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

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Background: Cystic fibrosis (CF) mice do not develop spontaneous lung disease. They are therefore a limited model for understanding CF lung pathogenesis and testing therapies. CF rats, already available in the USA, have shown an excessive mucus production in the lung, abnormal submucosal gland formation and impaired bacterial clearance (Birket, 2015, Tuggle 2016)

Aim: To establish a CF rat colony in Adelaide using CRISPR/Cas9 genome editing for the purpose of testing lentiviral-mediated gene therapy for CF airway disease

Methods: CF rat colony founders were generated via injection of guide and Cas9 RNA into Sprague Dawley rat oocytes 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 incorporate a TTT deletion corresponding to codon 508 in order to produce the CF-causing mutation that is most common in the human population; Phe508del.

Founders and offspring were genotyped using Sanger sequencing.

Results: Transplant of 90 genome edited oocytes into surrogate mothers resulted in 30 live-born pups. Two targeted CFTR mutations and 6 off-target CFTR mutations were reported by the APN. Three offspring were selected as founders with genotypes outlined below. Each founder rat was transferred to Adelaide and paired with locally sourced normal Sprague Dawley rats, and the genotype of the first generation offspring was analysed. Identified heterozygous rats were then bred to produce second generation offspring, including CF animals.

FOUNDERS

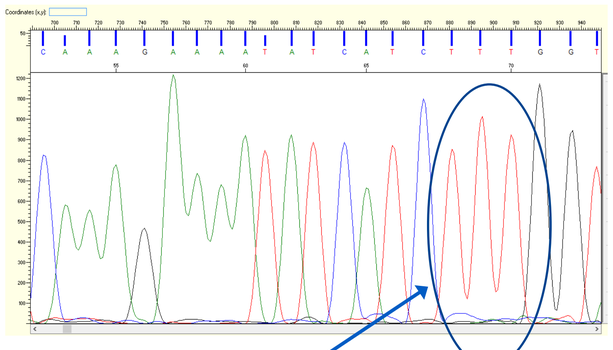
♂ **Targeted mutation: Phe508del/wild-type**

♂ **Off-target mutation: 510X/wild type**

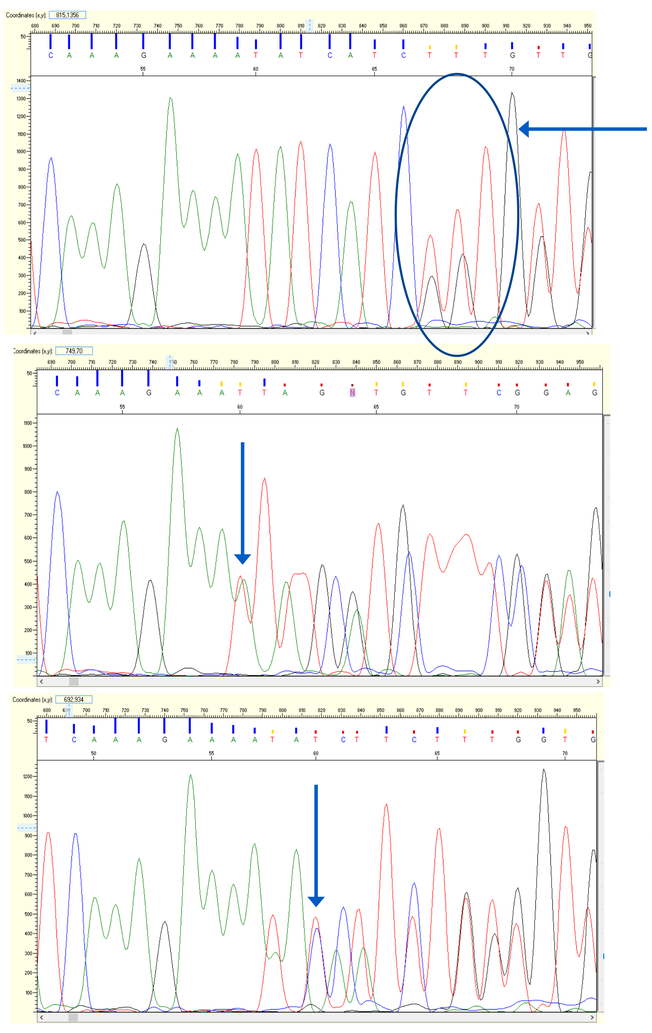
♀ **Targeted mutation/mosaic: Phedel508/wild-type/C-T silent mutation**

FIRST GENERATION OFFSPRING: CF heterozygote's

Genotype	Male	Female	Total
Normal (wt/wt) (A)	34	25	59
Phe508del/wt (B)	7	4	11
510X/wt (C)	2	2	4
512X/wt (D)	3	4	7



A) Wt/wt. TTT codon 508 present in both alleles



B) Phe508del/wt. Allelic sequence misalignment shows TTT codon deletion in one allele.
Predicted outcome: Translational loss of phenylalanine

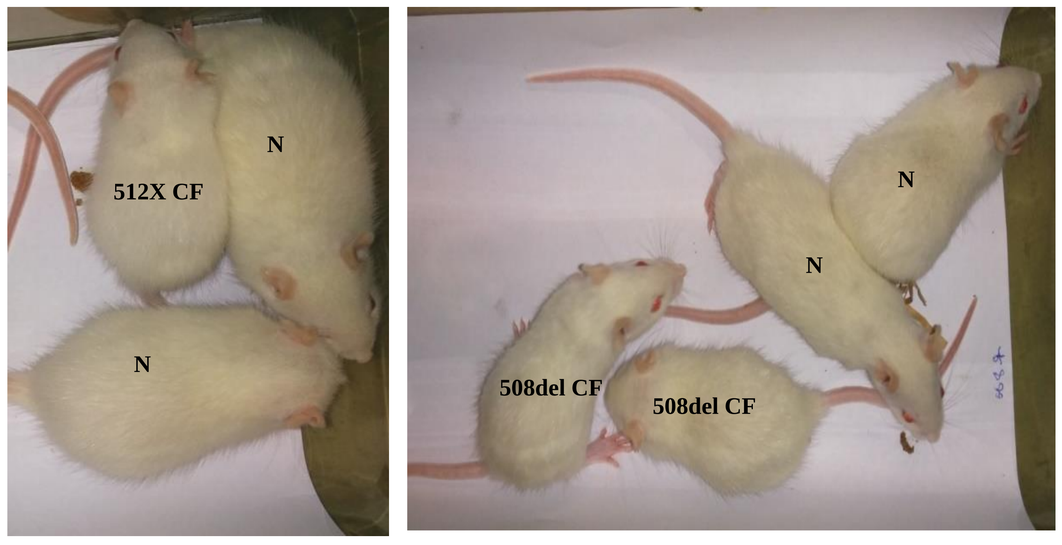
C) 510X/wt. Allelic sequence misalignment from codon 505 (arrow) occurs due to an 8 bp deletion in one allele.
Predicted outcome: Translational frameshift with introduction of a stop codon at 510

D) 512X/wt. Allelic sequence misalignment from codon 506 (arrow) due to 1 bp deletion (T) in one allele.
Predicted outcome: Translational frameshift with introduction of stop codon at 512

**** The 512X genotype appeared in the offspring of the female mosaic founder; she produced normal (wt/wt), Phe508del/wt and 512X/wt pups**

SECOND GENERATION OFFSPRING: CF homozygote's

	Phe508del	510X	512X
CFTR mutation class	II (defective protein processing)	I (lack of CFTR synthesis)	I (lack of CFTR synthesis)
n to date	6 (≥ 8 weeks)	2 (≥ 16 days)	9 (≥ 8 weeks)
Weight (% normal)	70-80	50	50-70
Activity level	Normal	Weak	Weak-normal
Mortality	Normal	High (100%)	Increased. 1 weaner death



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

Conclusion: CRISPR/Cas9 genome editing was used to create CF rat colony founders carrying type I (Phe508del) and type VII (510X and 512X) CFTR mutations. Breeding of CF heterozygous rats produced CF rats homozygous for each of these CFTR mutations, with different weights, activity levels and mortality evident for the each genotype. Complete characterisation of the CF rats is underway.