Phenotype characterisation of two CF rat models

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Introduction

- Cystic fibrosis (CF) rats are promising models for therapeutics development as they 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 two genotypes.
- A **Phe508del** CF rat model (class II) was generated using CRISPR-Cas9 gene editing, with a guide template containing a TTT deletion at amino acid position 508. We predict a CFTR protein folding impairment and increased CFTR protein degradation matching the human phenotype.
- An alternative genotype was also created that contains a 5 bp DNA insertion and an accompanying 7 bp deletion (1515_1521delinsAACAT KO rat). This 512X nonsense mutation (class I) predicts an impairment of CFTR protein synthesis, with characteristics expected to be similar to a ***CFTR* knockout** (**KO**).
- The aim of this study was to characterise the phenotypes of both CF rat models.

Methods

Animal husbandry

- KO rat production was maintained through breeding heterozygous pairs, while Phe508del animals were maintained using homozygous Phe508del females and heterozygous Phe508del/wild-type (WT) males.
- All CF animals receive a high-fat diet and ColonLytely in drinking water to reduce gastrointestinal complications.

Nasal potential difference (PD) measurements

- All rats were anaesthetised with an intraperitoneal injection of domitor:ketamine mixture 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 nasal PD measurements were recorded in mV and once a plateau of 1-2 mins was obtained, solutions were then changed.

Histology

- Tissues were harvested, immersion fixed in 10% neutral buffered formalin, and then embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) for evaluation by a veterinary pathologist.

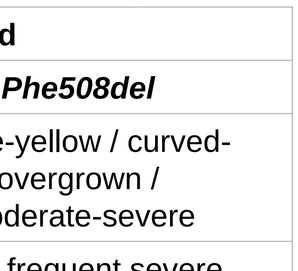
Results

Table 1: Summary of phenotypes observed up to 6 months of age.

Organ	Phenotype observed	
	Knockout	***Phe508del***
Dentition	White / curved-overgrown / severe	White-yellow / curved-overgrown / moderate-severe
Gastrointestinal tract	Frequent severe obstruction / increased gut motility /	Less frequent severe obstruction / increased gut motility
Male reproductive tract	Poorly developed vas deferens, seminal vesicles & epididymis	Poorly developed vas deferens, seminal vesicles & epididymis
Female reproduction	Fertile with small litters	Fertile with normal size litters
Nose	CF-like ΔPD	Mild CF-like ΔPD
Trachea and lungs	No significant findings	No significant findings
Liver and pancreas	Normal	Normal

Table 2: Mortality rates observed in the two CF rat genotypes. KO rats demonstrate higher mortality rates compared to Phe508del. *Image:* Knockout rat pups *(left)* are significantly smaller than aged-matched littermates *(right)*.

	Mortality pre-weaning	Morality post-weaning
Knockout	68.4%	21.4%
Phe508del	10%	13.9%



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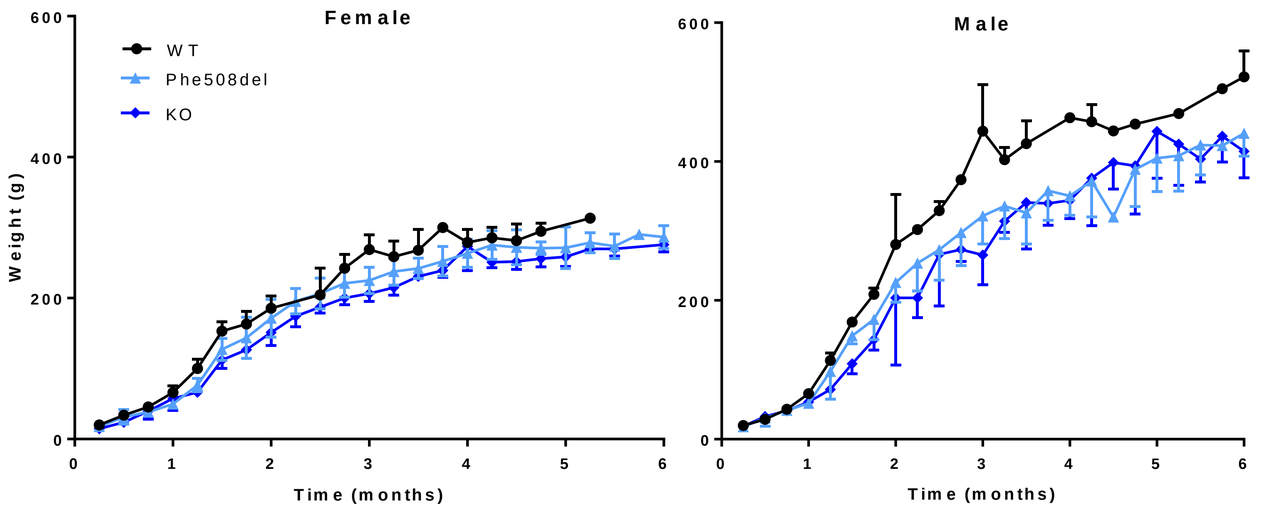

Figure 1: Body weight values (mean ± SD) from WT, Phe508del and KO rats from postnatal day 1 - 6 months of age. KO and Phe508del rats have a lower average body weight compared to wild-type, though this effect appears to be more pronounced in male animals.

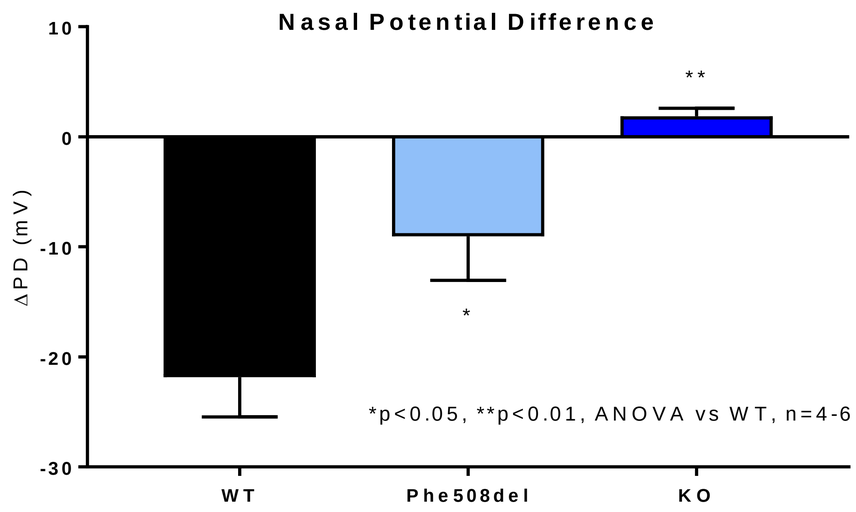

Figure 2: Nasal potential difference measurements in 2 - 9 month-old rats. The change in nasal PD (ΔPD) demonstrates a classic CF phenotype for KO rats, with a milder phenotype for Phe508del when compared to wild-type rats.

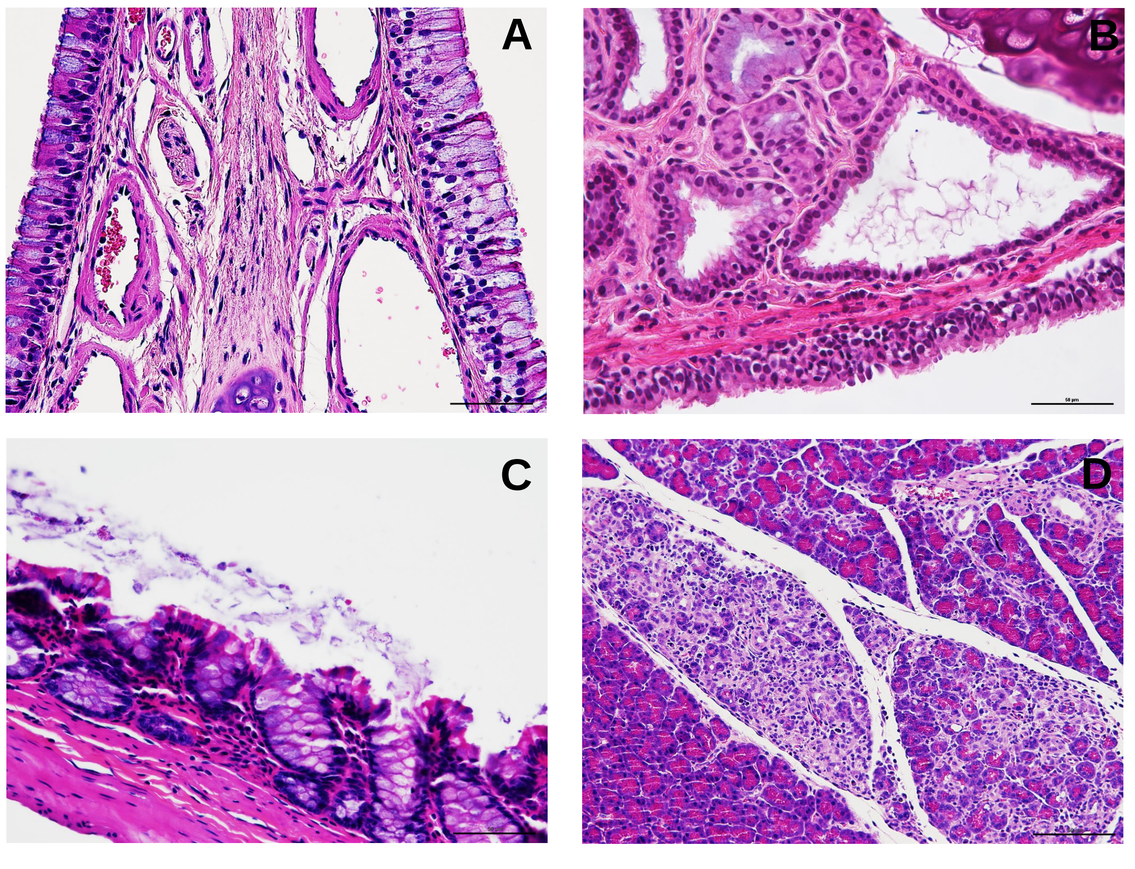

Figure 3: H&E stained sections from 6 month old Phe508del and KO rats demonstrates no significant CF-related histopathology to date in some organs. (A) Nasal epithelium from Phe508del rat. **(B)** Tracheal epithelium from KO rat shows presence of naturally occurring protein-like material in the submucosal glands. **(C)** Colon from Phe508del rat exhibits mucus extrusion from the crypts. **(D)** Pancreas from Phe508del rat shows multi-focal exocrine pancreatic degeneration. Scale bar = 50 µm, 400x magnification.

Conclusions

- Characterisation to date indicates Phe508del and KO rats exhibit mild features of the human CF phenotype in some organs.
- CF disease may be more severe in those animals that die early, so further analyses and quantitative data are required to gain a more comprehensive understanding.
- In the future we will investigate whether CF rats are more susceptible to bacteria-induced lung disease development.

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