and LV plus septum to determine Fulton's index (RV/LV+S).

To interrogate key downstream developmental pathways, pro-angiogenic mediators VEGF, KDR, and eNOS, and inflammatory mediators IL-1 and TNF-a were evaluated via PCR.

*Figure 1. Experimental methodology.*

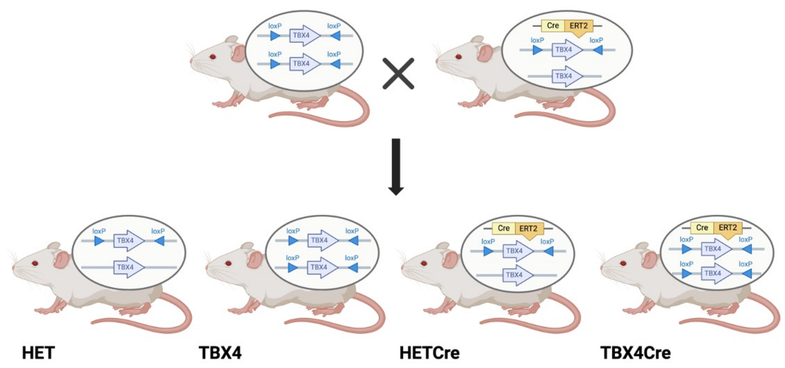
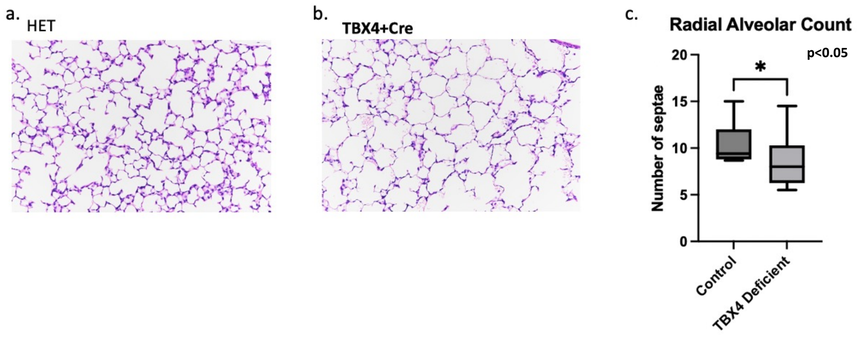



*Figure 2. RAC data and histology.*




## Results

Distal lung development, as measured by RAC, was reduced in the TBX4 deficient mice by 47% compared with controls ($p < 0.05$) (Figure 2). Additionally, TBX4 deficient mice exhibited significantly increased resistance ($p < 0.05$) and decreased compliance ($p < 0.01$) of the total respiratory system when compared with controls (Figure 3). Vessel density was reduced by 77% in the TBX4 deficient group compared with controls ($p < 0.01$) (Figure 4). Additionally, RVH, as measured by Fulton's index was increased by 15% in the TBX4 deficient group compared with controls ($p < 0.01$) (Figure 5).

TBX4 deficient mice exhibited an 82% reduction in lung VEGF expression ($p < 0.001$) (Figure 6a) and a 64% reduction in KDR expression ($p < 0.01$) (Figure 6b). Lung expression of eNOS was also significantly reduced ($p < 0.05$) (Figure 6c) relative to controls. Regarding inflammatory mediators, the TBX4 deficient group exhibited a 93% reduction in IL-1 ($p < 0.001$) and a 54% reduction in TNF-a expression ($p < 0.05$) compared with controls.

*Figure 3. Resistance and compliance data.*

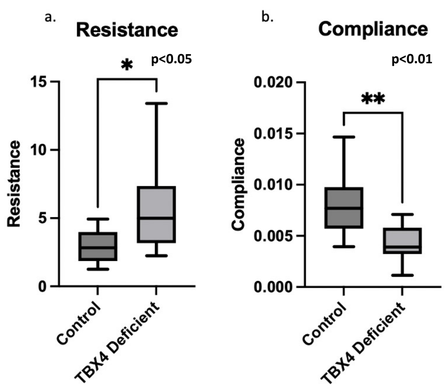
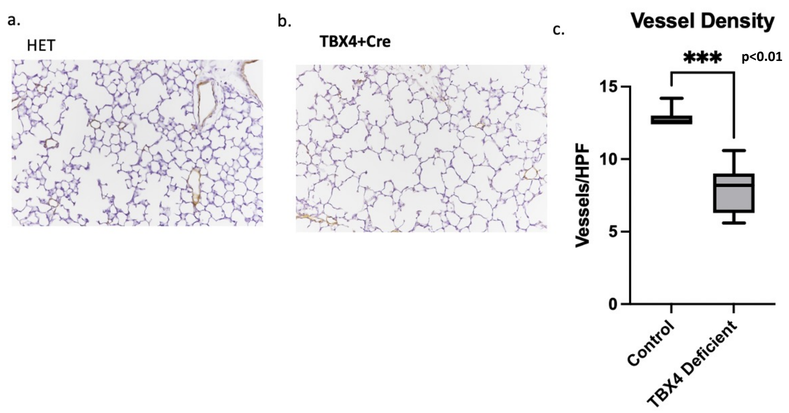



*Figure 4. Vessel Density.*




## Discussion

We found that the postnatal disruption of TBX4 expression in infant mice during the alveolar stage of development was sufficient to impair alveolar growth and lung function, reduce vessel density, and cause RVH. We also found reduction in pro-angiogenic mediators (VEGF, KDR, and eNOS) but not enhanced inflammation.

Previous studies have shown that gestational TBX4 disruption is lethal. However, we found that the postnatal deletion of TBX4 is compatible with life and confers pulmonary hypertension. These findings are interesting in the setting of clinical variability associated with TBX4 related disease. Early onset disease often presents in cyanotic newborns with severe hypoxemic respiratory failure and PPHN, with poor or partial responsiveness to aggressive treatment [3]. Other patients present in childhood with late onset PH with lung disease who may come to clinical attention due to associated skeletal abnormalities, especially small patella syndrome [4]. Mechanisms of the late disruption of TBX4 for future study could relate to epigenetic modification.

We additionally explored downstream mediators of disease and found that decreased lung expression of pro-angiogenic mediators but not enhanced inflammation. These findings support the speculation that disruption of the nitric oxide-guanylyl cyclase-cGMP pathway may in part modulate TBX4 related PH.[5]

## Conclusion

Previous literature shows that TBX4 deletion is lethal. We found that compared to littermate controls, postnatal TBX4 insufficiency decreases distal lung alveolarization, impairs lung function, decreases pulmonary vessel density, and causes right ventricular hypertrophy. As such, early TBX4 insufficiency after birth is capable of disrupting postnatal distal lung airspace and vascular growth and causes RVH in infant mice and is associated with decreased lung expression of pro-angiogenic mediators.

*Figure 6. Pro-angiogenic mediators.*

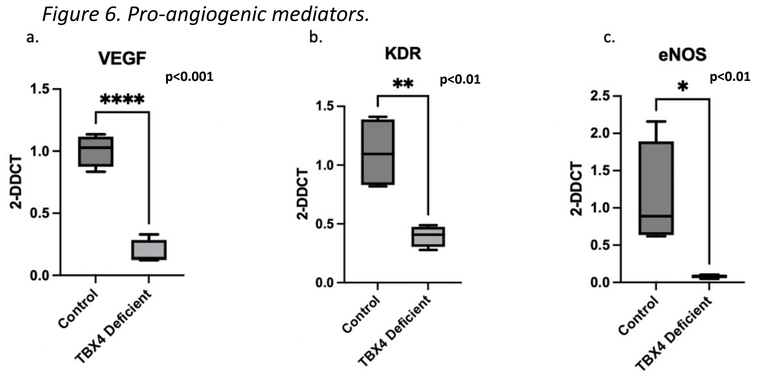
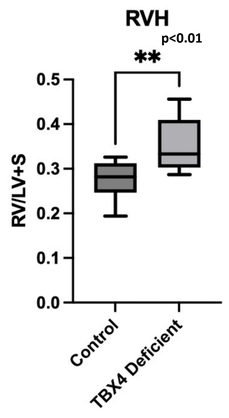



*Figure 5. Right Ventricular Hypertrophy.*




## References

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