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Three-Dimensional Assessment of Bronchiectasis in a Mouse Model of Mucociliary Clearance Disorder

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Abstract: Bronchiectasis is a chronic pulmonary disease affecting the conducting air ways of the lung, which may result from congenital disorders that affect ciliary motility. The disease is being recognized with increasing frequency around the world. Patients with bronchiectasis show permanent enlargement of peripheral bronchi accompanied by repeated respiratory infections, disabling productive cough and shortness of breath, resulting in loss of lung function. Mouse models of reduced mucociliary clearance have failed to display signs of bronchiectasis in multiple studies, hindering the development of targeted therapies. In this brief report we present the detection and quantification of bronchiectasis in *TAp73* knockout mice using synchrotron radiation-based free-propagation phase contrast CT imaging, allowing the study of bronchiectasis in a pre-clinical and translational setting for the assessment of novel treatment strategies.

Clinical Relevance: Murine models of reduced mucociliary clearance have been established to investigate their pathobiology and develop novel treatment approaches but failed to develop visible airway enlargements. Synchrotron free-propagation phase contrast CT imaging is an innovative, sensitive, non-destructive in-situ technique, that allows for three-dimensional ultra-high-resolution detection of bronchiectasis in murine models of impaired mucociliary clearance.

Keywords: Bronchiectasis, Mouse Model, Mucociliary Clearance Disorder, 3D Imaging

Bronchiectasis is a chronic pathological condition characterized by abnormal enlargement of the lung`s conductive airways. It is associated with a lack of ciliary motility and restricted mucociliary clearance in diseases such as Primary Ciliary Dyskinesia (PCD) or “immotile cilia syndrome.” Recent studies have shown an increase in the prevalence of bronchiectasis, causing a significant burden on public healthcare systems [1,2]. The mechanisms that trigger and drive the development of bronchiectasis have yet to be fully elucidated. Murine models of immotile cilia or reduced mucociliary clearance failed to display signs of bronchiectasis in multiple studies, raising questions about the suitability of murine models for non-CF bronchiectasis and hindering the development of targeted therapies [3].

The influence of age and size has been discussed since the duration of mucociliary clearance defects could significantly impact airway remodeling and many murine models of PCD do not survive long enough for the development of relevant airway remodeling [4]. Moreover, the murine airway anatomy poses significant challenges to the employed imaging modality since most of the conducting airways measure under 1mm in diameter [5]. Motion artifacts from ventilation and the beating heart further limit the imaging results; they were named the number one technical pitfall in the CT evaluation of bronchiectasis [6]. Given these limitations, the failure in developing suitable murine models of immotile cilia raises the question: “*Could we have simply missed it?*”

To answer the question of whether we can detect a phenotype of bronchiectasis or bronchial enlargement under pathogen-free conditions in laboratory mice with mucociliary clearance defects, we generated a cohort of *TAp73* wildtype (WT, N=6) and knockout (KO, N=4) mice. All experimental procedures were approved and performed in accordance with the requirements set forth by Ethics Committee of the University Medical Centre Goettingen (application number: 18/2/16).

TP73, a p53 homolog, has been identified in key steps during the differentiation of motile multiciliated cells and the regulation of airway multiciliogenesis, and TAp73 KO mice have strongly reduced mucociliary clearance [7,8,9]. Mice were evaluated at 10 months of age (308 days), corresponding to a human age of approximately 34 years [10]. At this age, most human patients with severe PCD have already developed visible bronchiectasis on chest CT. Synchrotron-based imaging has been shown to increase structural detectability and permit exact quantification of tissue parameters in small rodents in a variety of applications and organ systems. The high degree of coherence of synchrotron x-ray light allows for phase sensitive imaging strategies that cannot be applied using commonly available x-ray tubes [11]. One of those is the so-called synchrotron free propagation phase contrast CT (SR-pCT), which is characterized by a larger contrast-to-noise ratio in soft-tissue application than classical CT and has been successfully applied for lung imaging in a multitude of studies

[12,13]. The ability of SR-pCT to study the mouse lung *in-situ* in great detail enables reliable depiction of subtle anatomical alterations in their original context [13].

Here we utilized SR-pCT to analyze the lung structure at a spatial resolution of ~9µm. To maintain the lung architecture, sacrificed mice were tracheotomized and the lungs were inflated with a constant air pressure of 30cmH_2O. The trachea was tied and the entire mouse was embedded in 1% agarose gel in 50ml falcon tubes to prevent *post-mortem* deformation or lung deflation [14]. Imaging was performed at the SYRMEP beamline of the Italian synchrotron light source "Elettra" Trieste, Italy using a sample-to-detector distance of 30cm, a quasi-monochromatic x-ray beam with an energy of 22keV, and a full 360° off-center scan was performed yielding 1800 angular projections by an effective detector pixel size of 9x9µm^2. Single distance phase retrieval was applied using the transport of intensity equation [15]. 3D reconstructions were performed utilizing a classical filtered back projection algorithm. The delta-to-beta ratio between the decrement of the real part of the complex refractive index and the imaginary part was set to 1950 based on Mohammadi et al. [16]. In the obtained 3D data sets 5-10 volumes-of-interest of a size of 22mm^3 were randomly selected in the periphery of the lung. The average pore size of small conducting airways was then quantified in three dimensions utilizing the software "Pore3D", resulting in a list of pore descriptions for each volume-of-interest. To focus the analysis to smaller bronchi rather than larger airways or parenchymal alveoli, only pores with a size between 0.2 and 0.75mm were used to assess bronchial dilation. To minimize the influence of differences in the size and weight of the analyzed mice we normalized the values with the 3rd root of the weight.

Using SR-pCT we were able to analyze the entire mouse lung *in-situ* on the anatomical level of small conducting airways and found significantly larger normalized airway diameters in the *TAp73* KO compared to that found in the WT mice. Side-by-side comparison of a WT control (Figure 1a) and a *TAp73* KO mouse (Figure 1b) shows enlarged small airways in the KO, suggesting the appearance of bronchiectasis-like phenotype in those mice. Statistical evaluation of the cohorts revealed higher porosity of the *TAp73* KO lungs with reduced mucociliary clearance.

Our study demonstrates that SR-pCT is a sensitive, non-destructive *in-situ* technique for three-dimensional ultra-high-resolution detection of bronchiectasis in mouse models of immotile cilia and restricted mucociliary clearance. Additionally, we show that mice with defective mucociliary clearance do develop measurable airway enlargements at 10 months, corresponding to the mid-thirties in humans. Therefore, SR-pCT may be a useful tool for pre-clinical and translational evaluation of novel treatment strategies and may allow for small rodent research to broaden our understanding of the mechanisms involved in onset and development of airway remodeling and bronchiectasis.

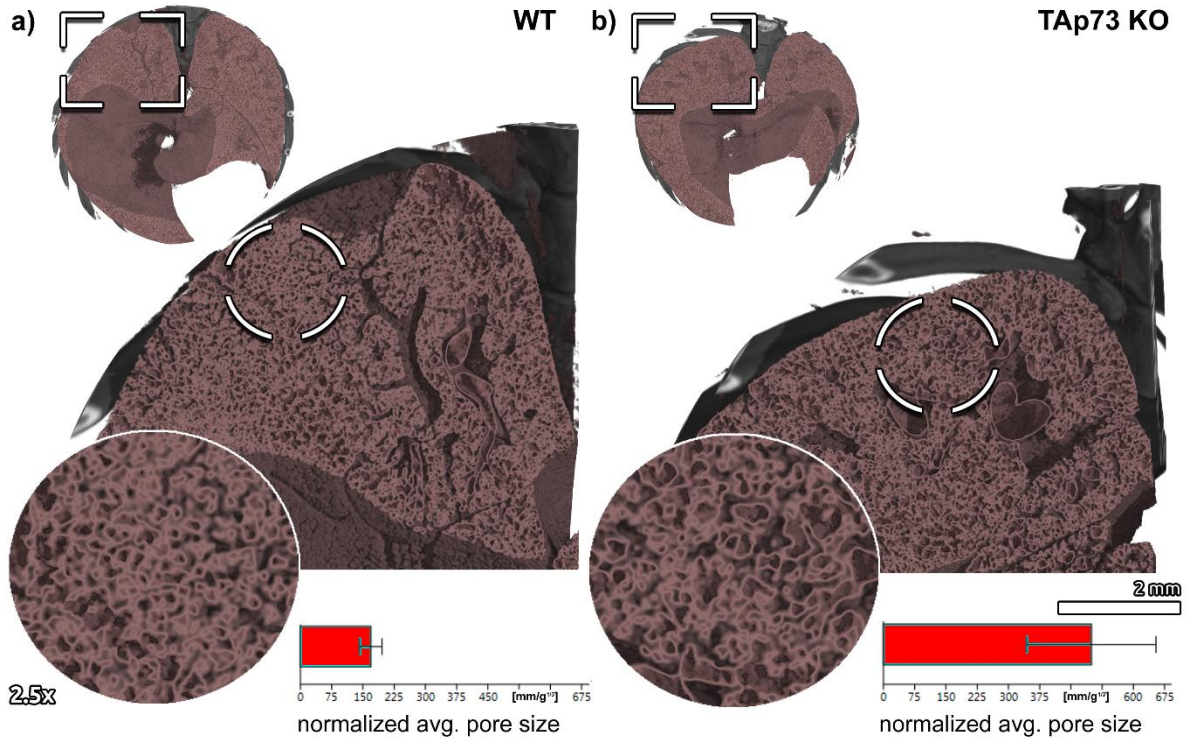

Figure 1 Legend

SR-pCT *in-situ* analysis (spatial resolution of ~9µm) of a WT control (Figure 1a) and a *TAp73* KO mouse with mucociliary clearance defects (Figure 1b). Cross-sections of the entire lung are displayed with two levels of magnification of the left lower lobe. ($p<0.05$). The depicted bars present the results for *TAp73* KO (N=4) 499±310mm/g$^{1/3}$ and WT (N=6) 169±66mm/g$^{1/3}$ (mean STD) ($p<0.032$).

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