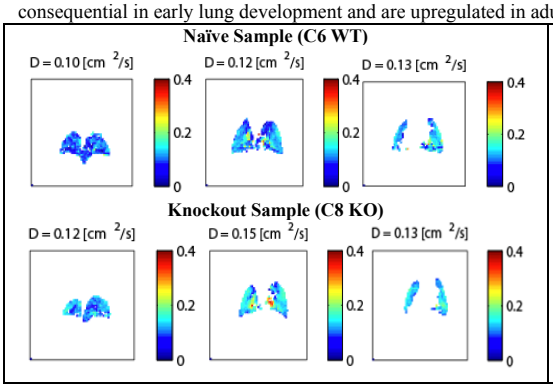
Non-invasive Assessment of Pulmonary Developmental Deficiency in a Model of Transgenic Mice using Hyperpolarized Gas Diffusion MRI

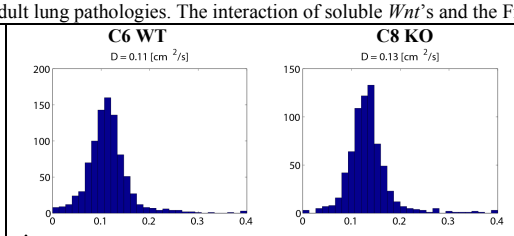
A. Barulic[1], K. Emami[2], Y. Xin[1], P. Mongkolwisetwara[1], H. Profka[1], N. N. Kuzma[1], J. M. D'Armiento[3], T. Shiomi[4], S. J. Kadlecek[1], Y. Xu[1], H. Hamedani[1], B. M. Pullinger[1], R. Ghosh[1], J. Rajaei[1], S. Pickup[1], M. Ishii[5], and R. R. Rizi[1]

[1]Radiology, University of Pennsylvania, Philadelphia, PA, United States, [2]Radiology, University of Pennsylvania, PA, Pennsylvania, United States, [3]Departments of Medicine and Surgery, Columbia University, New York, NY, United States, [4]Department of Molecular Medicine, Columbia University, New York, NY, United States, [5]Otolaryngology–Head & Neck Surgery, Johns Hopkins University, Baltimore, MD, United States

INTRODUCTION: The *Wnt* signaling pathway represents a network of proteins noted for their fundamental role in embryogenesis and cancer. These molecules are particularly consequential in early lung development and are upregulated in adult lung pathologies. The interaction of soluble *Wnt*'s and the Frizzled family of receptors is inhibited by the binding of secreted Frizzled related proteins (SFRPs). At present, the direct function of SFRPs during embryogenesis has yet to be fully discerned; likewise, their presence in a disease state of the lung is also a source of speculation. In order to elucidate the capacity in which SFRPs function during early morphogenesis of the lung, Foronjy *et al.*[1] examined SFRP1 knockout mice. These mice demonstrated abnormal *Wnt* signaling, proliferation of the mesenchymal tissue, and debilitated formation of the alveoli indicating that SFRP1s are fundamental to successful alveolar formation. In this study hyperpolarized (HP) ^{3}He MRI was used to assess the SFRP1 knockout mice lung microstructure. This technique is a non-invasive and quantitative probe of pulmonary airways based on imaging of the respiratory gas diffusivity with sub-millimeter resolution**.** The mobility of ^{3}He atoms is restricted by impermeable lung tissue barriers thereby establishing a monotonic relationship between the apparent diffusion coefficient (ADC) of ^{3}He atoms, a parameter that can be measured using hyperpolarized gas MRI, and the airway size. We hypothesize that a marked degradation in alveolar structure in SFRP1 mice should exhibit measurable increases in ^{3}He gas diffusion rates within the acinus.



← Figure 1. Representative ADC maps of naïve (bottom row) and SFRP1 knockout (top row) mouse lungs. Images in columns 1-3 correspond to anterior, middle, and posterior lung slices.



↑ Figure 2. Respective ADC histograms of naïve and SFRP1 knockout mouse lungs.

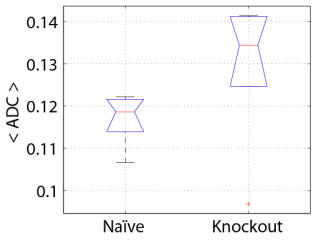


Figure 3. Analysis of variance of group mean ADC values.

	No.	ADC [cm^2/s]		
SFRP1 Knockout Group (KO)	C3	0.13	±	0.05
	C4	0.14	±	0.06
	C7	0.12	±	0.04
	C8	0.12	±	0.05
	C11	0.14	±	0.06
	C12	0.13	±	0.05
		0.13	±	**0.01**
Naïve Wild-type Group (WT)	C1	0.12	±	0.04
	C2	0.11	±	0.05
	C5	0.12	±	0.04
	C6	0.10	±	0.04
	C9	0.11	±	0.04
	C10	0.12	±	0.04
		0.11	±	**0.01**

Table 1. Group ADC statistics

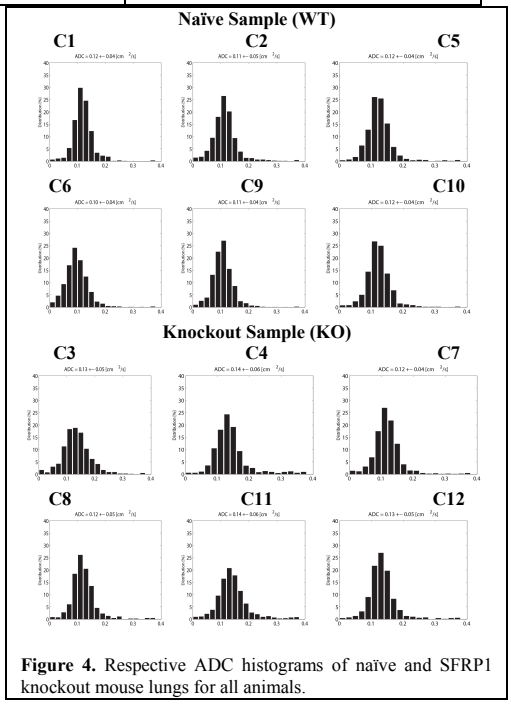


Figure 4. Respective ADC histograms of naïve and SFRP1 knockout mouse lungs for all animals.

METHODS: This study involved two mice cohorts: (i) SFRP1 knockout mice (n=6, body weight(BW)=26±6g), and (ii) naïve wild-type mice (n=6, BW=24±4g). The generation of the SFRP1 knockout mice in the C57BL/6(albino)-129SvEv^{brd} background was previously reported by Bodine *et al.* [2]. Mice were sedated with 100 mg/kg IP ketamine and 10 mg/kg xylazine, and tracheotomized with a 1.5-mm endotracheal tube before MRI. Heart rate, blood oxygenation, and body temperature were continuously monitored throughout the imaging session. The mice were connected to a custom-designed small animal ventilator equipped with real-time monitoring of peak inspiration pressure (PIP) and capable of delivering the breathing gas with an accuracy of ±100µL/breath (breathing air ventilation parameters: V_T=1.2ml/100g-BW; 110 BPM; I:E=1:2; FIO_2=20%). A 50-cm bore, 4.7-T MRI scanner (Varian Inc.) was fitted with 12-cm, 25-G/cm gradients and a 1-1/2"-ID quadrature 8-leg birdcage body coil (Stark Contrast). The mice were placed in a supine position in the magnet. A mixture of He:O_2 (4:1) was delivered with consistent ventilation parameters during the imaging session, with ^{4}He substituted by HP ^{3}He immediately before image acquisition. During an induced three-second breath-hold, ^{3}He ADC images were obtained utilizing a double acquisition diffusion-weighted gradient echo imaging pulse sequence [3], using diffusion time Δ=1ms and b-values = 0.00 and 2.18 s/cm^2 along the phase encoding direction with FOV=3×3cm^2, NS=3 (anterior, middle and posterior coronal slices), THK=4mm, α=45°, MS=64×64 (planar resolution of ~470 µm). For each animal, pulse width calibration was performed on the loaded RF coil to estimate the associated B1 field.

RESULTS AND DISCUSSION: ADC maps and respective histograms were computed for three imaged slices in each animal. **Figure 1** shows representative ADC maps of the three slices for one animal from each cohort. These images illustrate that diffusion is less restricted in the SFRP1 knockout group as indicated by the lighter areas on the maps compared to the darker areas of wild-type cohort lungs. The respective histograms for these two animals are shown in **Figure 2**. **Table 1** shows the mean and standard deviation of ADC distribution across the three slices for each animal as well as the group statistics. The SFRP1 knockout group, on average, shows a significantly higher ADC value than the wild-type group (0.13±0.1 vs. 0.11±0.1, P<0.012 as shown in **Figure 3**). **Figure 4** displays respective histograms for all 12 animals. The distribution histograms of ADC in the SFRP1 knockout group demonstrates a larger dispersion than the wild-type group (as measured by ADC standard deviation, P<0.0035).

CONCLUSION: SFRP1 knockout mice demonstrated a significant increase in overall ^{3}He ADC value compared to their naïve counterparts as demonstrated by HP gas diffusion MRI in three coronal slices of the mice lungs. The heterogeneity of ADC distribution also showed a significant increase in the knock-out group compared the wild-type mice. These observations are in agreement with histological results and suggest that HP ^{3}He MRI can serve as a sensitive and non-invasive *in vivo* imaging tool for detecting alterations in the alveoli size due to the SFRP1 knockout in mice. Measurements of this type can serve as a platform for longitudinal study of development and repair response in pulmonary cells, as well as for the study of lung embryogenesis and monitoring the progression of therapeutic interventions for various lung pathologies.

REFERENCES: [1] Foronjy *et al.*, AJP 104(2) (2010), 598-607; [2] Bodine *et al.*, Mol Endocrinol. 18 (2004), 1222-1237; [3] Emami *et al.* Proc Intl Soc Mag Res Med. (2007).