Histologic Features and Decreased Lung FOXF1 Gene Expression in Severe Bronchopulmonary Dysplasia without a Genetic Diagnosis of Alveolar Capillary Dysplasia

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Abstract

Severe BPD can be associated with clinical and histologic features that are similar to ACD without evidence of FOXF1 genetic disease. Importantly, lung FOXF1 and TMEM100 gene expression is markedly decreased in severe BPD, suggesting that impaired FOXF1 signaling may contribute to abnormal lung growth and refractory pulmonary hypertension in BPD.

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INTRODUCTION:

Bronchopulmonary dysplasia (BPD), the chronic lung disease of prematurity, contributes to significant morbidities and mortality, especially in extremely low gestational-age newborns. Despite advances in perinatal care, BPD remains a major sequel of prematurity, often leading to the prolonged need for mechanical ventilation, pulmonary hypertension (PH), other co-morbidities, and mortality.[1,2] Further, there has been a growing recognition that some preterm infants develop severe BPD (sBPD), and this subgroup has been identified as having a much higher risk for poor outcomes, including late mortality and PH.[3-5]

Compromised alveolar development with impaired lung vascular growth and hypertensive remodeling are histologic hallmarks of sBPD, as well as genetic lethal lung developmental disorders (LLDD), including alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV).[6,7] Extensive work has shown that loss of*FOXF1* expression is the genetic basis for ACDMPV,.[8] As with sBPD, ACDMPV is characterized by marked disruption of the alveolar capillary network, which causes severe neonatal respiratory distress and PH, leading to nearly uniform mortality despite aggressive cardiopulmonary therapies.[6,7] Additional studies of ACDMPV have shown that the "MPV," are actually bronchial veins and represent recruitment of intrapulmonary bronchopulmonary anastomoses (IBA), which connect the pulmonary vasculature with bronchial vessels.[8] The presence of prominent IBAs have similarly been identified in fatal sBPD,[11] suggesting that overlapping features between ACDMPV and sBPD exist, and that these conditions may share pathogenetic mechanisms. Such a link has been suggested in extensive laboratory studies that demonstrated impaired *FoxF1* expression in neonatal mice exposed to hyperoxia, a common experimental model for BPD.[12]

Although multiple mechanisms are associated with high risk for BPD, outcomes remain highly variable and mechanisms that increase susceptibility for this challenging subgroup of preterm infants with sBPD and PH remain poorly understood.[2] In this report, we present the case of a preterm infant who died with sBPD and PH, andhad striking histologic features compatible with the diagnosis of ACDMPV but had negative genetic findings for *FOXF1* . We further demonstrate dramatic reductions in lung *FOXF1* content in sBPD, suggesting common mechanistic links between ACDMPV and sBPD with impaired *FOXF1* signaling.

CASE PRESENTATION:

This 530 gm female newborn was delivered to a G_1P_0 female by an emergency cesarean-section due to eclampsia at 25 weeks' gestation. She was rapidly intubated at birth, treated with exogenous surfactant, placed on conventional mechanical ventilation in the NICU and subsequently transferred to a level III medical center. Her early course was characterized by profound hypoxemic respiratory failure with severe PH. Throughout her course, serial echocardiograms demonstrated signs of supra-systemic PA pressures with

RV hypertrophy and dilation. The infant was managed with high frequency and conventional ventilation initially but failed to tolerate extubation and non-invasive respiratory support at 45 weeks GA. Despite aggressive therapy which included use of PH-targeted therapies, her hypoxemia continued to worsen along with frequent cyanotic episodes. A tracheostomy was performed to support longer-term invasive ventilation. Serial chest radiographs revealed progressive hyperinflation with heterogenous regions of patchy atelectasis and infiltrates with regional over-distension. , Her course worsened with increased severity of cyanotic episodes due to progressive worsening of her chronic lung disease and PH. After extensive discussions with the family, the decision to redirect care due to futility was made and the child died. The family agreed to a limited heart and lung autopsy as well as clinical exome sequencing (ES) to identify genetic variants associated with known developmental lung diseases.

ES at Prevention Genetics revealed the following heterozygous variants of uncertain significance: c.2929G>A (p.Glu977Lys) and c.3019G>A (p.Gly1007Ser) in *APC2* , c.4451C>G (p.Ser1484Cys) in *KAT6B* , and c.664G>A (p.Ala222Thr) in *KMT2D* . Histopathology findings were consistent with sBPD with marked pulmonary hypertensive remodeling and prominent IBA. (**FIGURE 1 A+B**) As these features are also compatible with the diagnosis of ACDMPV, additional genetic and molecular analyses were performed from the lung tissue using Sanger sequencing, genome wide array CGH, and RT-qPCR. No evidence of point mutations nor CNV deletions involving *FOXF1* or *TMEM100* was found.

Real Time-qPCR measurements were performed in triplicates using as a control normal lung tissue specimen from three age-matched individuals. RNA from the frozen lung autopsy specimen was extracted using miRNeasy Mini Kit Isolated RNA was reverse-transcribed using SuperScript III First-Strand Synthesis System TaqMan gene expression assays were obtained from Applied Biosystems. RT-qPCRs were done using TaqMan Universal PCR Master Mix For relative transcript quantification, the comparative CT method was used. *FOXF1* and *TMEM100*transcript levels were normalized to that of *GAPDH* . The expressions were calculated relative to one of the controls. Remarkably, transcript levels of *FOXF1* and *TMEM100* are significantly reduced in the lungs of the proband, resembling those seen in patients with *FOXF1* deficiency due to pathogenic CNV deletion. (**FIGURE 1 C+D**)

DISCUSSION

We report the case of an extremely preterm infant who developed progressive ventilator-dependent sBPD with refractory PH, who died at 10 months of age despite aggressive interventions after a prolonged course in the NICU. Clinical and radiographic features were characteristic of sBPD, as classified in the category of type 2 sBPD[3] or Grade 3 BPD,[4] which is associated with poor outcomes. Lung histology demonstrated classic features of sBPD, including increased interstitial thickening, decreased alveolarization, and hypertensive pulmonary arterial remodeling with reduced vessel density. The lungs further demonstrated striking findings associated with ACDMPV.[7] These include the presence of prominent thin-walled and engorged vascular structures, which have been characterized as IBA, that generally share a common sheath with small pulmonary arteries, and have been shown by 3D reconstruction and Synchotron imaging to represent connections between the pulmonary and bronchial ciruclations.[9-11] Due to these features, additional genetic studies were performed but these failed to render the diagnosis of ACDMPV. However, measurements of lung *FOXF1* and*TMEM100* expression were dramatically reduced to levels observed in ACDMPV patients. These findings suggest that disruption of*FOXF1* and *TMEM100* signaling, as observed in ACDMPV,[13,14] may also contribute to the pathobiology of disruption of lung development in sBPD.

Recent advances have identified the genetic basis for diverse LLDD, including point mutations or copy-number variant deletions involving*FOXF1* , *TBX4* , and other genes, which have enabled clinicians to better discriminate these disorders by identifying factors beyond clinical and histopathologic features alone.[7]Past studies have shown that expressions of *FOXF1* and*TMEM100* are decreased in lung tissue in infants dying with proven genetic cases of ACDMPV.[13,14] Dysregulation of genes from *FOXF1* -related pathways are essential for epithelial branching and vascular patterning, including the lung-specific *TMEM100* gene, which is known to be crucial in vascular morphogenesis.[13] Most recently, these data were corroborated in the *Foxf1* mouse model, which further demonstrated the role of *FOXF1* in mediating endothelial progenitor cell

stimulation of angiogenesis via BMP9 signaling[15]Findings from this report support the proposal that in some infants with sBPD, down-regulation of *FOXF1* and *TMEM100* signaling may contribute to the pathogenesis of severe impairment of lung alveolar and vascular growth, including prominent IBAs with PH.

Genetic deletion of *Foxf1* reduces pulmonary endothelial cell growth and angiogenesis during development and increases susceptibility to lung injury in mice.[12] Past animal studies have demonstrated that early disruption of angiogenesis, including decreased VEGF signaling, impairs airspace development, suggesting an important role of angiocrine signaling for promoting alveolar as well as vascular growth.[16] Overall, these findings remind us that the pathogenesis of ACD and other rare but lethal LLDD in term neonates is highly relevant to more common multifactorial disorders of lung hypoplasia, such as sBPD.[12] This case report supports the proposal that pulmonary vascular growth is a critical driver of lung maturation and suggests that therapeutic strategies to preserve the survival and function of endothelial cells may effectively stimulate lung vascular growth, improve alveolarization, and reduce the risk of PH in preterm infants. Future preclinical and clinical studies are needed to define the potential roles of impaired *FOXF1* and*TMEM100* signaling in the subgroup of preterm infants with severe BPD and PH, which will likely enhance our understanding of early pathogenetic mechanisms underlying sBPD and the failure of recovery from severe lung injury.

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Figure Legends:

Figure 1 : Upper panel: Lung histopathology: A: Medium power view shows underdeveloped lung parenchyma with many enlarged and simplified alveoli and thick interstitium. The enlarged alveoli are associated with double capillary layers (arrows). B: Markedly dilated and congested thin walled vessels are present within the bronchoarterial bundles (Br: bronchiole, PA: pulmonary arteries) consistent with dilated bronchial veins of IBA and resembling those of misaligned pulmonary veins (MPV) seen in ACD (arrows). Lover panel: Expression of*FOXF1* (C) and *TMEM* (D)A decrease in the expression of*FOXF1* and *TMEM* in the proband similar to that observed in ACDMPV patients due to *FOXF1* haploinsufficiency is seen (One-way ANOVA with Tukey Test: $p < 0.01$). ACDMPV 179.3: paternal deletion of the *FOXF1* enhancer. Error bars represent standard deviation.

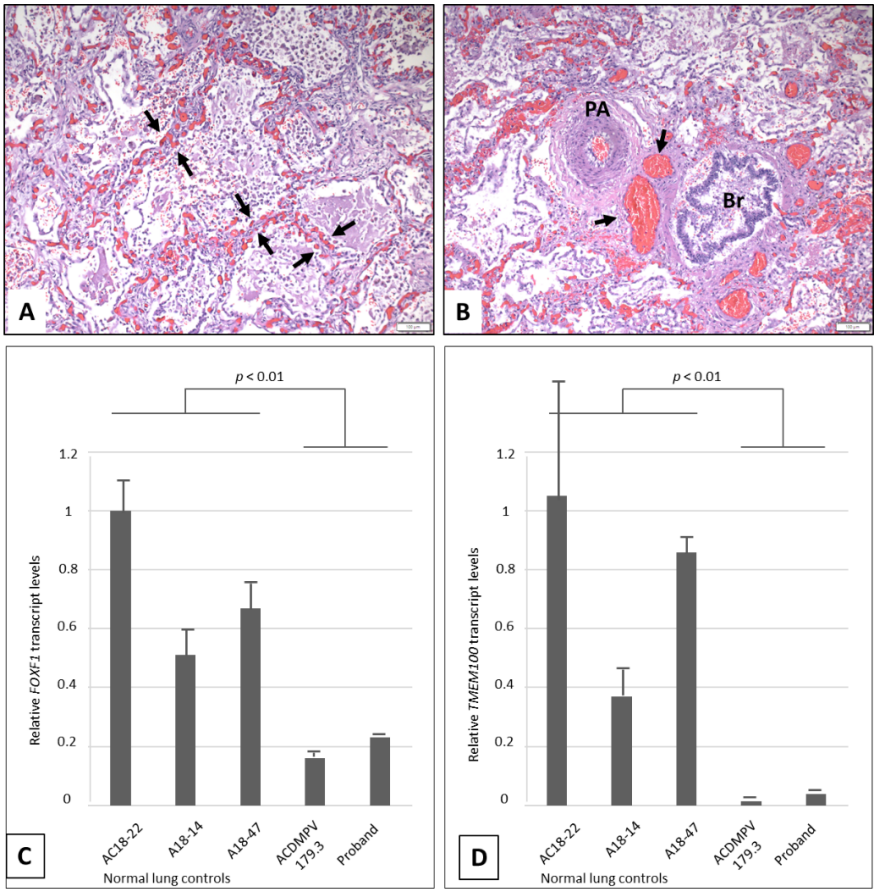

FIGURE 1