Generation of new cystic fibrosis rat models in Australia using CRISPR/Cas9 genome editing

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Background: Cystic fibrosis (CF) mice do not develop spontaneous CF-like lung disease, and so they are unsuited to understanding CF lung pathogenesis and testing therapies. The first CF rats produced in the USA (Birket 2015, Tuggle 2014) have shown an excessive mucus production in the lung, abnormal submucosal gland formation and impaired bacterial clearance

Aim: To establish and characterise a CF rat model on a Sprague Dawley background for use in Australia in studies of CF airway gene correction.

Methods: Founder animals were generated through the CRISPR services of the Australian Phenomics Network (APN) at Monash University (www.australianphenomics.org.au). A homology-directed-repair (HDR) template was used to delete the codon aligning to 508 in the human CFTR gene sequence (Phe508del, the most common human CF-causing mutation).

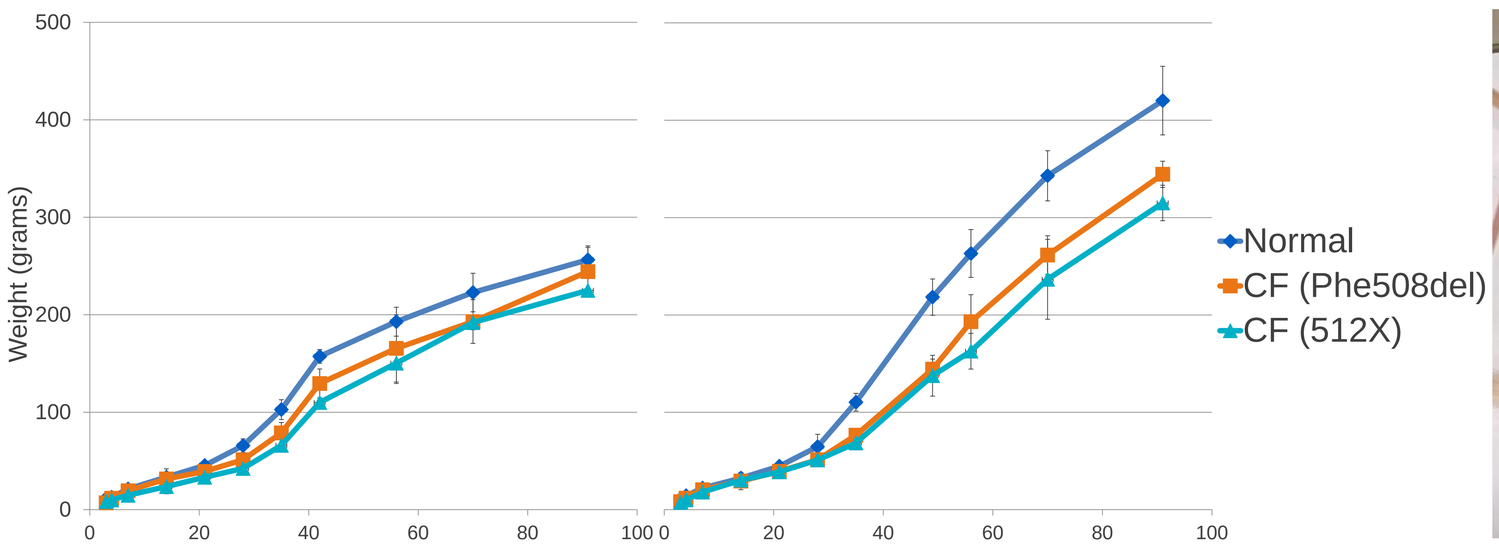
Two animals harbouring the targeted mutation and one animal harbouring an off-target mutation (causing termination early in the coding sequence at codon 512; 512X) were used as colony founders.

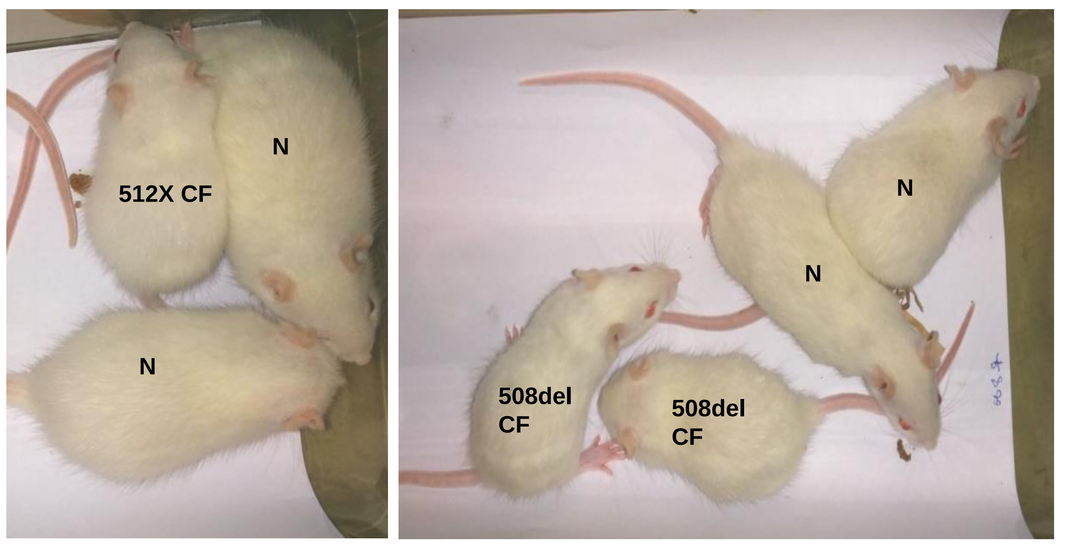
CF rat production is maintained through the breeding of mutation-matched CF-heterozygous pairs an currently all animals receive ColonLytely-water to prevent gastrointestinal obstruction .

Results: The Adelaide CF rat colony has generated more than 70 CF individuals to date and characterisation is underway. Light microscopic observations of lung and trachea from a limited number of CF rats has not revealed clear respiratory differences compared with normal rats at 8 weeks of age (data not shown). However, differences in weight, mortality, tooth development and gut histology are observed by 8 weeks of age and earlier.

CF rats, early observations

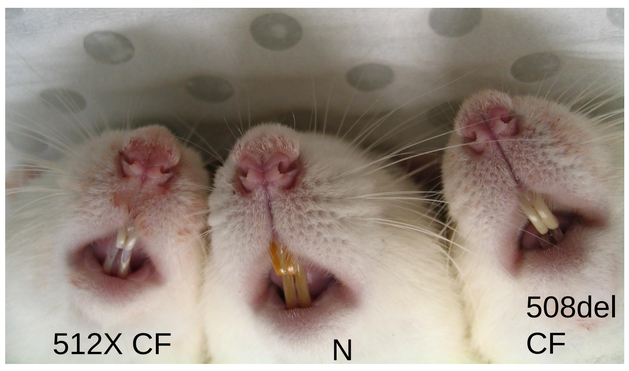
•CF rats are smaller than normal rats





Four week old normal (N), 512X CF and Phe508del CF rats

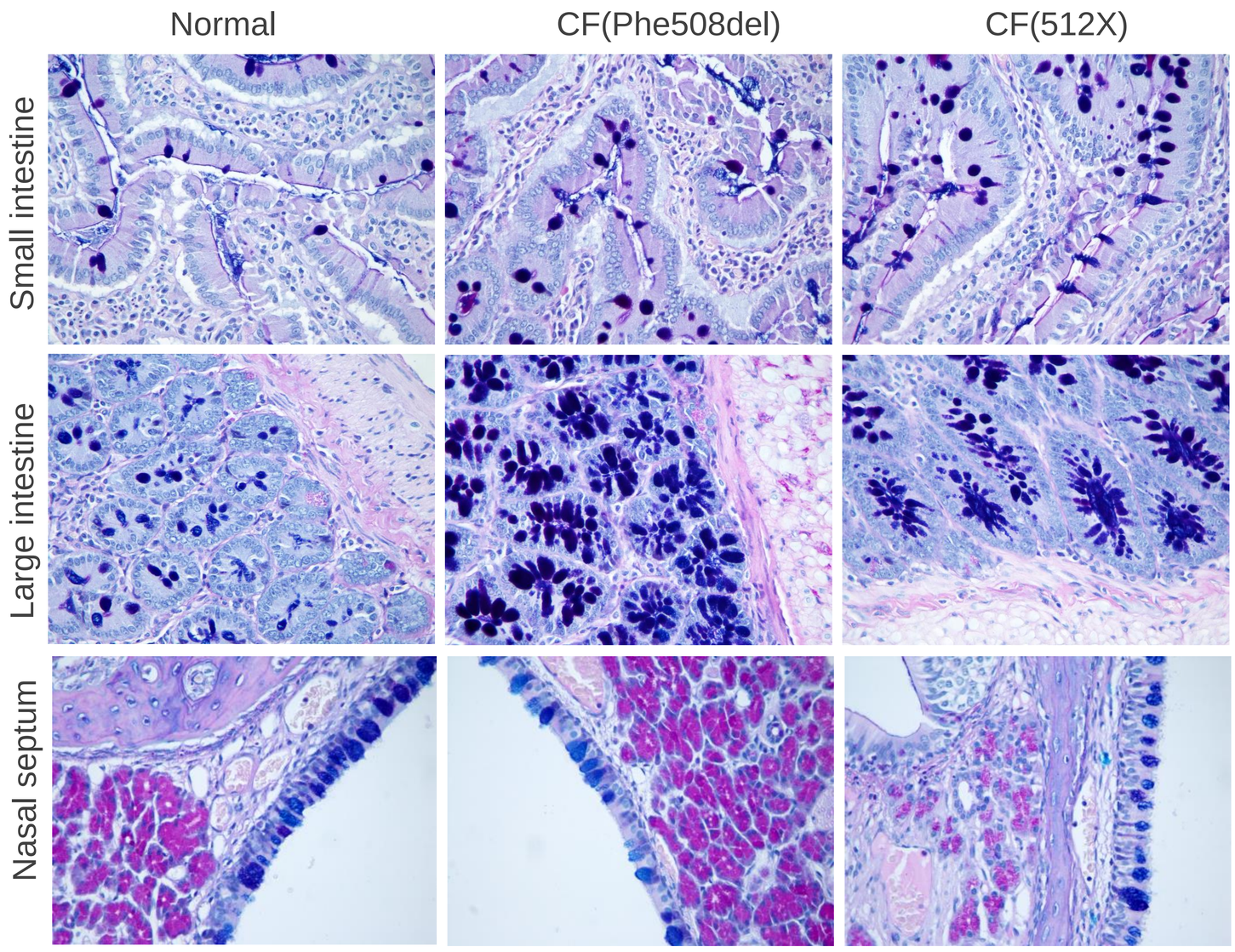
•CF teeth abnormalities are visible by 8 weeks of age



•Phe508del and 512X CF rats are genetically different and early data suggest phenotypically different too

	Phe508del	512X
CFTR mutation class	II (Defective protein processing)	VII (Frameshift leading to lack of CFTR synthesis; knockouts)
Number of births	34 (≤ 17 weeks)	37 (≤ 17 weeks)
Number of unexpected deaths	None known	At least 10 between 0-3 weeks of age
Weight (Ave % normal)	~80	~70

•AB-PAS staining shows increased amounts of mucin in the upper and lower intestines, but not in the airways, at 8 weeks of age



400x original magnification

Conclusion: Similar to the USA-developed CF rats, the Australian CF rat has not shown clear respiratory abnormalities by 8 weeks of age, although we anticipate changes to occur at later time points. Characterisation of the rats is ongoing and will utilise nasal and tracheal potential difference measurements, immunohistochemistry, and computed tomographic x-ray velocimetry, among other methods, for the assessment of CF abnormalities in animals at more advanced ages than those currently reported.

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