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Original Article

# Animal and model systems for studying cystic fibrosis☆,☆☆

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**Abstract**

The cystic fibrosis (CF) field is the beneficiary of five species of animal models that lack functional cystic fibrosis transmembrane conductance regulator (CFTR) channel. These models are rapidly informing mechanisms of disease pathogenesis and CFTR function regardless of how faithfully a given organ reproduces the human CF phenotype. New approaches of genetic engineering with RNA-guided nucleases are rapidly expanding both the potential types of models available and the approaches to correct the CFTR defect. The application of new CRISPR/Cas9 genome editing techniques are similarly increasing capabilities for in vitro modeling of CFTR functions in cell lines and primary cells using air-liquid interface cultures and organoids. Gene editing of CFTR mutations in somatic stem cells and induced pluripotent stem cells is also transforming gene therapy approaches for CF. This short review evaluates several areas that are key to building animal and cell systems capable of modeling CF disease and testing potential treatments.


*Keywords:* Animal models; Cellular systems; CF lung disease; Pancreatic disease; Gene editing

## 1. Introduction

Animal models of disease are indispensable for understanding disease pathogenesis and critical for developing new therapies. The cystic fibrosis (CF) field is unusually fortunate in this regard with multiple species that offer unique advantages (Fig. 1). While the CF mouse lacks a spontaneous lung and pancreatic phenotype, it has provided a wealth of insight into the biological underpinnings of CFTR-deficient epithelia in other organs such as the intestine, and lessons learned from CF mice have laid a rich foundation for other CF animal models [1,2]. The CF ferret and pig recapitulate the full spectrum of the CF phenotype observed in human patients, from inflammatory and infectious lung disease to in utero meconium ileus and CF-related diabetes (CFRD). However, the ferret and pig models are resource intensive and pose some challenges that limit broad utilization by the field. The CF rat acquires gut obstruction at weaning and while it remains unclear if the CF rat lung will become spontaneously infected, the tracheal surface has a reduced airway surface liquid layer (ASL) but normal mucociliary clearance at birth [3]. The rabbit is the newest addition to the Noah's Ark of CF models [4], however, other than gut

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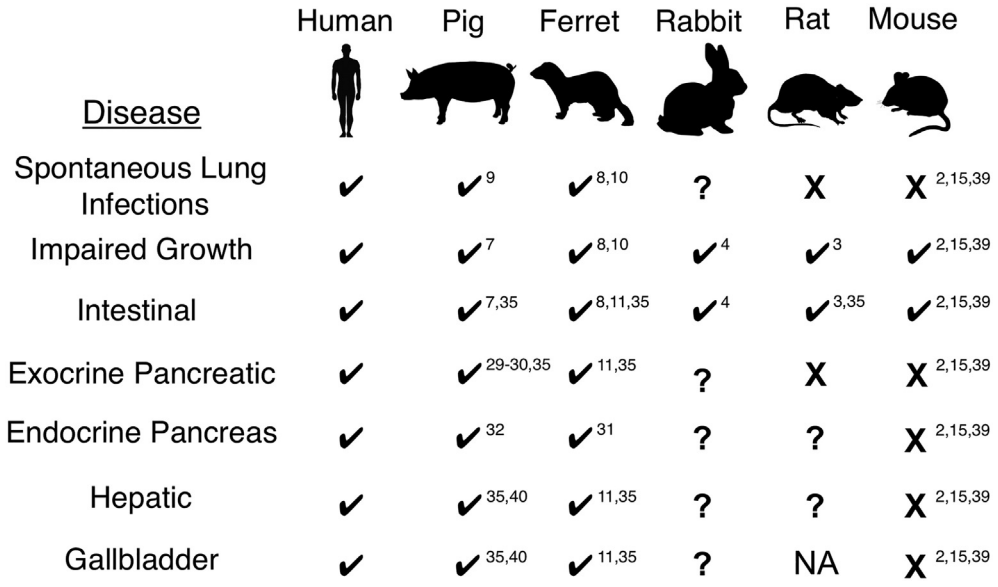


Fig. 1. Organ disease presence or absence in various CFTR-knockout (CF) animal models. Organs affected in humans with CF are listed on the left. Check marks below each CF model species indicates disease presence, while question marks denote disease has yet to be evaluated. Organs marked by an X indicate lack of overt disease. NA, not applicable as rats do not have a gallbladder. Superscript denotes references for the relevant studies in the literature.

obstruction at weaning, the phenotype remains unclear and the model is awaiting publication in a peer-reviewed forum. CRISPR/Cas9-targeting technologies used to disrupt the *CFTR* gene in rabbits also has significant potential for generating in vitro models of epithelia for the study of cellular processes impacted by CFTR. This brief review will discuss salient features of both in vitro and in vivo CF model systems.

## 2. Animal models of CF

The CFTR-knockout pig and ferret were first generated nearly 10 years ago [5,6]. These species were chosen because of their conserved lung cell biology with humans (Table 1). For example, ferrets and pigs contain submucosal glands (SMGs) throughout the cartilaginous airways, while in mice and rats SMGs are limited to the trachea and rabbits lack SMGs. Organs affected by the lack of CFTR in pigs and ferrets include the lung, pancreas, intestine, liver, gallbladder, and vas deferens [7–11]. We will focus this portion of the review on several of these organs and compare across the most described model species.

### 2.1. Lung disease

CFTR-knockout mice were engineered soon after the discovery of the gene [13,14] and while they have an intestinal phenotype, it is generally accepted that CF mice do not spontaneously develop lung infections like CF humans [2,15]. Nonetheless, certain strains of CF mice have an elevated proinflammatory response to *Pseudomonas*-laden beads agar bead instilled in the lung when compared to control animals [16]; however, this abnormal response is thought to stem from CFTR function within the hematopoietic compartment since bone marrow transplants between CF → WT and WT → CF can either produce or reverse the phenotype, respectively [17]. The lack of substantial and spontaneous lung disease in CF mice has prompted the creation of alternative mouse models that exhibited the classic muco-obstructive and inflammatory pathology observed in human CF lungs. Targeted overexpression of a subunit of the epithelial sodium channel (β-ENaC) in mouse airways produced a model strikingly similar to CF lung disease, including reduced mucociliary transport (MCT), airway obstruction with mucus, neutrophil dominant immune responses, increased susceptibility to bacterial infections, and shortened

Table 1
Cellular anatomy of the airways in CF animal models.

| Species | Submucosal glands (airway level) | Secretory cell type (proximal airways) | Secretory cell type (distal airways) | Respiratory bronchioles |
|---|---|---|---|---|
| Mouse | Proximal Trachea | Club | Club | Absent |
| Rat | Trachea | Serous | Club | Absent |
| Rabbit | Absent | Club | Club | Absent |
| Ferret | Trachea/bronchial | Goblet | Club | Several generations |
| Pig | Trachea/bronchial | Goblet | Club | Single generation |
| Human | Trachea/bronchial | Goblet | Club | Several generations |

The information in this table was largely extracted from the following book [12].

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