Phenotype characterisation of *Phe508del* and *CFTR* knockout cystic fibrosis rats

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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.
- 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 *Phe508del* and KO rat models.

METHODS

Nasal potential difference (PD) measurements

- Rats were anaesthetised with domitor:ketamine and non-surgically intubated with a 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 changed. NPD tracings were interpreted by an experienced assessor blinded to the animal genotype.

RNAscope® in situ hybridisation

- *In situ* RNA detection was performed using the RNAscope® 2.5 Red HD Detection kit (Advanced Cell Diagnostics). The integrated probe design with signal amplification and detection were used to achieve single-molecule detection of *CFTR* mRNA (labeled magenta).

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

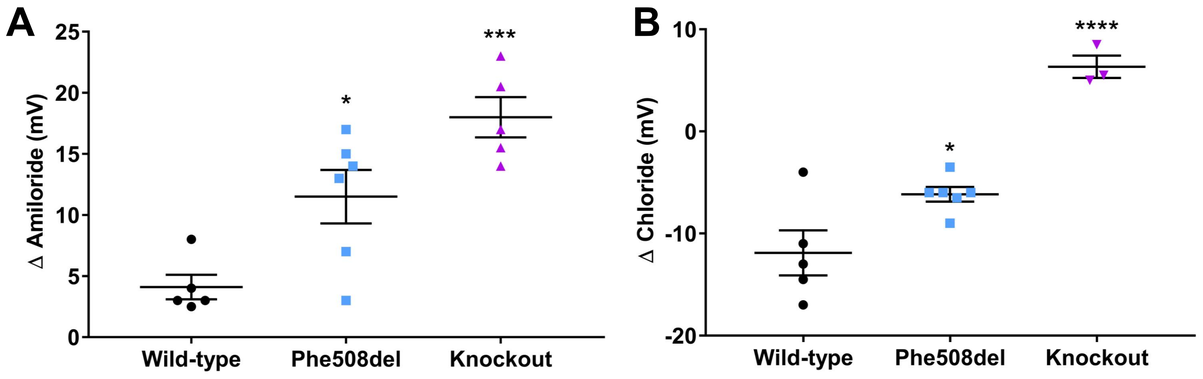

Figure 1. Nasal potential difference measurements in *Phe508del* and KO rats. (A) ΔPD response to Krebs-Ringer buffer and amiloride. **(B)** ΔPD response to low chloride Krebs-Ringer buffer and amiloride. When compared to wild-type controls, KO rats demonstrate a classic CF bioelectrical profile while *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).

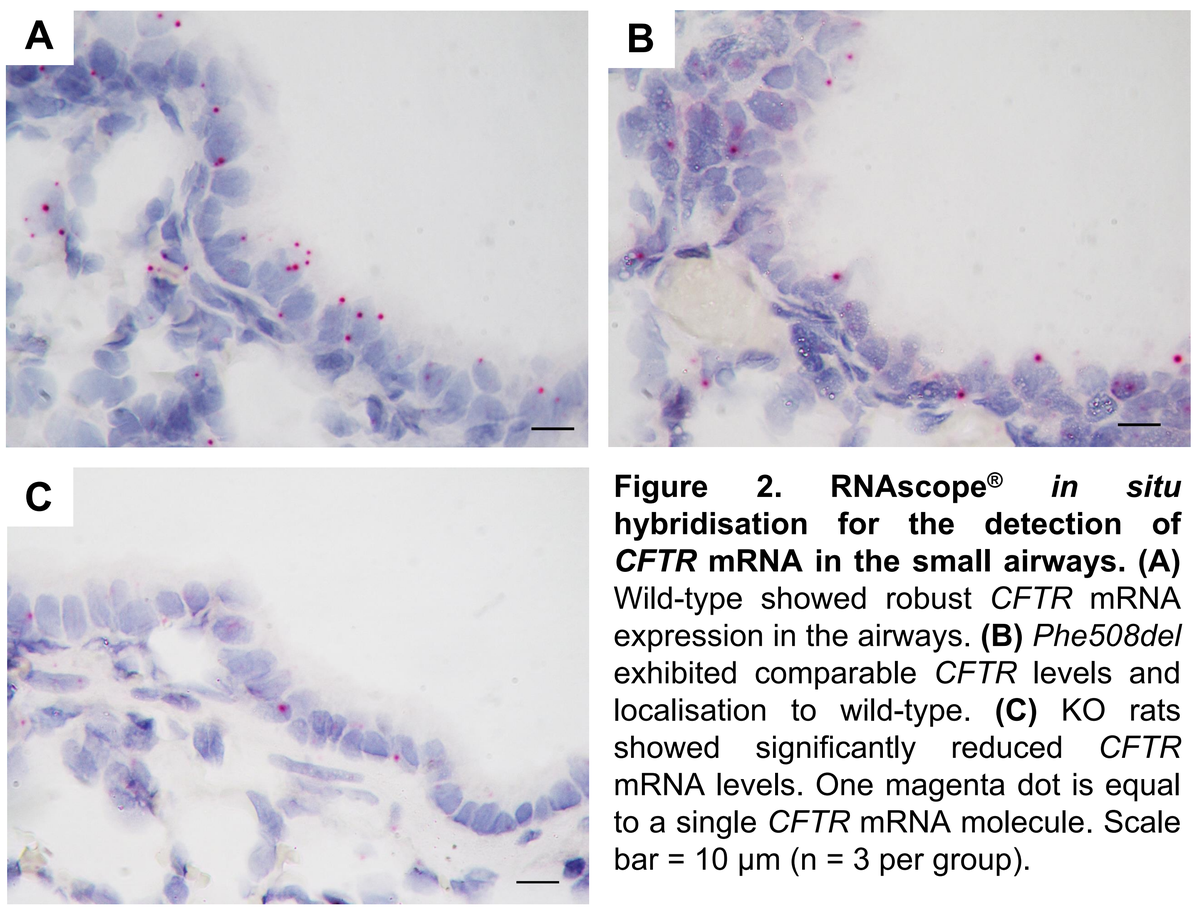

Figure 2. RNAscope® *in situ* hybridisation for the detection of *CFTR* mRNA in the small airways. (A) Wild-type showed robust *CFTR* mRNA expression in the airways. **(B)** *Phe508del* exhibited comparable *CFTR* levels and localisation to wild-type. **(C)** KO rats showed significantly reduced *CFTR* mRNA levels. One magenta dot is equal to a single *CFTR* mRNA molecule. Scale bar = 10 µm (n = 3 per group).

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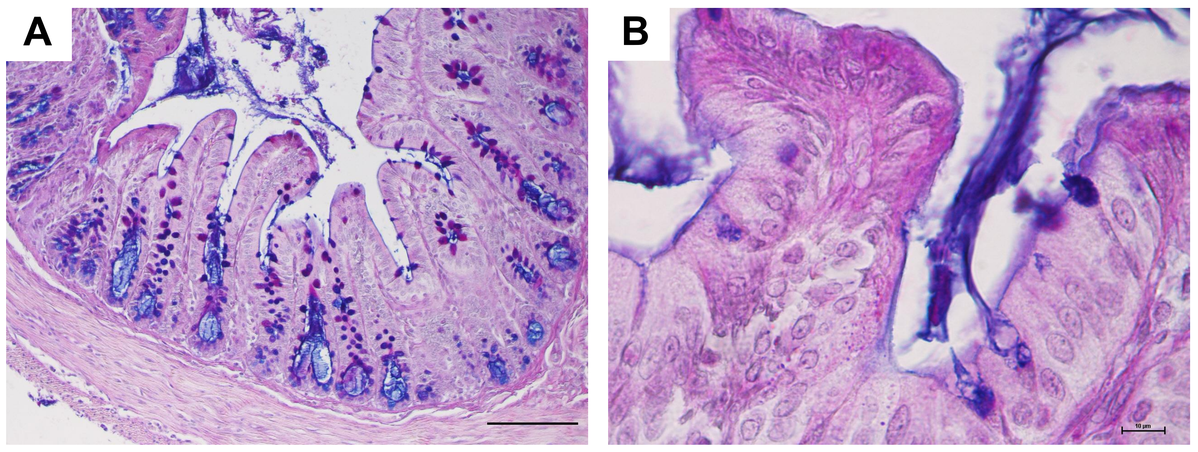

Figure 3. AB-PAS stained large intestine sections from CF rats. *Phe508del* and KO rats exhibited distended colonic crypts due to excess extruded mucus. This intestinal pathology was more pronounced in KO rats. Scale bar A = 100 µm, B = 10 µm (n = 8 per group).

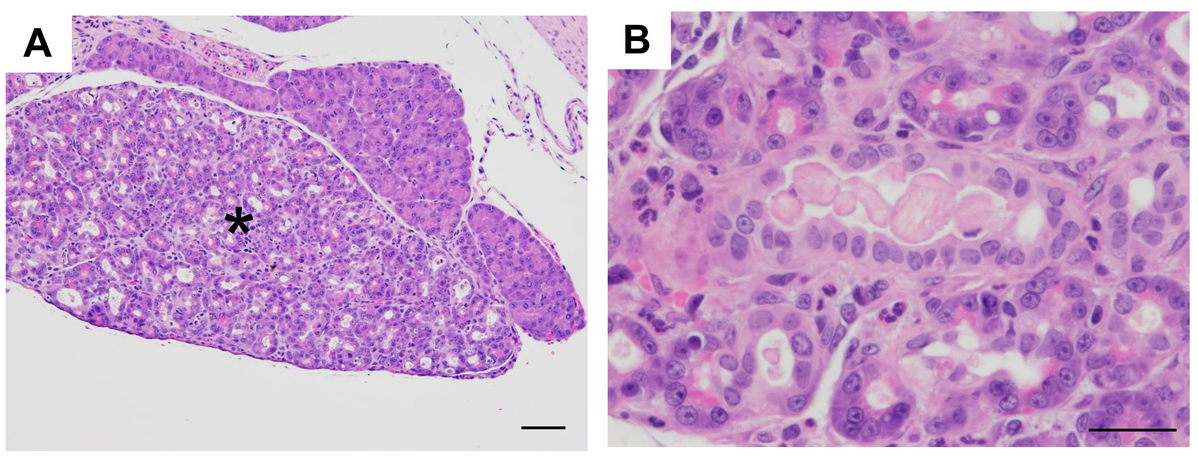

Figure 4. H&E stained pancreatic sections from KO rats. A proportion (~25%) of KO rats demonstrated multi-focal degeneration of the exocrine pancreas, while *Phe508del* rats exhibited normal pancreas histology. **(A)** Pancreas from KO rat with focal degeneration of the exocrine pancreas (*) adjacent to normally developed pancreatic tissue. **(B)** Pancreatic duct filled with eosinophilic secretions. Scale bar = 50 µm (n = 8 per group).

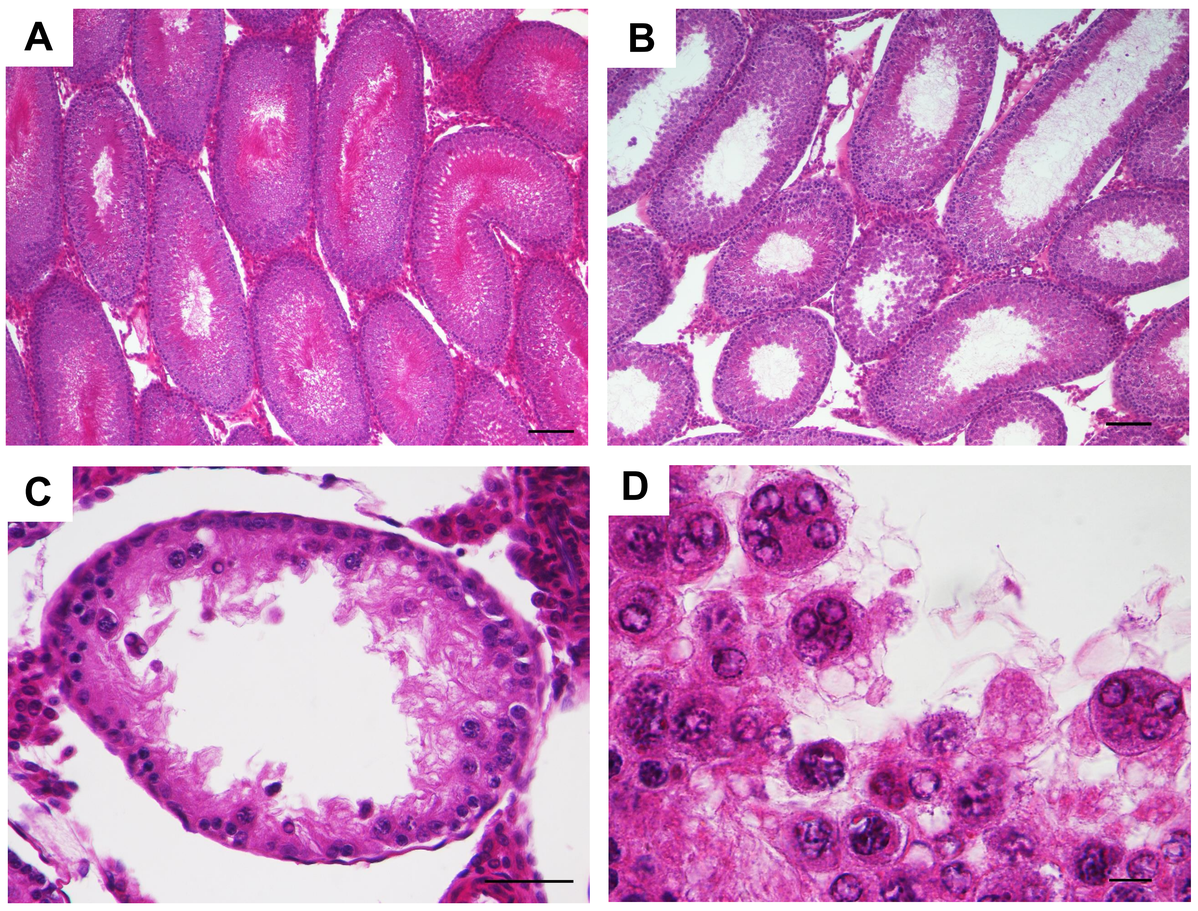

Figure 5. H&E stained testis sections from CF rats. (A) Normally developed testis tissue in wild-type rat. *Phe508del* and KO rats demonstrated **(B)** testicular degeneration and reduced sperm abundance, **(C)** diminished germinal epithelium in the seminiferous tubules, **(D)** multinucleated giant cells in the lumina of some tubules. Scale bar A, B = 100 µm, C = 50 µm, D = 10 µm (n = 3 per group).

CONCLUSIONS

- Nasal PD measurements showed a severe CF bioelectric profile in KO rats and a milder phenotype in *Phe508del* rats.
- RNAscope® revealed robust *CFTR* mRNA expression in the bronchi and small airways of wild-type animals, with *Phe508del* rats showing similar levels and localisation. Comparatively, KO rats had a significant reduction in mRNA.
- Both rat models demonstrated colonic crypt dilation with KO rats more severely affected.
- KO rats exhibited multifocal degeneration of the exocrine pancreas.
- *Phe508del* and KO males demonstrated testicular degeneration.