Phenotype characterisation of Phe508del and CFTR knockout cystic fibrosis rats

Alexandra McCarron[1-3], Patricia Cmielewski[1-3], Nicole Reyne[1-3], John Finnie[2,4], Nathan Rout-Pitt[1-3], Chantelle McIntyre[2,4], Fiona Craig[2,4], David Parsons[1-3], Martin Donnelley[1-3]

1. Robinson Research Institute, University of Adelaide, South Australia
2. Adelaide Medical School, University of Adelaide, South Australia
3. Department of Respiratory & Sleep Medicine, Women's & Children's Hospital, South Australia
4. SA Pathology, Adelaide, South Australia

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.
- CF rats were primarily generated for the pre-clinical development of airway-directed gene therapies.
- The aim of this study was to characterise the phenotypes of both Phe508del and KO rat models.

Methods

CFTR mRNA quantification

- RNA was isolated from lung samples using the Qiagen RNeasy® PowerLyzer® Tissue & Cells Kit. cDNA was synthesised using the Qiagen QuantiTect reverse transcription kit. CFTR mRNA levels were quantified by qPCR using SYBR® green. CFTR expression was normalised to Cyclophilin A mRNA expression.

Nasal potential difference (PD) measurements

- Rats were anaesthetised with domitor:ketamine and non-surgically intubated with a 16 G cannula to permit normal breathing during nasal perfusions of solutions (10-25 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. NPD tracings were interpreted by an experienced assessor blinded to the animal genotype.

Histology

- Tissues were fixed in 10% neutral buffered formalin, paraffin embedded, sectioned and stained with hematoxylin and eosin (H&E) or alcian blue periodic acid–Schiff (AB-PAS) for evaluation by a veterinary pathologist.

Results

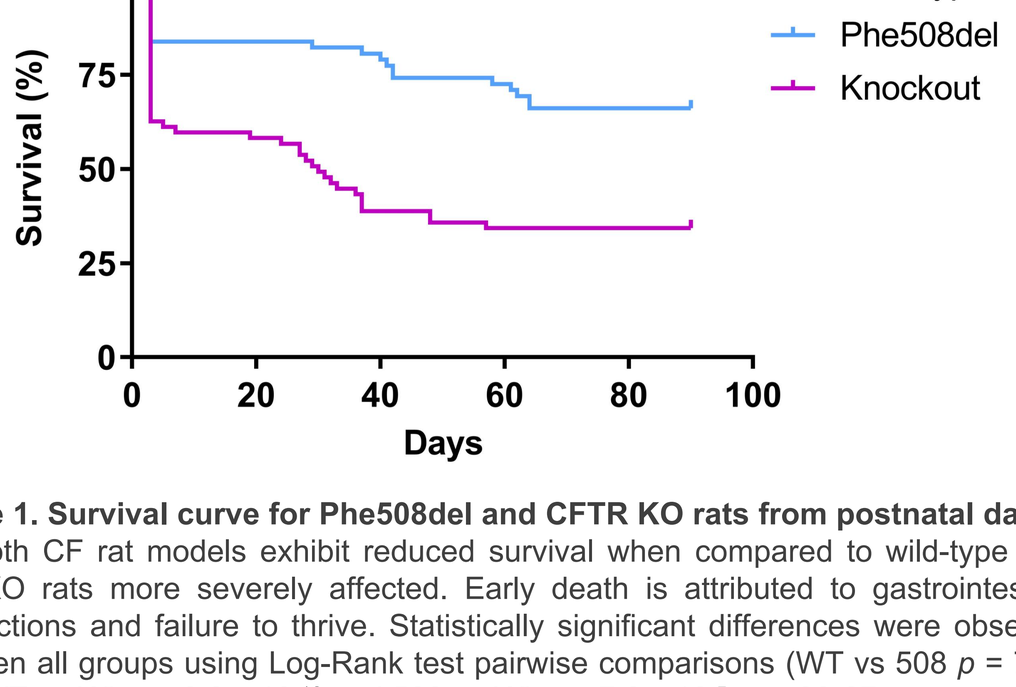

Figure 1. Survival curve for Phe508del and CFTR KO rats from postnatal day 1 - 90. Both CF rat models exhibit reduced survival when compared to wild-type rats, with KO rats more severely affected. Early death is attributed to gastrointestinal obstructions and failure to thrive. Statistically significant differences were observed between all groups using Log-Rank test pairwise comparisons (WT vs 508 p = 7.6 x 10^{-5}, WT vs KO p = 3.3 x 10^{-10} and 508 vs KO p = 7.6 x 10^{-5}, n = 38-67 per group).

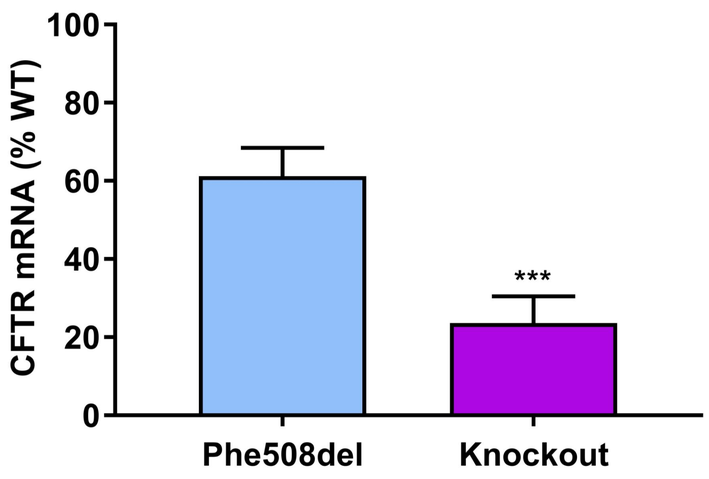

Figure 2. CFTR mRNA expression levels in Phe508del and CFTR KO rat lungs relative to wild-type. Phe508del rats had only a small reduction in mRNA levels while KO rats had significantly lower CFTR mRNA expression when compared to wild-type rats (mean with SEM, ***p<0.001, one-way ANOVA vs WT, n = 7-9 per group).

Acknowledgments: The CF rat colony was set up with funding provided by CF South Australia. Project funding was provided by the Fay Fuller Foundation, NHMRC GNT1098127 and Cure4CF Foundation. AM was supported by a MS McLeod PhD Scholarship and MD by a Robinson Research Institute Career Development Fellowship. Thanks to Emma Knight for providing statistical support. Thanks also to the University of Adelaide Laboratory Animal Services (LAS) and Histology Services for providing technical assistance.

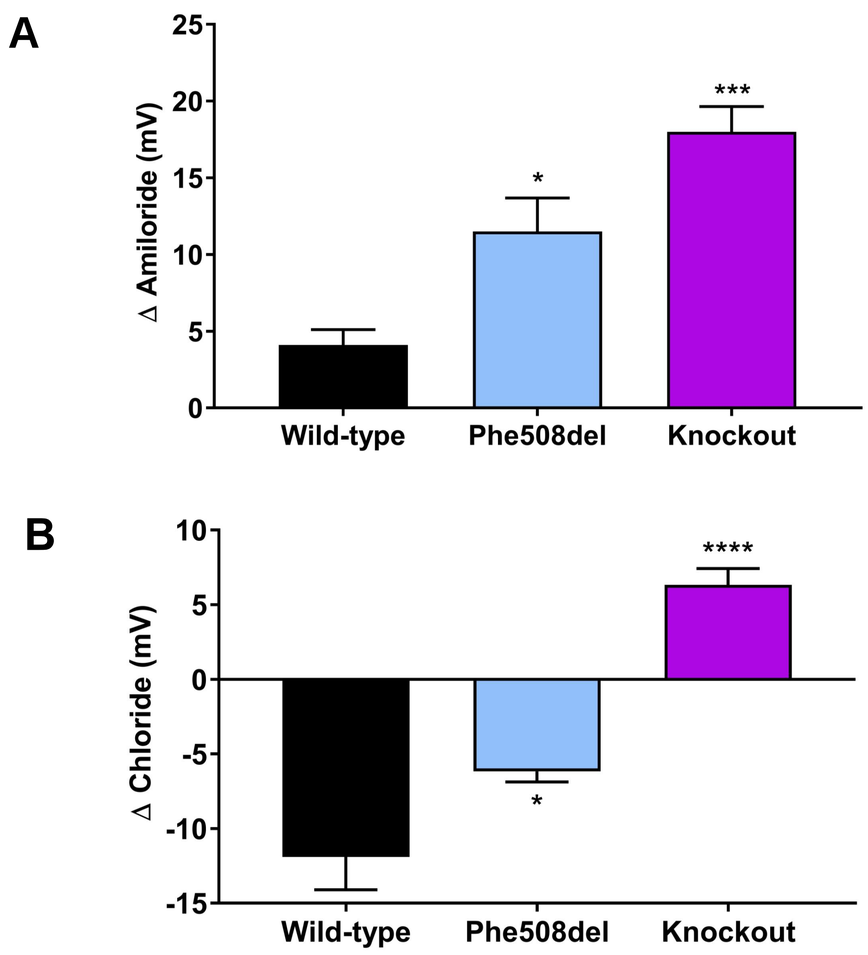

Figure 3. Nasal potential difference measurements in Phe508del and CFTR KO rats. (A) ΔPD response to Krebs-Ringer buffer and amiloride. **(B)** ΔPD response to low chloride Krebs-Ringer buffer and amiloride. When compared to wild-type controls, KO rats demonstrate a classic CF bioelectrical profile while Phe508del rats exhibit a milder phenotype (mean with SEM, *p<0.05, ***p<0.001, ****p<0.0001, ANOVA vs WT, n = 3-6 per group).

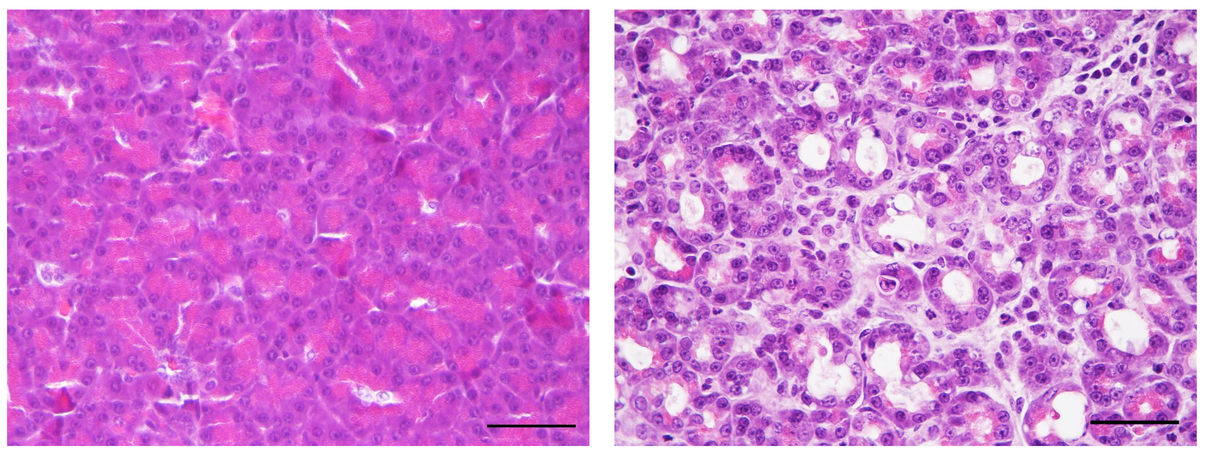

Figure 4. H&E stained pancreatic sections from 1 month old Phe508del (left) and CFTR KO (right) rats. A proportion (~25%) of KO rats demonstrate multi-focal degeneration of the exocrine pancreas, while Phe508del rats exhibit normal pancreas histology. Scale bar = 50 µm (n = 8 per group).

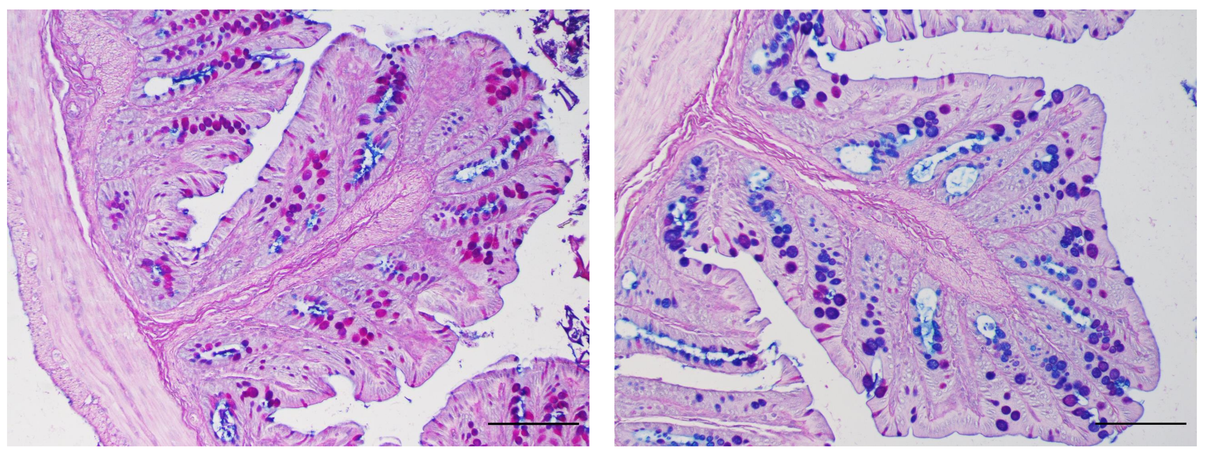

Figure 5. AB-PAS stained large intestine sections from 1 month old Phe508del (left) and CFTR KO (right) rats. Phe508del and KO rats exhibit distended intestinal crypts that are often dilated with mucins. This intestinal pathology is more pronounced in KO rats. Scale bar = 100 µm (n = 8 per group).

Conclusions

- CF rats demonstrate reduced survival due to gastrointestinal obstructions.
- Lung CFTR mRNA expression is significantly reduced in KO rats when compared to wild-type rats.
- Nasal PD shows a severe CF-like bioelectric phenotype in KO rats and a mild phenotype in Phe508del rats.
- Pancreas and intestine tissues demonstrate abnormal histopathology in both Phe508del and KO rats.