Detection of regional lung disease in β-ENaC mice using a laboratory x-ray source

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1. Introduction

- Sensitive measures of lung structure and function are necessary to track the progression of Cystic Fibrosis (CF) lung disease, and to assess the local and global effects of genetic and pharmaceutical therapies.
- X-ray velocimetry (XV) combines CT techniques with volumetric particle image velocimetry (PIV). PIV tracks motion of lung speckle—produced when x-rays pass through alveoli—to infer information about airflow.
- The magnitude and direction of the motion of the speckle pattern between frames allows the expansion across all areas of the lung, and the amount of air displaced by each section to be determined. Areas where airflow is restricted manifest as reduced expansion.

2. Methods

- β-ENaC mice (n=17, bred on a C57Bl/6N background) were imaged along with littermate controls (n=13)[1].
- Mice were anaesthetized with domitor/ketamine and surgically intubated for mechanical ventilation (AccVent200, NHD, Melbourne) (PIP=12cmH_2O; PEEP=2cmH_2O). Breathing rate was set to 120 bpm with inspiration/expiration time at 150ms/350ms.
- The mice were mounted on a rotary stage (Zaber Technologies, Vancouver, Canada) in front of a high-brightness liquid-metal-jet x-ray beam (Excillum AB, Kista, Sweden).
- A flat-panel detector (PaxScan, Varian Medical Systems, Palo Alto, CA, USA) captured images at 30 Hz with an exposure time of 15 ms as the mouse was rotated through 360^0.
- Imaging was synchronized with ventilation to capture images at 15 time points throughout the breath — accumulating 400 projections per time point for CT reconstruction [2].
- 15 separate CT constructions, each representing a different point in the breath, were prepared for frame-by-frame analysis.
- Using PIV, the speckle pattern across the lungs was analyzed over each time point for local volume expansion [2].
- The airway tree was segmented from the CT reconstruction [3] and used to assign a value for airflow for each terminal airway point, and for calculation of a regional respiratory time constant.

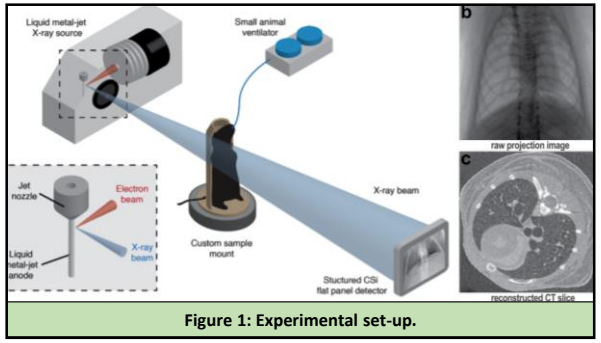

Figure 1: Experimental set-up.

3. Results

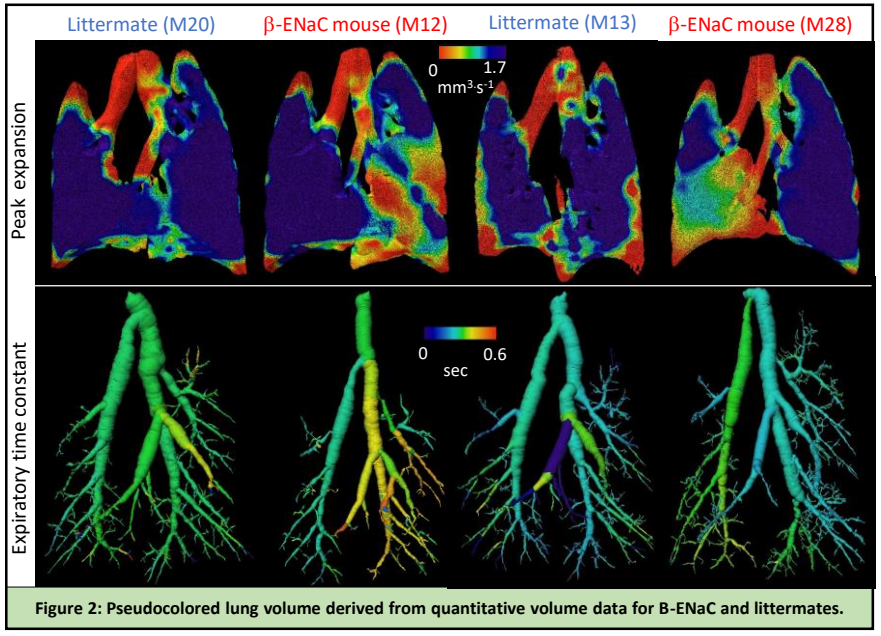

Figure 2: Pseudocolored lung volume derived from quantitative volume data for B-ENaC and littermates.

- Two typical examples of the heterogeneity seen in CF-like disease in β-ENaC mice are shown in Fig 5 alongside healthy littermates.
- The first row of images in Fig. 5 shows expansion across the lung at peak inspiration. The second row shows the regional expiratory time constant.
- Both examples of disease, M12 and M28, show heterogeneity.
- The dynamics of the lung tissue and airflow are captured using CTXV. Additionally, we are able to see the exact locations of damage areas.
- A numerical analysis of CTXV data can be used to quantify symptoms of lung disease.

4. Analysis and Discussion

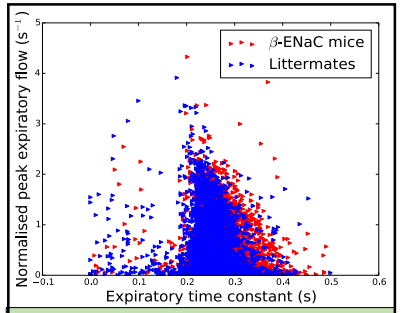

Figure 3: Plot of quantitative time constant and flow data across all data sets.

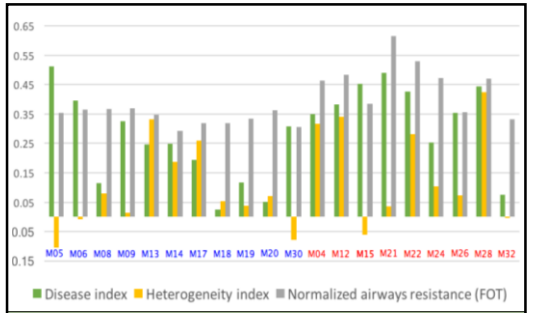

Figure 4: FOT data alongside numerical CTXV data for β-ENaC (red) mice and littermates (blue).

- Figure 3 shows the regional expiratory time constant from all β-ENaC and littermate data sets plotted against the normalized peak expiratory flow. The data from littermates is more concentrated around the median whereas the data from diseased mice is more dispersed—a statistical representation of heterogeneity.
- The amount of dispersion present in the numerical data can indicate the presence of concentrated areas with a higher value than the median i.e. areas with restricted airflow. The heterogeneity index (HI), derived from dispersion, is shown along data obtained using force oscillation techniques (FOT) in Figure 4.
- Additionally, the fraction of the data that is considered "unhealthy"—a significantly higher time constant than the median for healthy littermates, gives a disease index (DI) , shown along side FOT and HI data for each mouse in Figure 4. Both M12 and M28 from Figure 2 show high levels of heterogeneity and disease to correlate with the FOT data.
- The CTXV data shows a range of symptoms in diseased β-ENaC mice, as well as the variability of lung disease, providing the tools for a sophisticated lung disease model. This information is not accessible from the single, global measure taken with FOT.
- CTXV can also be used for targeted treatment due to the ability to identify locations of functional changes.

5. Conclusion

- This rigorous modelling and analysis of CF lung disease provides detailed regional lung health information that is not possible with existing techniques—such as spirometry—which supply a single, global measure. This non-invasive method can provide a novel method of assessment of disease and treatment in CF lung disease.

[1] Mall, M et al., Increased airway epithelial Na+ absorption produces cystic fibrosis-like lung disease in mice. *Nat. Med.* **10**, 487–493 (2004).
[2] Samarage, C.R. et al., Contrast free angiography of the pulmonary vasculature in live mice using a laboratory x-ray source. *Med Phys.* **43**, 6017—6023 (2016).
[3] Frangi, A.F. et al., Medical Image Computing and Computer-Assisted Intervention in *MICCAI* (ed. Wells, W.M., Colchester, A. & Delp, S.) 130–137 (Springer Berlin Heidelberg, 1998).

Supported by NHMRC (APP1079127); USA CF Foundation (FOURAS 1510); Cure 4 Cystic Fibrosis Foundation.