Phenotype characterisation of two CF rat models: an update

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Introduction

- Cystic fibrosis (CF) rats have previously been shown to recapitulate important features of human CF disease (Tuggle et al. 2014, Birket et al. 2018).
- Over the last two years we have developed and bred CF rats with Phe508del and CFTR knockout (KO) genotypes, primarily for the pre-clinical development of airway-directed gene therapies.
- The aim of this study was to characterise the phenotypes of both CF rat models.

Methods

CFTR mRNA quantification

- RNA was isolated from lung samples using the Qiagen RNeasy® PowerLyzer® Tissue & Cells Kit. cDNA was made using the Qiagen QuantiTect reverse transcription kit and CFTR gene expression was quantified using RT-PCR and a SYBR™ green mastermix.

Nasal potential difference (PD) measurements

- Rats were anaesthetised with domitor:ketamine and non-surgically intubated with a 16G cannula to permit normal breathing during nasal perfusions of normal and low chloride Krebs-Ringer buffer solutions (50 µl/min). Rats were suspended by their incisors and PD measurements were recorded. Once a plateau of 1-2 minutes was obtained, solutions were then changed.

Pulmonary function testing (PFT)

- Anaesthetised rats were tracheostomised and a 14G cannula was inserted. Each rat was connected to a small animal ventilator (flexiVent, Scireq) with the following parameters used: tidal volume of 10 ml/kg, I:E ratio of 67%, a respiratory rate of 80 breaths/min, PEEP of 3 cmH_2O, and a maximum pressure of 30 cmH_2O. A script was used to perform a 6 second broadband perturbation (Quick Prime-6) to fit the Constant Phase Model and separate the airway and tissue mechanics.

Histology

- Lungs were inflation fixed with 10% neutral buffered formalin, while all other tissues were immersion fixed. Tissues were paraffin embedded, sectioned and stained with hematoxylin and eosin (H&E) for evaluation by a veterinary pathologist.

Results

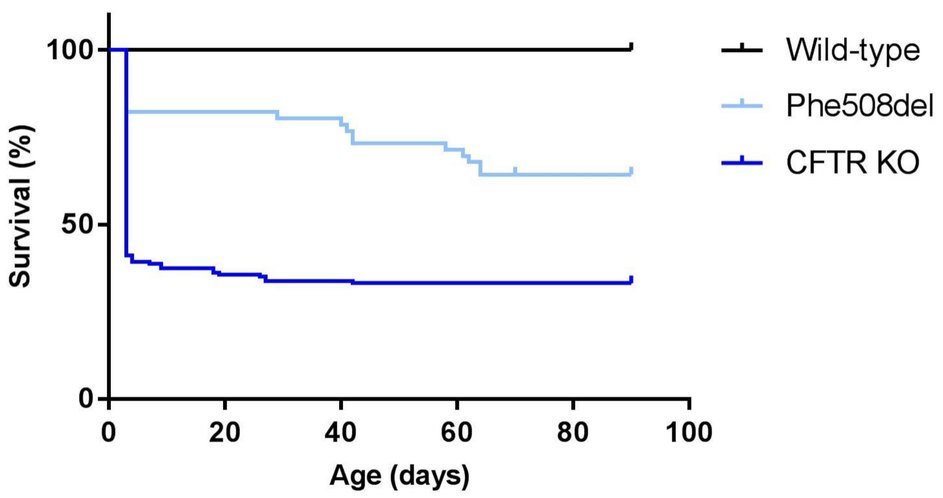

Figure 1: Survival curve for Phe508del and CFTR KO rats from postnatal day 1 - 90. Phe508del rats demonstrate higher survival compared to CFTR KO rats. Early death is attributed to gastrointestinal (GI) complications and failure to thrive. Statistically significant differences were observed between all groups using a Log-rank (Mantel-Cox) test: Phe508del vs WT p<0.001, KO vs WT p<0.0001, Phe508del vs KO p<0.0001, n = 28-56.

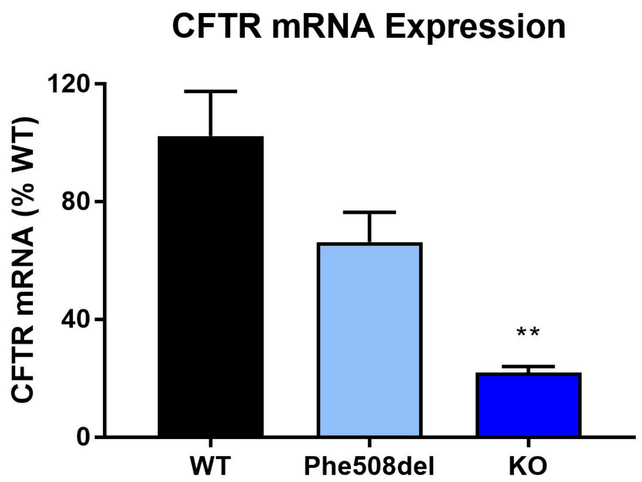

Figure 2: CFTR mRNA expression levels in Phe508del and CFTR KO rat lungs relative to wild-type. KO rats had significantly lower CFTR mRNA expression compared to WT (mean with SEM, **p<0.01, ANOVA, KO vs WT, n = 3 per group).

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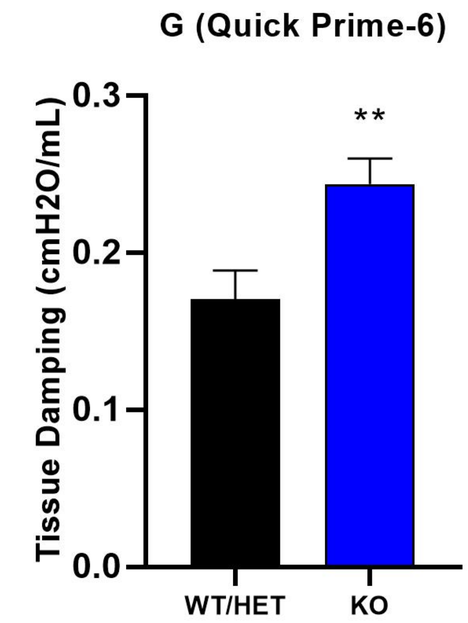

Figure 3: Pulmonary function testing. There was a statistically significant difference in the tissue damping (measured by Quick Prime-6 perturbation), between WT/heterozygous (HET) and CFTR KO animals (mean with SEM, **p<0.01, t-test, n =9-14). PFT has not yet been performed on Phe508del rats.

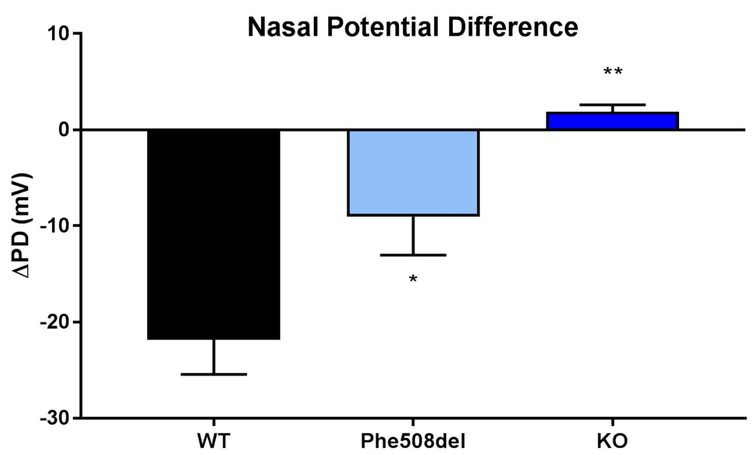

Figure 4: Nasal potential difference measurements. The change in nasal PD (ΔPD) demonstrates a classic CF bioelectrical phenotype for KO rats, with a milder phenotype for Phe508del rats when compared to wild-type (mean with SEM, *p<0.05, **p<0.01, ANOVA vs WT, n = 4-6).

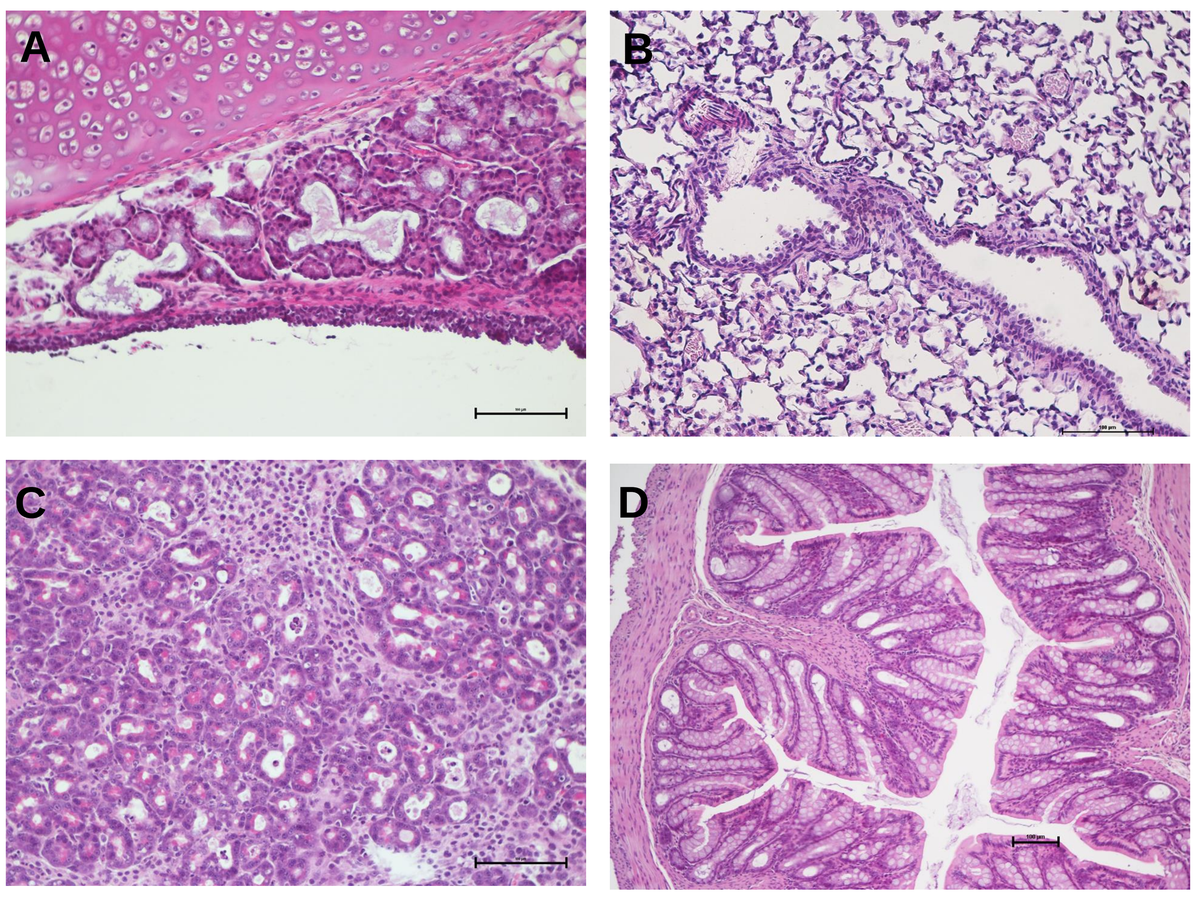

Figure 5: H&E stained sections from 1 month old Phe508del and CFTR KO rats. **(A)** Tracheal epithelium from Phe508del rat shows normal histology. **(B)** Lungs from a KO rat show clear airways. **(C)** Pancreas from KO rat exhibits multifocal exocrine degeneration. **(D)** Distal colon from KO rat shows mucus extrusion into the lumen. Scale bar = 100 µm.

Conclusions

- CF rats demonstrate reduced survival due to GI complications.
- CFTR mRNA expression in the lung is lowered compared to wild-type in both Phe508del and KO rats.
- Nasal PD and small airway function (tissue damping) show a more severe CF-like phenotype in KO rats.
- Pancreas and colon in CF rats demonstrate abnormal histopathology.

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