Published in final edited form as:
Curr Opin Pulm Med. 2011 November ; 17(6): 478–483. doi:10.1097/MCP.0b013e32834b14c9.

New animal models of cystic fibrosis: what are they teaching us?

Nicholas W. Keiser[a] and **John F. Engelhardt**[a,b,c]

[a]Department of Anatomy and Cell Biology, University of Iowa Carver College of Medicine, Iowa City, Iowa, USA

[b]Department of Internal Medicine, University of Iowa Carver College of Medicine, Iowa City, Iowa, USA

[c]Center for Gene Therapy of Cystic Fibrosis and Other Genetic Diseases, University of Iowa Carver College of Medicine, Iowa City, Iowa, USA

Abstract

Purpose of review—Cystic fibrosis is the first human genetic disease to benefit from the directed engineering of three different species of animal models (mice, pigs, and ferrets). Recent studies on the cystic fibrosis pig and ferret models are providing new information about the pathophysiology of cystic fibrosis in various organ systems. Additionally, new conditional cystic fibrosis transmembrane conductance regulator (CFTR) knockout mice are teaching unexpected lessons about CFTR function in surprising cellular locations. Comparisons between these animal models and the human condition are key to dissecting the complexities of disease pathophysiology in cystic fibrosis.

Recent findings—Cystic fibrosis pigs and ferrets have provided new models to study the spontaneous development of disease in the lung and pancreas, two organs that are largely spared overt spontaneous disease in cystic fibrosis mice. New cystic fibrosis mouse models are now interrogating CFTR functions involved in growth and inflammation at an organ-based level using conditional knockout technology. Together, these models are providing new insights on the human condition.

Summary—Basic and clinical cystic fibrosis research will benefit greatly from the comparative pathophysiology of cystic fibrosis mice, pigs, and ferrets. Both similarities and differences between these three cystic fibrosis models will inform pathophysiologically important mechanisms of CFTR function in humans and aid in the development of both organ-specific and general therapies for cystic fibrosis.

Keywords

cystic fibrosis; ferret; mouse; pathology; pathophysiology; pig

Correspondence to Professor John F. Engelhardt, PhD, Department of Anatomy and Cell Biology, University of Iowa, Room 1-111 Bowen Science Building, 51 Newton Road, Iowa City, IA 52242-1109, USA, Tel: +1 319 335 7753; fax: +1 319 335 6581; john-engelhardt@uiowa.edu.

Conflicts of interest

There are no conflicts of interest.

Introduction

The development of animal models for human genetic disorders is essential to study pathophysiology and develop therapies. In the case of cystic fibrosis, past research has been limited to direct studies on human patients and mouse models for the disease. Although invaluable information has been gained using cystic fibrosis mouse models, they do not fully recapitulate the natural progression of cystic fibrosis lung and pancreatic disease seen in human patients [1]. For these reasons, two additional cystic fibrosis models in the pig [2,3] and ferret [4••,5] were recently generated. Findings from these larger cystic fibrosis animal models have begun to demonstrate many phenotypic similarities to the human disease [4••, 6,7••]. Additionally, refinements in the cystic fibrosis mouse models using organ-specific conditional cystic fibrosis transmembrane conductance regulator (CFTR) ablation are beginning to inform new functions of CFTR in tissues not previously appreciated [8••, 9–11]. Major insights about the pathobiology of cystic fibrosis are surfacing through the comparisons of similarities and differences between these three animal models and the human disease. This review will concentrate on recent advances and studies in the pig and ferret cystic fibrosis models, with a focus on specific organ systems and how well they recapitulate human cystic fibrosis disease. As cystic fibrosis mouse models have been discussed in detail in recent reviews [12], we will focus discussion on cystic fibrosis mouse models in a comparative context to the newer cystic fibrosis pig and ferret models.

Lung and trachea

Lung infections are the major cause of morbidity and mortality in cystic fibrosis patients [13]. Although cystic fibrosis mice have been shown to acquire some degree of pathological abnormalities in the lung following bacterial challenge, it is generally accepted that cystic fibrosis mice do not acquire spontaneous and chronic bacterial infections as seen in cystic fibrosis patients [12]. Initial analysis of $CFTR^{-/-}$ pigs demonstrated no signs of lung disease, infection, or inflammation in the first day of life [6]. However, both $CFTR^{-/-}$ and $CFTR^{F508del/F508del}$ pigs developed lung disease within the first few months of life, characterized by airway inflammation, airway remodeling, mucus accumulation, and infection with multiple bacterial species [7••,14•]. Much like the cystic fibrosis pigs, $CFTR^{-/-}$ ferrets also show evidence of lung infections early in life [4••]. The severity of early lung infection within the first weeks of life for the cystic fibrosis ferrets necessitates their rearing on antibiotics prior to weaning.

Despite the fact that cystic fibrosis pigs develop spontaneous lung infections, neutrophil counts and interleukin (IL)-8 levels in neonatal bronchial alveolar lavage (BAL) fluid do not show signs of inflammation at birth, lending support to the hypothesis that infection precedes inflammation in cystic fibrosis [7••]. In addition, upon bacterial challenge, $CFTR^{-/-}$ pigs fail to effectively kill bacteria, suggesting that the basic defect in bacterial clearance is caused by impaired innate immunity in the lung. Similar defects in bacterial clearance have been observed in newborn cystic fibrosis ferrets challenged with bacteria (unpublished observation, J. F. Engelhardt, University of Iowa). Bacterial species in both cystic fibrosis pig and ferret BAL were quite diverse, with common pathogens between the two models including *Streptococcus, Staphlyococcus,* and *Enterococcus* species [4••,7••]. The absence of *Pseudomonas aeruginosa* in the lungs from both models is notable, given that this is one of the more common pathogens in the human cystic fibrosis lung. These findings suggest that the innate immunity defect in cystic fibrosis may be a general one and not specific to particular types of bacteria. Such findings support a growing body of work using high-density 16S rRNA gene sequencing that also demonstrates a high degree of complexity in bacterial communities in the cystic fibrosis lung [15].

CFTR is highly expressed in serous cells of submucosal glands in the cartilaginous airways of humans, pigs, and ferrets [16,17]. These structures have been proposed to be an important source of antibacterial factors that protect the airways from infection [18,19•]. Defects in submucosal gland secretion exist in cystic fibrosis mice [20], pigs [21,22], and ferrets [4••]. As submucosal glands are only present in the proximal trachea of mice, these structures have been thought to play less of a role in innate immunity in the mouse lung. The lack of submucosal glands throughout the proximal airways of mice has been hypothesized to be a contributing factor as to why cystic fibrosis mice fail to develop spontaneous lung disease. Further analysis of submucosal gland secretions in cystic fibrosis pigs and ferrets prior to overt lung disease may help to unravel their contribution to innate immune defects in the cystic fibrosis airway.

Electrophysiological analysis of tracheal epithelium from cystic fibrosis pigs [23••] and ferrets [4••] demonstrates that CFTR is the primary pathway for cyclic AMP (cAMP)-mediated chloride transport in these species, as in humans. This is not the case in cystic fibrosis mice, where an alternative non-CFTR, cAMP-activated, chloride channel(s) exists [24,25]. Recent data from the cystic fibrosis pig model have also raised interesting questions regarding sodium transport by the epithelial sodium channel (ENaC) and its relationship to cystic fibrosis disease [23••]. One prevailing hypothesis for pathophysiology in the cystic fibrosis lung has been the disruption of CFTR-mediated negative regulation of ENaC, leading to sodium hyperabsorption and dehydration of the airway [26,27]. However, studies from the cystic fibrosis pigs have challenged this hypothesis by demonstrating a lack of CFTR-dependent changes in sodium absorptive flux, fluid absorption, and depth of periciliary fluid in cystic fibrosis pig airway epithelia [23••]. These findings contradict earlier studies with human patient tissue and cell-based models [26,27], and may reveal species differences between the human and pig, or illustrate important distinctions between methods when studying ion and fluid transport in airway epithelia [23••].

The recent generation of a conditional CFTR-knockout mouse model [9] has yielded some interesting findings concerning the role of CFTR in lung inflammation. For example, myeloid-specific CFTR-knockout mice have impaired bacterial clearance and fail to resolve inflammation in the lung following challenge with agar beads impregnated with *Pseudomonas aeruginosa* [10]. Additionally, mice with the specific ablation of CFTR in $CD3^+$ lymphocytes displayed inflammatory defects, including augmented IgE production in response to pathogens, altered Ca^{2+} flux in response to T-cell receptor activation, and increased IL-13 secretion [8••]. Taken together, these results support an intrinsic role for CFTR in immune cells that impacts regulation of lung inflammatory responses.

Gastrointestinal tract

Meconium ileus is an in-utero intestinal obstruction that presents at birth in ~15% of newborn infants with cystic fibrosis [13,28]. To date, all cystic fibrosis animal models present with intestinal obstructive phenotypes to varying extents. Most cystic fibrosis mice typically die from mucus-mediated intestinal or colonic obstruction by 40 days of age unless weaned onto special liquid diets [29,30]; the frequency and age of onset of this pathology varies significantly depending on the genetic background and CFTR mutation [31]. Although cystic fibrosis mouse intestinal pathology is similar to meconium ileus, it develops postnatally and thus is distinctly different from the failure to pass meconium at birth. In contrast, both cystic fibrosis pigs and ferrets demonstrate a phenotype of meconium ileus that is extremely similar to that observed in cystic fibrosis infants. Meconium ileus occurs in 100% of $CFTR^{-/-}$ piglets, which is fatal within the first 48 h if surgery is not performed [6]. In contrast, 75% of $CFTR^{-/-}$ ferrets present with meconium ileus at birth with a similar time course to cystic fibrosis pigs [4••]. Intestinal atresias, diverticulosis, and microcolon, all

conditions that can occur in cystic fibrosis infants, are also seen in newborn $CFTR^{-/-}$ pigs and ferrets [4••,32•].

Several additional findings on meconium ileus in the cystic fibrosis pig and ferret models are worth noting. First, the frequency of this condition in both species is significantly higher than that seen in humans. The reason for this difference is currently unclear, but it suggests that pig and ferret CFTR plays a much more important role in hydration of the in-utero intestine than human CFTR. Second, meconium ileus in the cystic fibrosis ferret has a significant genetic influence [4••], a finding also observed in cystic fibrosis infants [33,34]. Third, the recent characterization of a $CFTR^{F508del/F508del}$ pig model also demonstrates 100% penetrance of meconium ileus [14•]. This is particularly revealing, as the pig CFTR-F508del protein demonstrates residual processing to the plasma membrane and partial function [35]. Thus, the pig likely requires high levels of functional CFTR in the intestine to clear meconium following birth.

Pancreas

The pancreas is a severely affected organ in humans with cystic fibrosis, and exocrine pancreatic insufficiency occurs in a vast majority of cystic fibrosis patients [13,28,36]. Although some pancreatic pathology has been noted in cystic fibrosis mice, in general this model is not thought to develop similar pancreatic disease to cystic fibrosis patients. Research has suggested that alternative chloride channels in the mouse pancreas compensate for the lack of CFTR [37–39]. In contrast, both the cystic fibrosis pig and ferret models demonstrate good parallels to disease in the human cystic fibrosis pancreas, but with varying degrees of severity [4••,6]. $CFTR^{-/-}$ pigs are born with severe exocrine pancreas destruction and rapidly develop pancreatic insufficiency after surgery to resolve meconium ileus [6,32•]. Despite these changes, islet structure is preserved in newborn $CFTR^{-/-}$ pigs. About 75% of cystic fibrosis infants have microscopic changes in the pancreas at birth, and a fraction of these (3%) display severe pancreatic damage [28,36]. The destruction of the exocrine pancreas in newborn $CFTR^{-/-}$ pigs is comparable to these cases on the severe end of the spectrum [28,36]. Interestingly, the $CFTR^{F508del/F508del}$ pig appears to have a slightly less severe exocrine pancreatic phenotype than the knockout animal [14•], suggesting that residual function of the pig CFTR-F508del protein may partially attenuate disease progression.

Pathology in the newborn $CFTR^{-/-}$ ferret pancreas is seen in 100% of animals, and characterized by dilation of most acini and ductules with inspissated, eosinophilic zymogen secretions [4••]. This relatively minor level of histopathology in the exocrine pancreas of $CFTR^{-/-}$ ferrets is similar to what is seen in 72% of cystic fibrosis infants with microscopic lesions in the pancreas [28,36]. Thus, at birth, the severity of exocrine pancreas disease in newborn cystic fibrosis pigs is significantly greater than that seen in the newborn cystic fibrosis ferret. The exocrine pancreas of cystic fibrosis ferrets appears to undergo rapid destruction over the first months of life, leading to pancreatic insufficiency and the need for pancreatic enzymes in the rearing process [4••]. In general, the ferret and pig models of cystic fibrosis may prove to be extremely useful for studying the mechanisms responsible exocrine pancreas decline in cystic fibrosis patients.

Liver and gallbladder

A common cause of morbidity in humans with cystic fibrosis is biliary cirrhosis [13,36,40]. Cystic fibrosis mice are generally thought to not develop significant hepatobiliary pathologies [12]. In contrast, $CFTR^{-/-}$ and $CFTR^{F508del/F508del}$ pigs develop moderate hepatic lesions, and exhibit common signs of biliary cirrhosis, such as cellular inflammation, ductal hyperplasia, and fibrosis [6,14•,32•]. In the newborn $CFTR^{-/-}$ ferret, the liver is

histopathologically indistinguishable from non-cystic fibrosis animals [4••]. However, newborn cystic fibrosis ferrets demonstrate abnormally elevated plasma alanine aminotransferase and bilirubin levels suggestive of liver disease [4••]. This finding is similar to children with cystic fibrosis, who often have abnormally elevated liver enzymes in the blood in the absence of liver histopathology [41]. Interestingly, oral administration of ursodeoxycholic acid (a hydrophilic dihydroxylated bile acid) normalizes liver function tests in neonatal cystic fibrosis ferrets [4••] similarly to what has been observed in cystic fibrosis infants [42]. These findings suggest similar pathophysiologic mechanisms in the processing of bile acids between cystic fibrosis humans and ferrets.

Gallbladder disease is observed in 15–30% of older cystic fibrosis patients at the time of autopsy [13,36,43]. Gall-bladder abnormalities are generally mild in cystic fibrosis mice, and vary with background strain and CFTR mutation [12]. However, disease of the gallbladder is severe in CFTR knockout and $CFTR^{F508del/F508del}$ pigs, with micro-gallbladder occurring in 100% of newborns [6,14•,32•]. Pathologies noted in the gallbladder of cystic fibrosis pigs include luminal obstruction by bile and mucus, focal aggregates of neutrophils, and mild instances of mononuclear inflammation. In contrast, the gallbladder of newborn CFTR knockout ferrets is indistinguishable from non-cystic fibrosis animals at the histologic level [4••].

Growth and nutrition

Nutritional deficits are important considerations in humans with cystic fibrosis [44,45]. Cystic fibrosis mice, pigs, and ferrets demonstrate growth impairment to varying degrees [4••,46•,47]. The cystic fibrosis ferret has the most severe growth impairment, and this is thought to be primarily due to reduced gastrointestinal pH, as improved weight gain is observed when animals are reared on the proton-pump inhibitor omeprazole [4••]. Other findings have begun to suggest that neuroendocrine defects may also contribute to growth impairment in cystic fibrosis. For example, a recent report found that chloride secretion is defective in the thyroids of $CFTR^{-/-}$ pigs, providing a possible mechanism to cystic fibrosis-linked hypothyroidism [48]. Additionally, analysis of insulin-like growth factor 1 (IGF-1) levels in the serum of newborn cystic fibrosis pigs and infants demonstrates reduced levels of this hormone [46•]. Cystic fibrosis mice also have similar reductions in serum IGF-1 at older ages [47].

The recent generation of a conditional CFTR knockout mouse [9] has yielded some extremely interesting findings concerning tissue-specific functional roles for CFTR in growth. For example, specific deletion of CFTR in the intestinal epithelium does not lead to growth impairment [49]. Of note, the gastrointestinal obstructive phenotype in these mice was much less severe than their systemic knockout counterparts. Neuronal-specific CFTR knockouts display major reductions in growth compared with wild-type littermates, but improved body-mass index compared with complete CFTR knockouts [11]. Interestingly, these neuronal-specific CFTR knockout mice also show a decrease in serum IGF-1 levels [11] and low bone mass [50]. These results suggest new roles for CFTR in the neuroendocrine system that control growth.

Reproductive system

The vast majority of males with cystic fibrosis (~97%) are sterile due to the progressive deterioration of the vas deferens [51]. Interestingly, although male cystic fibrosis mice are generally thought to lack fertility defects [12], certain strains of female cystic fibrosis mice have reduced fertility due to inadequate sperm transport within the female reproductive tract [52]. Both cystic fibrosis pig and ferret male newborns demonstrate a severely degenerated vas deferens at birth [4••,53]. These interesting findings make both the cystic fibrosis pig and

ferret excellent models for the study of reproductive defects in males with cystic fibrosis and the role of CFTR in the early development of the male reproductive organs.

Conclusion

Difference in disease of various organs between cystic fibrosis mice, pigs, and ferrets provide unique opportunities to characterize how modifier genes and environmental factors influence the severity of disease onset and progression in the human condition. Like these models, cystic fibrosis patients also have wide ranging disease severities influenced by the CFTR mutation, modifier genes, and the environment. A more detailed understanding of organ physiology in the newer cystic fibrosis pig and ferret models – as done in the cystic fibrosis mouse models for the past two decades – will be needed to understand the molecular basis for species differences in disease phenotypes. Such studies will likely uncover new modifier genes and pathways that impact the severity of CFTR dysfunction and may be directly translatable to therapies.

One major challenge that remains to fully unlock the potential of cystic fibrosis pig and ferret models is the development of improved models that can be reared to weaning more easily. In this context, genetic complementation of the meconium ileus phenotype in the cystic fibrosis pigs and ferrets is an immediate need. Gut-specific CFTR expression in cystic fibrosis mice rescues obstructive bowel disease [54] and this same transgenic approach also prevents meconium ileus in CFTR knockout ferrets [4••]. However, it is important to recognize that correction of CFTR in the gut may also impact disease progression in other organs such as the lung and pancreas. Thus, cell-specific transgenic complementation approaches in cystic fibrosis pigs and ferrets will not only generate animal models which are easier to rear, but will likely also prove useful in dissecting the contribution of CFTR in different tissues to disease progression in the whole animal. Such approaches will complement studies in conditional CFTR knockout mice that benefit from widely available tissue-specific promoters.

In conclusion, cystic fibrosis research will benefit greatly from the wide array of cystic fibrosis animal models now available and under construction. Notably, the spontaneous lung and pancreatic phenotypes observed in the cystic fibrosis pig and ferret models now allow for more detailed mechanistic studies on disease progression in these organs less approachable in mice. However, a complete understanding of cystic fibrosis pathophysiology and the development of effective therapies will most likely emerge from the aggregate understanding of cystic fibrosis disease processes across species and its relationship to the human condition.

Acknowledgments

Authors thank Dr Mitchell Drumm for his advice regarding conditional cystic fibrosis mouse models. Funding sources are the NIH (DK047967, HL091842, R24DK091211, and RC1HL099516) and the Cystic Fibrosis Foundation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 488).

1. Fisher JT, Zhang Y, Engelhardt JF. Comparative biology of cystic fibrosis animal models. Methods Mol Biol. 2011; 742:311–334. [PubMed: 21547741]
2. Rogers CS, Abraham WM, Brogden KA, et al. The porcine lung as a potential model for cystic fibrosis. Am J Physiol Lung Cell Mol Physiol. 2008; 295:L240–263. [PubMed: 18487356]
3. Rogers CS, Hao Y, Rokhlina T, et al. Production of cftr-null and cftr-deltaf508 heterozygous pigs by adeno-associated virus-mediated gene targeting and somatic cell nuclear transfer. J Clin Invest. 2008; 118:1571–1577. [PubMed: 18324337]

4••. Sun X, Sui H, Fisher JT, et al. Disease phenotype of a ferret cftr-knockout model of cystic fibrosis. J Clin Invest. 2010; 120:3149–3160. First report of the phenotype of newborn CFTR knockout ferrets. Early lung infection and type of bacteria in the BAL suggest that aspiration may influence early colonization of the lung. Alterations in bile acid metabolism and gastrointestinal pH appear to influence early liver disease and growth of CFTR knockout ferrets. [PubMed: 20739752]

5. Sun X, Yan Z, Yi Y, et al. Adeno-associated virus-targeted disruption of the cftr gene in cloned ferrets. J Clin Invest. 2008; 118:1578–1583. [PubMed: 18324338]
6. Rogers CS, Stoltz DA, Meyerholz DK, et al. Disruption of the cftr gene produces a model of cystic fibrosis in newborn pigs. Science. 2008; 321:1837–1841. [PubMed: 18818360]

7••. Stoltz DA, Meyerholz DK, Pezzulo AA, et al. Cystic fibrosis pigs develop lung disease and exhibit defective bacterial eradication at birth. Sci Transl Med. 2010; 2:29ra31. First report of the lung phenotype in cystic fibrosis pigs. The absence of lung inflammation at birth in this model suggests that bacterial infection precedes inflammation.

8••. Mueller C, Braag SA, Keeler A, et al. Lack of cftr in cd3+ lymphocytes leads to aberrant cytokine secretion and hyper-inflammatory adaptive immune responses. Am J Respir Cell Mol Biol. 2010; 44:922–929. Cell-specific deletion of CFTR in CD3+ lymphocytes displayed inflammatory defects in mice. Phenotypes include augmented IgE production in response to pathogens, altered Ca2+ flux in response to T-cell receptor activation, and increased IL-13 secretion. [PubMed: 20724552]

9. Hodges CA, Cotton CU, Palmert MR, Drumm ML. Generation of a conditional null allele for cftr in mice. Genesis. 2008; 46:546–552. [PubMed: 18802965]
10. Bonfield TL, Hodges CA, Drumm ML. The epithelial cell contingency: myeloid cells and inflammation resolution. Pediatr Pulmonol. 2010; 45(S33):272.
11. Hodges CA, Grady BR, Palmert MR, Drumm ML. Loss of cftr function in neurons results in poor growth and possible endocrine dysfunction. Pediatr Pulmonol. 2010; 45(S33):290–291.
12. Guilbault C, Saeed Z, Downey GP, Radzioch D. Cystic fibrosis mouse models. Am J Respir Cell Mol Biol. 2007; 36:1–7. [PubMed: 16888286]
13. Welsh, MJ.; Ramsey, BW.; Accurso, F.; Cutting, GR. The metabolic and molecular basis of inherited disease. New York: McGraw-Hill; 2001.

14•. Ostedgaard LS, Meyerholz DK, Chen JH, et al. The deltaf508 mutation causes cftr misprocessing and cystic fibrosis-like disease in pigs. Sci Transl Med. 2011; 3:74ra24. Analysis of the CFTR-ΔF508 pig model demonstrates similarities in phenotype to the CFTR knockout model despite residual processing of CFTR-ΔF508 in certain organs.

15. Stressmann FA, Rogers GB, Klem ER, et al. Analysis of the bacterial communities present in lungs of patients with cystic fibrosis from american and british centers. J Clin Microbiol. 2011; 49:281–291. [PubMed: 21068277]
16. Engelhardt JF, Yankaskas JR, Ernst SA, et al. Submucosal glands are the predominant site of cftr expression in the human bronchus. Nat Genet. 1992; 2:240–248. [PubMed: 1285365]
17. Sehgal A, Presente A, Engelhardt JF. Developmental expression patterns of cftr in ferret tracheal surface airway and submucosal gland epithelia. Am J Respir Cell Mol Biol. 1996; 15:122–131. [PubMed: 8679216]
18. Dajani R, Zhang Y, Taft PJ, et al. Lysozyme secretion by submucosal glands protects the airway from bacterial infection. Am J Respir Cell Mol Biol. 2005; 32:548–552. [PubMed: 15746432]

19•. Widdicombe JH. Transgenic animals may help resolve a sticky situation in cystic fibrosis. J Clin Invest. 2010; 120:3093–3096. A commentary on species-specific differences in submucosal gland biology between cystic fibrosis models and recent findings on gland dysfunction in the CFTR knockout pig and ferret models. [PubMed: 20739746]

20. Ianowski JP, Choi JY, Wine JJ, Hanrahan JW. Mucus secretion by single tracheal submucosal glands from normal and cystic fibrosis transmembrane conductance regulator knockout mice. J Physiol. 2007; 580:301–314. [PubMed: 17204498]

21. Joo NS, Cho HJ, Khansaheb M, Wine JJ. Hyposecretion of fluid from tracheal submucosal glands of cftr-deficient pigs. J Clin Invest. 2010; 120:3161–3166. [PubMed: 20739758]

22. Lee RJ, Foskett JK. cAMP-activated Ca2+ signaling is required for cftr-mediated serous cell fluid secretion in porcine and human airways. J Clin Invest. 2010; 120:3137–3148. [PubMed: 20739756]

23••. Chen JH, Stoltz DA, Karp PH, et al. Loss of anion transport without increased sodium absorption characterizes newborn porcine cystic fibrosis airway epithelia. Cell. 2011; 143:911–923. This report challenges a major running hypothesis in the field that sodium hyperabsorption occurs in the cystic fibrosis airway and leads to dehydration. Studies demonstrated a lack of CFTR-dependent changes in sodium absorptive flux, fluid absorption, and depth of periciliary fluid in cystic fibrosis pig airway epithelia. The study concludes that defects in chloride and bicarbonate transport, but not sodium transport, are largely responsible for lung disease in cystic fibrosis. [PubMed: 21145458]

24. Grubb BR, Paradiso AM, Boucher RC. Anomalies in ion transport in CF mouse tracheal epithelium. Am J Physiol. 1994; 267:C293–C300. [PubMed: 8048488]

25. Liu X, Yan Z, Luo M, Engelhardt JF. Species-specific differences in mouse and human airway epithelial biology of recombinant adeno-associated virus transduction. Am J Respir Cell Mol Biol. 2006; 34:56–64. [PubMed: 16195538]

26. Boucher RC. Cystic fibrosis: a disease of vulnerability to airway surface dehydration. Trends Mol Med. 2007; 13:231–240. [PubMed: 17524805]

27. Boucher RC. Evidence for airway surface dehydration as the initiating event in CF airway disease. J Intern Med. 2007; 261:5–16. [PubMed: 17222164]

28. Oppenheimer EH, Esterly JR. Cystic fibrosis of the pancreas: morphologic findings in infants with and without diagnostic pancreatic lesions. Arch Pathol. 1973; 96:149–154. [PubMed: 4722876]

29. Snouwaert JN, Brigman KK, Latour AM, et al. An animal model for cystic fibrosis made by gene targeting. Science. 1992; 257:1083–1088. [PubMed: 1380723]

30. Harmon GS, Dumlao DS, Ng DT, et al. Pharmacological correction of a defect in PPAR-gamma signaling ameliorates disease severity in cftr-deficient mice. Nat Med. 2010; 16:313–318. [PubMed: 20154695]

31. Grubb BR, Gabriel SE. Intestinal physiology and pathology in gene-targeted mouse models of cystic fibrosis. Am J Physiol. 1997; 273:G258–266. [PubMed: 9277402]

32•. Meyerholz DK, Stoltz DA, Pezzulo AA, Welsh MJ. Pathology of gastrointestinal organs in a porcine model of cystic fibrosis. Am J Pathol. 2010; 176:1377–1389. An extensive study describing numerous pathological abnormalities in CFTR knockout pig intestine, pancreas, gallbladder, and liver. [PubMed: 20110417]

33. Blackman SM, Deering-Brose R, McWilliams R, et al. Relative contribution of genetic and nongenetic modifiers to intestinal obstruction in cystic fibrosis. Gastroenterology. 2006; 131:1030–1039. [PubMed: 17030173]

34. Dorfman R, Li W, Sun L, et al. Modifier gene study of meconium ileus in cystic fibrosis: statistical considerations and gene mapping results. Hum Genet. 2009; 126:763–778. [PubMed: 19662435]

35. Ostedgaard LS, Rogers CS, Dong Q, et al. Processing and function of cftr-deltaf508 are species-dependent. Proc Natl Acad Sci U S A. 2007; 104:15370–15375. [PubMed: 17873061]

36. Oppenheimer EH, Esterly JR. Pathology of cystic fibrosis review of the literature and comparison with 146 autopsied cases. Perspect Pediatr Pathol. 1975; 2:241–278. [PubMed: 1168897]

37. Clarke LL, Grubb BR, Yankaskas JR, et al. Relationship of a noncystic fibrosis transmembrane conductance regulator-mediated chloride conductance to organ-level disease in cftr(−/−) mice. Proc Natl Acad Sci U S A. 1994; 91:479–483. [PubMed: 7507247]

38. Gray MA, Winpenny JP, Verdon B, et al. Chloride channels and cystic fibrosis of the pancreas. Biosci Rep. 1995; 15:531–541. [PubMed: 9156582]
39. Pascua P, Garcia M, Fernandez-Salazar MP, et al. Ducts isolated from the pancreas of cftr-null mice secrete fluid. Pflugers Arch. 2009; 459:203–214. [PubMed: 19655163]
40. Wilschanski M, Durie PR. Pathology of pancreatic and intestinal disorders in cystic fibrosis. J R Soc Med. 1998; 91 (Suppl 34):40–49. [PubMed: 9709387]
41. Lindblad A, Glaumann H, Strandvik B. Natural history of liver disease in cystic fibrosis. Hepatology. 1999; 30:1151–1158. [PubMed: 10534335]
42. Scher H, Bishop WP, McCray PB Jr. Ursodeoxycholic acid improves cholestasis in infants with cystic fibrosis. Ann Pharmacother. 1997; 31:1003–1005. [PubMed: 9296240]
43. Chaudry G, Navarro OM, Levine DS, Oudjhane K. Abdominal manifestations of cystic fibrosis in children. Pediatr Radiol. 2006; 36:233–240. [PubMed: 16391928]
44. Salvatore D, Buzzetti R, Baldo E, et al. An overview of international literature from cystic fibrosis registries 2. Neonatal screening and nutrition/growth. J Cyst Fibros. 2009; 9:75–83. [PubMed: 19955022]
45. Michel SH, Maqbool A, Hanna MD, Mascarenhas M. Nutrition management of pediatric patients who have cystic fibrosis. Pediatr Clin North Am. 2009; 56:1123–1141. [PubMed: 19931067]
46•. Rogan MP, Reznikov LR, Pezzulo AA, et al. Pigs and humans with cystic fibrosis have reduced insulin-like growth factor 1 (igf1) levels at birth. Proc Natl Acad Sci U S A. 2010; 107:20571–20575. This report demonstrates interesting correlates between reduced IGF-1 levels in newborn cystic fibrosis pigs and humans. The authors suggest that early IGF-1 therapy may be beneficial for improved growth of cystic fibrosis children. [PubMed: 21059918]
47. Rosenberg LA, Schluchter MD, Parlow AF, Drumm ML. Mouse as a model of growth retardation in cystic fibrosis. Pediatr Res. 2006; 59:191–195. [PubMed: 16439577]
48. Li H, Ganta S, Fong P. Altered ion transport by thyroid epithelia from cftr(−/−) pigs suggests mechanisms for hypothyroidism in cystic fibrosis. Exp Physiol. 2010; 95:1132–1144. [PubMed: 20729267]
49. Hodges CA, Grady BR, Mishra K, et al. Cystic fibrosis growth retardation is not correlated with loss of cftr in the intestinal epithelium. Am J Physiol Gastrointest Liver Physiol. 2011 [Epub ahead of print].
50. Stalvey MS, Gaur T, Drumm ML, Hodges CA. Neuronal-specific knockout mice display low bone mass phenotype. Pediatr Pulmonol. 2010; 45(S33):430.
51. Jarzabek K, Zbucka M, Pepinski W, et al. Cystic fibrosis as a cause of infertility. Reprod Biol. 2004; 4:119–129. [PubMed: 15297887]
52. Hodges CA, Palmert MR, Drumm ML. Infertility in females with cystic fibrosis is multifactorial: evidence from mouse models. Endocrinology. 2008; 149:2790–2797. [PubMed: 18325992]
53. Pierucci-Alves F, Akoyev V, Stewart JC 3rd, et al. Swine models of cystic fibrosis reveal male reproductive tract phenotype at birth. Biol Reprod. 2011 [Epub ahead of print].
54. Zhou L, Dey CR, Wert SE, et al. Correction of lethal intestinal defect in a mouse model of cystic fibrosis by human cftr. Science. 1994; 266:1705–1708. [PubMed: 7527588]

Key points

- CFTR knockout pigs and ferrets develop cystic fibrosis disease in multiple organs, including the gastrointestinal tract, lung, pancreas, liver, gallblad-der, and male reproductive system.
- Disease of the gastrointestinal tract is universal to all cystic fibrosis animal models; however, the cystic fibrosis ferret and pig models appear uniquely suited to study the development of spontaneous disease in the lung and pancreas.
- Conditional CFTR knockout mice are providing unique insight on the functions of CFTR in neurons and T-cells.
- Key differences in the severity of organ disease in cystic fibrosis mice, pigs, and ferrets will inform pathophysiologic mechanisms of disease potentially translatable to therapies.