

# Recent advances in understanding the mechanisms of CFTR pathophysiology

Amanda Leah Neujahr

Animal Science and Zoology

Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078, USA

**Key Words:**

Cystic Fibrosis, CFTR gene, stem cells

---

**Cystic fibrosis (CF) remains a common, life-threatening, incurable genetic disease in the Caucasian population. CF is caused by mutations in the CFTR gene on the long arm of chromosome 7. There are several mutations known, and phenotypic effects are variable. Despite advances in understanding CF pathology, treatment is still limited to the amelioration of symptoms, leaving much to be desired in terms of available therapeutic strategies. In recent years, the pig model has yielded a better understanding of clinically relevant aspects of human CF. The primary complication in human CF is respiratory dysfunction, although many other organ systems are affected. Male fertility is known to be adversely affected by CF, although the specific role of CFTR in this defect is unclear. More work to determine the mechanisms of CFTR function and dysfunction is needed, and the need for widespread use of large animal models to better understand human CF has been expressed in current literature.**

---

## Introduction

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene are responsible for cystic fibrosis (CF). The CFTR gene is located in the long arm of chromosome 7 (7q31.2), and encodes a protein that functions as a cAMP-regulated chloride channel in a variety of epithelial cells. In the absence of mutations, the CFTR gene is transcribed and translated normally, yielding a CFTR protein that is fully functional as a chloride channel in the cell membrane. Six major mechanisms of CFTR function alteration have been identified, and range from normal apical membrane expression of a protein with reduced chloride conductance ability to the complete loss of protein synthesis (Lubamba *et al.* 2012). Lacking or inefficient chloride transport in cells causes dehydration of secretions, leading to hyperviscous mucus which causes some of the common manifestations of CF including airway obstruction,

pancreatic insufficiency, and nutrient malabsorption in the intestine.

Phenotypic abnormalities typical of CF patients include malformations in the respiratory tract, pancreas, liver, gallbladder, and male reproductive tract. The CFTR gene has been associated with male fertility, and most males with CF are infertile because of congenital bilateral absence of the vas deferens (CBAVD) or because of morphological abnormalities of the epididymis. A higher prevalence of CFTR mutations among infertile, otherwise healthy men suggests that, in addition to causing CBAVD or morphological alterations of the epididymis, CFTR defects might also be associated with spermatozoa with reduced fertilizing capability, which is acquired in the epididymis. The most common and life-threatening hallmark of CF is respiratory infection and inflammation, but other systems are adversely affected simultaneously. A better understanding of the clinical aspects of CF in humans is needed to improve therapeutic options for CF

patients. The pig is a suitable biomedical model for humans due to similarities in size, anatomy, and physiology. A major drawback to the establishment of pig and other large animal models is that there are no proven germ-line competent embryonic stem cells available, as there are for the mouse and rat. Therefore, mutations must be introduced into somatic cells. Long generation intervals also hinder the production of mutants in a timely manner. The information gained from the pig models; however, has given detailed insight regarding the effects of CFTR mutations on the respiratory tract, gastrointestinal tract, male reproductive organs, and tooth enamel.

**Recent Progress**

CFTR mutations, like most genetic mutations, have largely been studied by producing organisms in which the gene of interest has been knocked out. The murine model has been indispensable for studying CFTR pathomechanics and the mechanisms of CF pathogenesis, but alterations in many organs of CF mice differ from those seen in human CF. A major drawback is that the respiratory tract abnormalities seen in human CF are the major source of severe complications, but mice do not exhibit the same respiratory defects. Targeted inactivation of the CFTR gene in the ferret and pig has resulted in models that more closely resemble human CF pathology. Klymiuk *et al.* produced two CFTR knockout pig models using two different methods for inactivating the CFTR gene (2011). One pig model was created by inserting a STOP box downstream of the start codon in exon 1, and another was created by inserting a STOP codon into exon 11. Both methods of CFTR inactivation resulted in pigs with the complete absence of the CFTR protein and that closely resembled the hallmarks of human CF. CFTR<sup>+/-</sup>

offspring were produced by nucleofection of the CFTR targeting vector into primary kidney cells, identification of CFTR<sup>+/-</sup> cell clones using qPCR, somatic cell nuclear transfer of the cell clones, and embryo transfer to three recipients, resulting in two pregnancies. One was terminated at 58 days for studies of the fetuses, and the other was carried to term, resulting in five verified CFTR<sup>+/-</sup> offspring. At 18 months old, the pigs were reported to be healthy, fertile, and free from clinical abnormalities. One CFTR<sup>+/-</sup> pig was euthanized and primary cell cultures from the kidney were used to target the second CFTR allele. CFTR<sup>-/-</sup> (knockout) pigs were produced using the same method, and two litters were carried to term. Two of the eleven total born CFTR knockout piglets were stillborn, but all others were viable and able to suckle. CFTR knockout piglets began showing signs of discomfort after a few hours, and none survived for more than 37 hours. Piglets were euthanized as their health status deteriorated. Immunohistochemical analysis of tissue from CFTR knockout pigs did not detect any CFTR protein. All CFTR knockout piglets had a severe meconium obstruction in the colon. Glands in the duodenum were lined by atrophic epithelia. The tracheas of CFTR knockout piglets were triangular rather than circular, and the caudal trachea and large bronchi exhibited variable thickening of the posterior wall that was not seen in wild-type controls. Notably, the epididymis and vas deferens of CFTR knockout pigs showed alterations typical of human CF.

Another study using the pig model investigated how lung pH is related to bacterial killing. Bacterial lung infection results in inflammation, and these infections are the major causes of morbidity and mortality associated with CF. The study found that airway surface liquid (ASL) was more acidic in CF pigs. CFTR is an anion channel that facilitates HCO<sub>3</sub><sup>-</sup> transport. When CFTR is

Class of CFTR Gene mutation and examples	Phenotypic Consequences
Class I: G542X, W1282X, R553X, 3950delT	No synthesis of CFTR occurs because of premature stop codons or aberrant mRNA splicing
Class II: F508del*, N1303K	An immature form of CFTR is synthesized, and mostly degraded by the ubiquitin-proteasomal pathway
<sup>1</sup> Class III: G551D	CFTR is synthesized and transported to the plasma membrane, but does not respond to cAMP or ATP stimulation or regulation
<sup>1</sup> Class IV: R334W, G314E, R347P, D1152H	CFTR is synthesized and correctly trafficked to the cell membrane, but Cl <sup>-</sup> conductance is abnormal
Class V: 3849 + 10 kb C>T, 3272-26 A>G	Synthesis or processing of CFTR is partly defective
Class VI: 1811 + 1.6 kb A>G	CFTR is synthesized. Membrane stability or ion conductance (other than Cl <sup>-</sup> ) is affected

**Table 1.** Various phenotypes of CFTR

Modified from Lubamba *et al.*

\* denotes the most common and first identified CFTR gene mutation

<sup>1</sup>Class III and IV mutations are associated with a milder CF phenotype; all others are considered severe

absent or defective,  $\text{HCO}_3^-$  secretion in airway epithelia is defective, and ASL pH falls, limiting antimicrobial function and leaving the lung more susceptible to infection caused by bacteria that enter the lung (Pezzulo 2012). *Staphylococcus aureus* was used in this study, as it is the most common organism isolated from young children with CF. The methods for generating the CFTR knockout mutants were not outlined, but the authors of this study state that these mutants (CF pigs) developed hallmark features of CF lungs within months of birth, whereas no CFTR knockout pigs used by Klymiuk *et al.* were able to survive over 37 hours post-partum. A small tracheal incision was made in pigs 6-15 h old so that bacteria-coated grids, prepared by chemical modification of gold electron microscopy grids, could be placed on the airway surface (Pezzulo 2012). In addition to *in vivo* experiments, bacterial killing was linked to pH in experiments that used ASL removed from pigs. Moreover, ASL pH in primary airway epithelial cultures was measured using a fluorescent pH indicator. CF ASL was more acidic in these experiments as well. All results imply that bacterial killing is dependent upon the pH of ASL.

Ruan *et al.* studied the role of CFTR in ATP secretion in the male reproductive tract (2012). A previously established line of mouse epididymal principal cells, DC2, was used. RT-PCR detected a strong CFTR mRNA signal, and Western-blot analysis revealed bands consistent with the molecular size of CFTR in DC2 cells and mouse lung cells, which were used as a positive control. Intracellular pH ( $\text{pH}_i$ ) recovery following intracellular alkalinization was measured as an assessment of  $\text{HCO}_3^-$  efflux. DC2 cells were bathed in a  $\text{Cl}^-$  free solution to avoid any influence of  $\text{Cl}^-/\text{HCO}_3^-$  exchangers. A  $\text{pH}_i$  recovery was observed, implying that DC2 cells secrete  $\text{HCO}_3^-$ . Treatment of cells with forskolin, a known CFTR activator, increased the rate of  $\text{pH}_i$  recovery, and the CFTR inhibitor, CFTR<sub>inh172</sub>, eliminated the stimulatory effect of forskolin. The results show that DC2 cells have functional CFTR in their plasma membrane. Similar methods and reasoning were employed to show that ATP release from DC2 cells is influenced by CFTR. Forskolin and adrenaline (another known CFTR activator) increased the concentration of ATP in extracellular solution after 10 minutes of incubation of treated cells versus non-treated (control) cells. Pre-treatment of cells with CFTR<sub>inh172</sub> for 15-20 min decreased ATP concentration and negated the effects of forskolin. *In vivo* assessment of ATP secretion into the lumen of the cauda epididymal tubule further provided evidence of the participation of CFTR in ATP secretion. The cauda epididymis was perfused through the lumen, and luminal perfusate was collected via cannulation of the vas deferens. The lumen was washed free of spermatozoa to eliminate them as a potential source of ATP. Steady

ATP release was detected under control conditions. Introduction of CFTR<sub>inh172</sub> into the luminal perfusate reduced ATP concentrations in the collected samples, suggesting that CFTR mediates ATP release *in vivo*. The study provides insight into how CFTR defects can affect male fertility, given the known roles of ATP in sperm function and providing optimal epididymal luminal conditions for sperm maturation and storage.

## Discussion

Understanding the mechanisms of dysfunction of CFTR mutations may help improve therapy and treatment options for CF patients. Recent studies using the porcine model have provided valuable insight into clinically relevant aspects of human CF that could not be studied accurately in CF mice. Data imply that CFTR is crucial in regulating the pH of ASL, which is related to bacterial killing. It is possible that increasing ASL pH in CF patients could prevent initial bacterial infections in the lungs, which is a major cause of complications and mortality. Also, the role of CFTR defects in male infertility is becoming better understood. Not only do CFTR mutations cause infertility because they result in morphological abnormalities, but CFTR may be important in regulating ATP secretion in the epididymis. The phenotypic manifestations of CFTR mutations are variable. Advances in understanding the mechanisms of CFTR function and dysfunction could accelerate the development of new therapies for CF, which is the most common lethal genetic disease among Caucasians.

## References

- Klymiuk, N., L. Mundhenk, K. Kraehe, A. Wuensch, S. Plog, D. Emrich, M.C. Langenmayer, M. Stehr, A. Holzinger, C. Kröner, A. Richter, B. Kessler, M. Kurome, M. Eddicks, H. Nagashima, K. Heinritzi, A.D. Gruber, and E. Wolf. 2011. Sequential targeting of CFTR by BAC vectors generates a novel pig model of cystic fibrosis. *J Mol Med* 90:597-608.
- Lubamba, B., B. Dhooghe, S. Noel, and T. Leal. 2012. Cystic fibrosis: Insight into CFTR pathophysiology and pharmacotherapy. *Clinical Biochemistry* 45:1132-1144.
- Pezzulo, A.A., X. X. Tang, M.J. Hoegger, M.H. Abou Alaiwa, S. Ramachandran, T.O. Moninger, P.H. Karp, C.L. Wohlford-Lenane, H.P. Haagsman, M. van Eijk, B. Bánfi, A.R. Horswill, D.A. Stoltz, P.B. McCray Jr., M.J. Welsh, and J. Zabner. 2012. Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. *Nature* 487:109-114.
- Ruan, Y.C., W.W.C. Shum, C. Belleannée, N. Da Silva, and S. Breton. 2012. ATP secretion in the male reproductive tract: essential role of CFTR. *J Physiol* 590:4209-4222.

