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CFTR: A New Horizon in the Pathomechanism and Treatment of Pancreatitis

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Abstract Cystic fibrosis transmembrane conductance regulator (CFTR) is an ion channel that conducts chloride and bicarbonate ions across epithelial cell membranes. Mutations in the *CFTR* gene diminish the ion channel function and lead to impaired epithelial fluid transport in multiple organs such as the lung and the pancreas resulting in cystic fibrosis. Heterozygous carriers of *CFTR* mutations do not develop cystic fibrosis but exhibit increased risk for pancreatitis and associated pancreatic damage characterized by elevated mucus levels, fibrosis, and cyst formation. Importantly, recent studies demonstrated that pancreatitis causing insults, such as alcohol, smoking, or bile acids, strongly inhibit CFTR function. Furthermore, human studies showed reduced levels of CFTR expression and function in all forms of pancreatitis. These findings indicate that impairment of CFTR is critical in the development of pancreatitis; therefore, correcting CFTR function could be the first specific therapy in pancreatitis. In this review, we summarize recent advances in the field and discuss new possibilities for the treatment of pancreatitis.

Keywords CFTR • Cystic fibrosis • Epithelial transport • Pancreas • Pancreatitis

Contents

1 Basics of CFTR

1.1 Biosynthesis and Degradation

The cystic fibrosis transmembrane conductance regulator (CFTR) protein is a cyclic AMP (cAMP)-regulated chloride (Cl^-)/bicarbonate (HCO_3^-) channel, expressed in the apical plasma membrane (PM) of secretory epithelia in the airways, pancreas, intestine, reproductive organs, and exocrine glands (Riordan 2008). CFTR consists of two homologous halves, each containing a hexa-helical membrane-spanning

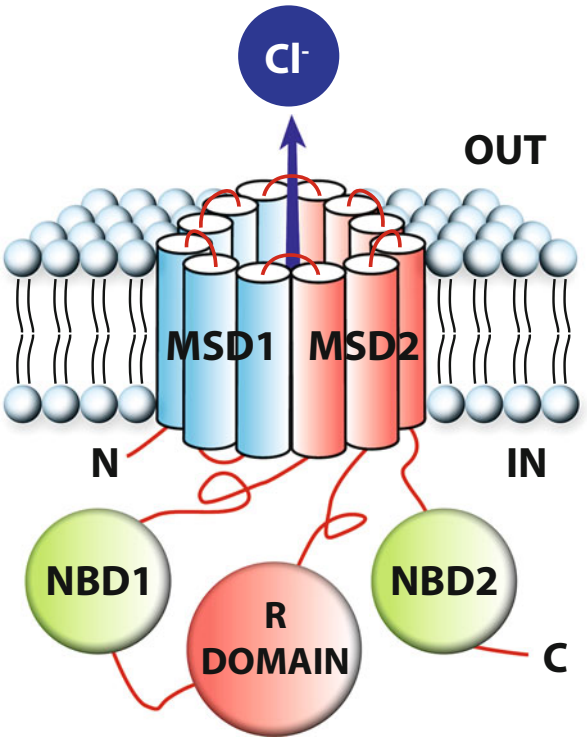

Fig. 1 Schematic structure of CFTR. The CFTR Cl^- channel consists of two homologous halves, each containing a hexa-helical membrane-spanning domain (MSD1 and MSD2) and a nucleotide-binding domain (NBD1 and NBD2). The two halves are connected by the R domain

domain (MSD1 and MSD2) and a nucleotide-binding domain (NBD1 and NBD2) (Fig. 1). The two halves are connected by the R domain (Riordan 2005). The NBDs contain conserved ATP-binding sequences: Walker A and B motifs, classifying CFTR as a member of the ATP-binding cassette (ABC) transporter family. Structural, biochemical, and functional evidence suggest that the two NBD domains interact and the ATP-binding site of one NBD is complemented by the ABC signature motif of the other (Riordan 2005).

While NBD1 folds largely cotranslationally, the native fold of NBD2 as well as CFTR is attained posttranslationally (Lukacs and Verkman 2012). Assembly of MSD1, NBD1, R domain, and MSD2 is necessary and sufficient to form the minimal folding unit of CFTR (Du and Lukacs 2009). These and other observations support the cooperative domain folding model and ensure the dynamic conformational coupling between the cytosolic NBDs and the pore-forming MSDs in the native molecule and provide a structural explanation for the cooperative domain *unfolding*, caused by cystic fibrosis (CF) mutations (Du and Lukacs 2009).

Despite interactions with several cytosolic and endoplasmic reticulum (ER) chaperones (heat shock proteins Hsp70 and Hsp90; co-chaperones, Hdj2 [DNAJ1], HsBp1, Hop, and p23; small Hsps, calnexin, and calreticulin), only 30–60% of the newly synthesized nascent CFTR attains folded conformation, presumably due to the metastable nature of NBD1 and NBD2, the slow kinetics of domain assembly, and the highly efficient ER quality control (Kim and Skach

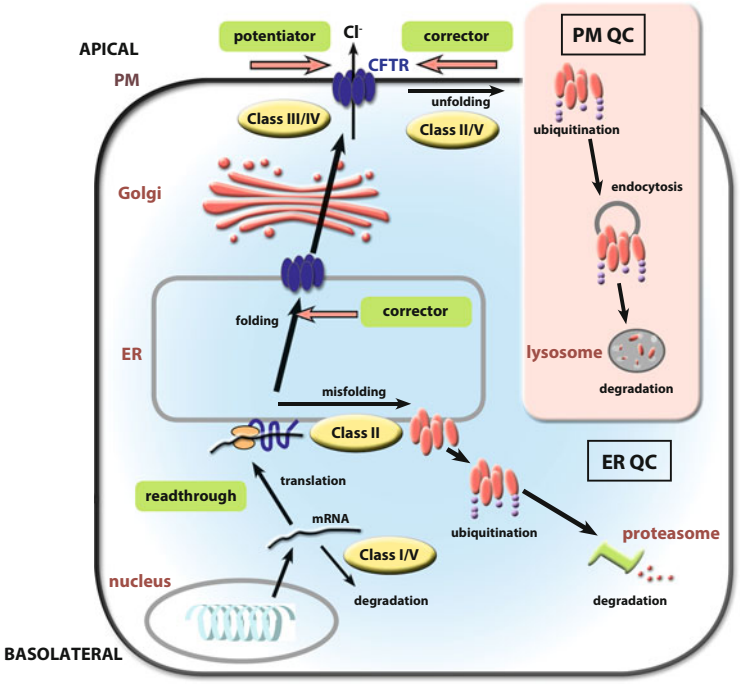

Fig. 2 Biosynthesis and maturation of CFTR. Only a fraction of newly synthesized and cotranslationally core-glycosylated channels undergoes conformational maturation in the endoplasmic reticulum (ER). The natively folded channel is then transferred by vesicular transport to the *cis*/*medial* golgi, where CFTR is complex-glycosylated before being delivered to the plasma membrane (PM). Unfolded CFTR, similar to other damaged proteins, is rapidly removed from the PM upon ubiquitination by the PM quality control (QC) system. CFTR ubiquitination accelerates internalization, impedes recycling, and facilitates preferential sorting toward lysosomal degradation. The six major classes of mutations are highlighted in *yellow* and treatment options in *green*

2012). The conformational maturation of CFTR is promoted by cytosolic ATP and the conjugation of *N*-glycan chains at the ER (Lukacs and Verkman 2012). Approximately 40–70% of the synthesized core-glycosylated channels are eliminated in the ER by the ubiquitin-proteasome system (UPS). The native CFTR that bypasses multiple checkpoints in the ER is packaged into COPII-coated transport vesicles and undergoes complex glycosylation in the *medial* Golgi before being delivered to the PM (Fig. 2) (Farinha et al. 2013).

At the PM, CFTR is constitutively internalized followed by efficient recycling from the endosomes back to the PM, which renders the channel metabolically stable (half-life [$T_{1/2}$] of ~12–14 h). In contrast, the most common mutation, p.F508del, has severalfold accelerated PM turnover ($T_{1/2} \sim 2$ h) relative to its wild-type counterpart similar to other conformationally damaged PM proteins (Okiyoneda et al. 2011). Damaged PM proteins are usually removed following their ubiquitination that confers accelerated internalization, impeded recycling, and

preferential sorting toward lysosomal degradation (Okiyoneda et al. 2011). Thus, it is plausible to assume that wild-type CFTR harboring various structural defects due to cellular stress and aging, similarly to that of the p.F508del mutant, is recognized by components of the protein homeostasis (proteostasis) machinery and is targeted by a ubiquitin- and ESCRT-dependent mechanism for lysosomal proteolysis. Multiple E3 ubiquitin ligases (CHIP, gp78, NEDD4, and c-Cbl) in cooperation with chaperones/co-chaperones (Hsc70, Hsp90, and co-chaperones, e.g., Hdj2 [DNAJ1], Bag1, HOP, and Aha1) have been implicated in the removal of damaged p.F508del and wild-type CFTR from the PM (Apaja and Lukacs 2014). Supporting the ubiquitin-dependent degradation of wild-type CFTR, the deubiquitinating enzyme (DUB) Usp10 stabilizes the channel at the PM by facilitating its recycling (Bomberger et al. 2009).

Presently, six major classes of mutations are distinguished based on their cellular pathophysiology (Fig. 2) (Zielenski 2000). Severely reduced cell surface CFTR expression is associated with (a) class I mutations which include frameshift, splicing, or nonsense mutations that introduce premature termination codons; (b) class II mutations, which lead to misfolding and impaired protein biogenesis at the ER; (c) class V mutations which result in reduced synthesis due to promoter or splicing abnormalities; and (d) class VI mutations that destabilize the channel in post-ER compartments and/or at the PM. CFTR function is selectively compromised by class III and IV mutations that can impair the gating and channel pore conductance, respectively.

1.2 Physiological Functions

In epithelial tissues, transport of anions by CFTR represents the rate-limiting step for anion (Cl^- and HCO_3^-) secretion, which ultimately controls transepithelial fluid secretion and hence hydration of the epithelial luminal surfaces (Fig. 3). In addition, through CFTR's ability to conduct HCO_3^-, as well as to regulate Cl^-/HCO_3^- exchangers belonging to the SLC26A family (Lee et al. 2012), CFTR also controls the pH of the secreted fluid. CFTR, therefore, controls both the amount and composition of epithelial secretions such as pancreatic juice, sweat, and airway surface liquid which play vital physiological roles in the innate defense of the lungs, digestion of foods, reproduction, and body temperature regulation.

A major interest in the channel stems from the fact that loss of function mutations in the gene encoding CFTR result in the inherited disease CF, one of the most common, life-shortening genetic diseases in the Caucasian population. At the other end of the spectrum and affecting far more people globally, overactive CFTR causes clinically important secretory diarrheas induced by toxins from pathogenic bacteria, such as *Vibrio cholerae*.

CFTR gating is regulated by the R domain which contains multiple PKA and PKC phosphorylation sites and which physically interacts with NBD1 (Baker et al. 2007; Gadsby et al. 2006; Hwang and Sheppard 2009; Chong et al. 2013)

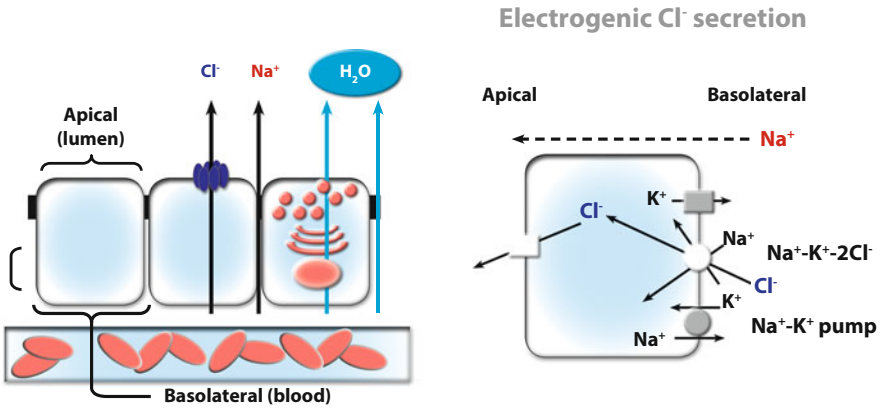

Fig. 3 Mechanism of transepithelial fluid secretion in the gastrointestinal tract. In epithelial tissues CFTR is usually expressed on the apical PM mediating the rate-limiting step for anion (chloride and bicarbonate) secretion, which ultimately controls transepithelial fluid secretion and hence hydration of the luminal surface. During electrogenic Cl^- secretion, Cl^- is transported into the cells via the basolateral membrane by the $Na^+/K^+/2Cl^-$ cotransporter that is followed by the Cl^- transport through the apical membrane via the CFTR channel

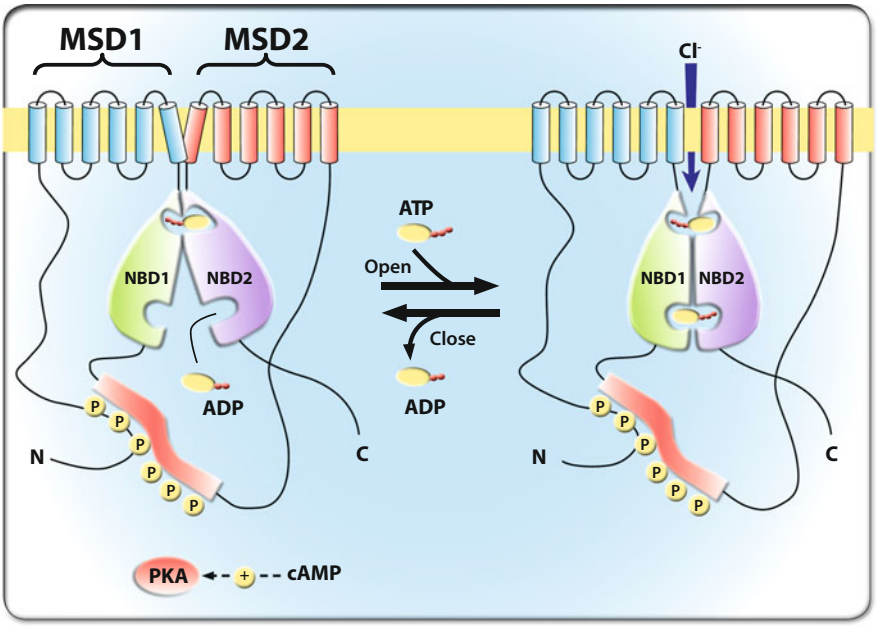

Fig. 4 Regulation of CFTR gating. CFTR gating is regulated by the R domain which contains multiple PKA and PKC phosphorylation sites and which physically interacts with NBD1. CFTR uses the energy of ATP binding and hydrolysis to drive ligand-induced conformational changes in the protein that lead to the regulated opening and closing (gating) of the channel "pore"

(Fig. 4). Uniquely, CFTR uses the energy of ATP binding and hydrolysis to drive ligand-induced conformational changes in the protein that lead to the regulated opening and closing (gating) of the channel "pore." In this way, CFTR gating tightly "regulates" the flow of anions in and out of the cell, with the anion being transported down its prevailing electrochemical gradient by a purely facilitative diffusional process.

For the channel to open, it must be first phosphorylated by PKA, and then ATP must also bind to CFTR's NBDs. Extensive biochemical, electrophysiological, structural, and molecular modeling studies support a scheme whereby following PKA phosphorylation of the R domain; CFTR gating is induced by ATP binding and dimerization of its two NBDs (Mense et al. 2006). This event is transmitted to the MSDs that form the "pore" of the channel through a complex and dynamic interaction between their cytoplasmic linker loops (coupling helices) and the dimerized NBDs (He et al. 2008). ATP (ligand) binding therefore drives the structural rearrangement of the transmembrane helices that result in the opening of the channel pore (Fig. 4). CFTR is also an ATPase (Cheung et al. 2008); hydrolysis of ATP and the release of ADP and P_i disrupt the NBD dimer thereby terminating channel opening. Further rounds of channel opening can continue if ATP binds again, but the gating cycle can be stopped by the removal of phosphate groups from the R domain by intracellular phosphatases (Gadsby et al. 2006; Hwang and Sheppard 2009). It is apparent then that CFTR gating is a complex process involving a variety of intracellular factors that regulate multiple inter- and intra-domain interactions within the protein.

1.3 Genetic Mutations

Genetic variations in *CFTR* that affect membrane levels or channel activity lead to various pancreatic phenotypes (Ooi and Durie 2012). CF with pancreatic insufficiency develops when both *CFTR* alleles harbor "severe" mutations resulting in loss of CFTR function. CF can also present with pancreatic sufficiency provided a severe mutation on one *CFTR* allele is compounded by a "mild" or "variable" mutation on the other allele, with some preservation of CFTR function. Whereas CF patients with pancreatic insufficiency do not develop acute pancreatitis, about 15–20% of pancreatic sufficient CF patients may present with acute or recurrent acute pancreatitis. The combination of a severe and a mild *CFTR* mutation can also result in idiopathic recurrent acute or chronic pancreatitis, and some of these patients exhibit abnormal sweat Cl^- or nasal potential difference (NPD) tests. Thus, a clear diagnostic line between CF-related pancreatitis and CFTR-related idiopathic chronic pancreatitis cannot be drawn. Finally, heterozygous carrier status for a CF-causing mutation increases the risk for recurrent acute and chronic pancreatitis. The discovery that *CFTR* variants are overrepresented in chronic pancreatitis was first published in 1998 (Sharer et al. 1998; Cohn et al. 1998) and was confirmed by a large number of follow-up studies (e.g. Weiss et al. 2005; Cohn

et al. 2005; Bishop et al. 2005; Noone et al. 2001; Ockenga et al. 2000; Truninger et al. 2001). However, many of these were limited by the use of small cohorts, the lack of screening unaffected controls, and incomplete analysis of the *CFTR* gene resulting in some controversy regarding the extent of disease risk, the role of non-CF-causing variants and the significance of common polymorphic variants, and their associated haplotypes. Recently published analyses of large German (660 cases), French (253 cases), and North-American (984 cases) cohorts (Masson et al. 2013; Rosendahl et al. 2013; LaRusch et al. 2014) confirmed earlier findings and strengthened the consensus on the role of *CFTR* variants in chronic pancreatitis.

Heterozygous carriers of the severe p.F508del *CFTR* mutations are overrepresented in patients with chronic pancreatitis (~7%) relative to healthy controls (~3%). Thus, CF carrier status confers a small risk for chronic pancreatitis (odds ratio ~2.5). Other types of severe mutations are less frequently found in chronic pancreatitis but seem to be associated with similar disease risk as p.F508del.

Heterozygous carriers of the mild *CFTR* mutations p.R117H are enriched among cases with chronic pancreatitis (~2.5%) relative to controls (~0.6%). Mutation p.R117H appears to cause comparable or even slightly higher risk (odds ratio ~4) than the severe p.F508del mutation, for reasons that are not readily apparent. With respect to the CF phenotype, the penetrance of p.R117H is influenced by the length of the poly-T tract in intron-8 (legacy numbering); however, such conclusion cannot be drawn for chronic pancreatitis on the basis of available data. Other mild or variable CF mutations are rare in chronic pancreatitis but are likely to confer similar risk as p.R117H.

Compound heterozygous genotypes composed of severe and mild *CFTR* alleles are strong risk factors for chronic pancreatitis and may be considered causative (Masson et al. 2013). The odds ratio of 16 described for a large German cohort is likely a conservative estimate for disease risk (Rosendahl et al. 2013). Due to some uncertainty which mutations can be considered mild, the reported frequency of compound heterozygotes in chronic pancreatitis cases has been highly variable. After exclusion of T5 allele carriers (see below), neutral mutations as given in the CFTR2 database (http://cftr2.org), and mutations of unknown clinical significance, the prevalence of compound heterozygotes was 1.4% in a German cohort (Rosendahl et al. 2013) and 2.4% in a French cohort (Masson et al. 2013). Compound heterozygosity for two mild *CFTR* mutations is rare and probably increases pancreatitis risk strongly, although solid data are lacking.

Non-CF-causing *CFTR* variants are not overrepresented in chronic pancreatitis cases. A recent study suggested that some non-CF-causing variants (e.g., p.R75Q, p.L997F) may be risk factors for chronic pancreatitis due to a selective defect in HCO_3^- conductance (LaRusch et al. 2014). While functional measurements seem to support this contention, a genetic association between these variants and chronic pancreatitis cannot be confirmed (Masson et al. 2013; Rosendahl et al. 2013; LaRusch et al. 2014; Martinez et al. 2014).

Common polymorphic *CFTR* alleles do not modify pancreatitis risk. There are two adjacent polymorphic regions (poly-T and poly-TG tracts) in intron-8 which can alter inclusion of exon-9 (legacy numbering) during splicing (Chu et al. 1993;

Cuppens et al. 1998; Groman et al. 2004). A short poly-T tract (c.1210-12T[5], legacy name T5) in combination with a longer poly-TG tract (c.1210-34TG[12], legacy name TG12) results in more transcripts lacking exon-9 that give rise to inactive CFTR protein. A T5 allele combined with a severe *CFTR* mutation may cause CF and increases the penetrance of the mild p.R117H mutation. Studies on smaller cohorts reported enrichment of the T5 allele in chronic pancreatitis (Weiss et al. 2005; Bishop et al. 2005; Noone et al. 2001; Arduino et al. 1999); however, more recent analyses on larger cohorts did not find such overrepresentation (Rosendahl et al. 2013; LaRusch et al. 2014). When analyzed separately, the distribution of the TG12 allele between chronic pancreatitis cases and controls was also equal (Rosendahl et al. 2013). Whether or not the T5-TG12 complex allele confers a small risk in chronic pancreatitis remains to be determined. Curiously, small but measurable differences were reported for the distribution of the TG10 and TG11 alleles between chronic pancreatitis cases and controls, either analyzed separately or as part of conserved haplotypes (Rosendahl et al. 2013; Steiner et al. 2011). In two studies, TG10 appeared to increase disease risk slightly, while TG11 seemed to be protective. Since the two studies used overlapping cohorts and the results clearly contradict the expected functional effects of the TG10 and TG11 tracts, cautious interpretation of the findings is warranted until independent confirmation (Table 1).

Chronic pancreatitis is a complex genetic disease, and mutations in different risk genes are often found in cases. Transheterozygosity of *CFTR* variants with mutations in *PRSS1*, *SPINK1*, and *CTRC* was reported (Weiss et al. 2005; Noone et al. 2001; Rosendahl et al. 2013; LaRusch et al. 2014; Masson et al. 2013). The combined effects of multiple pathogenic mutations result in strongly amplified disease risk, best documented for the co-occurrence of *SPINK1* and *CFTR* mutations. There is no compelling evidence for epistasis between *SPINK1* and *CFTR* mutations; the two susceptibility genes seem to act independently in determining the pancreatitis phenotype (Rosendahl et al. 2013). *CFTR* mutations can also contribute to the increased clinical penetrance of anatomical risk factors for chronic pancreatitis such as pancreas divisum (Bertin et al. 2012).

Table 1 The effect of CFTR mutations on the risk for chronic pancreatitis

CFTR alleles	Pancreatitis risk	Examples
Severe/mild	High risk, causative	p.F508del/p.R117H
Mild/mild	Likely high risk	p.R117H/p.R117H
Severe/–	Low risk, 2.5-fold	p.F508del/–
Mild/–	Low risk, 4-fold	p.R117H/–
Non-CF causing	No risk	p.R75Q, p.L997F
T5	No risk	
TG12	No risk	
T5-TG12	Limited data	

2 Pancreatic Damage in CF

2.1 Experimental Observations in CF Models

A CF animal model that recapitulates the human findings provides a powerful tool to study disease pathophysiology and design therapies. There are five main CF animal models with pancreatic manifestations (Table 2).

2.1.1 Mice

CF mouse models were generated by disrupting the endogenous *Cftr* gene in embryonic stem cells using the homologous recombination technology (Snouwaert et al. 1992; Clarke et al. 1992). In general, intestinal disease is the hallmark of CF mouse models with minimal or no pathological changes in the pancreas. The lack of pancreatic disease may be due to developmental/structural differences in the rodent pancreas, relatively low CFTR expression with higher expression of alternative anion channels, modifier genes, and/or the short life-span of CF mice (Gray et al. 1994; Olivier et al. 2015). This model is an important tool to study the intestinal disease in CF without the confounding effects of pancreatic disease.

2.1.2 Rats

Recently, a CF rat model was generated using zinc finger endonuclease technology (Tuggle et al. 2014). Animals demonstrated CFTR channel dysfunction in airway epithelia, histological abnormalities in the ileum, and absence of vas deference, all characteristics of CF. The CF rats did not have any evidence of pancreatic involvement within the first 6 weeks of life.

2.1.3 Pigs

The CF porcine model was generated by disrupting the *CFTR* gene in pig fibroblasts followed by somatic cell nuclear transfer (Rogers et al. 2008). CF pigs have multisystem disease that recapitulates the human disease and exhibit a severe pancreatic phenotype. The pancreatic lesions start in utero and progress over time in this model (Abu-El-Haija et al. 2012), in concordance with the previous autopsy studies of humans with CF (Andersen 1938). The newborn CF pig pancreas has acinar cell loss, duct proliferation, dilated acini/ducts with eosinophilic material, expansion of interlobular connective tissue, and scattered inflammatory cell aggregates (Rogers et al. 2008; Abu-El-Haija et al. 2012). Mucous cell metaplasia is a late finding. In older animals, the exocrine pancreatic lesions progress and the pancreas is mostly replaced by fat and fibrosis. The islets are morphologically

Table 2 Characteristics of pancreatic lesions in CF animal models and humans

	In utero	Birth	Juvenile/adult
Mouse	Unknown	No or minimal disease; high mortality at weaning if intestinal obstruction is left untreated	No or minimal disease
Rat	Unknown	Unknown but probably normal	Normal pancreas histology at 22–44 days of age
Pig	Inflammation, dilated acini/ducts with eosinophilic periodic acid-Schiff (PAS)-positive material, acinar cell loss	Acinar atrophy progresses; acini/ducts are filled with eosinophilic material; duct cell proliferation, mucus cell metaplasia, increased loose connective tissue; normal islet morphology but abnormal glycemic response and decreased insulin secretion; high mortality from meconium ileus and intestinal obstruction/perforation, ameliorated in gut-corrected animals	Acinar cell loss, dilated ducts with inspissated material, duct cell proliferation, mucus cell metaplasia, fatty infiltration and fibrosis; normal islet morphology but animals develop spontaneous hyperglycemia
Ferret	Unknown	Minimal disease; dilation of most acini and ductules with inspissated, eosinophilic secretions, no inflammation; high mortality from intestinal disease, gut-corrected CF ferrets are available	Rapid destruction of the pancreas with progressive inflammation, acinar atrophy, fibrosis, islet cell loss
Zebrafish	Not applicable	Acinar cell loss starts at 14–16 days postfertilization (dpf), neutrophil infiltration is present and seems to have an impact on acinar damage, high mortality	Acinar atrophy, duct dilatation with PAS-positive material, fibrosis; disorganized islets appearing smaller and more numerous than those in wild type
Humans	Pancreatic phenotype correlates with the severity of *CFTR* mutation. First lesions identified ~ 17 weeks of gestation as deposits of PAS-positive material within some acini and ductules. Pancreatic lesions progress to include degeneration of acinar	Depending on the severity of *CFTR* mutation, pancreatic lesions progress to acinar atrophy, fatty infiltration, and fibrosis	Depending on the severity of *CFTR* mutation, pancreatic lesions progress to acinar atrophy, fatty infiltration, and fibrosis. Partial islet cell loss is a late finding. With increased age, CF-related diabetes develops. Life expectancy is ~37 years

(continued)

Table 2 (continued)

	In utero	Birth	Juvenile/adult
	cells and lack of zymogen granules, dilatation of acini and ducts filled with inspissated PAS-positive material and ductular proliferation. Inflammation is mild to moderate. Islets are normal morphologically		

intact but functionally abnormal (Uc et al. 2015). As in humans (Kopelman et al. 1985), the pancreatic fluid is acidic, low in volume and high in protein, and concentrated in CF pigs at birth (Uc et al. 2012). The proinflammatory, complement cascade, proapoptotic, and profibrotic pathways are activated in CF pig pancreas and likely contribute to the destructive process (Abu-El-Haija et al. 2012).

2.1.4 Ferrets

CF ferrets were generated using homologous recombination as described for CF pigs. The pancreatic lesions are mild in CF ferrets at birth compared to CF pigs (dilation of most acini and ductules with inspissated, eosinophilic secretions). Interestingly, the exocrine pancreas undergoes rapid destruction over the first month of life (Sun et al. 2010), leading to acinar loss, fibrosis, and pancreatic insufficiency. Even though CF ferrets have only mild exocrine pancreatic disease at birth, they still manifest significant problems in glucose tolerance and insulin secretion (Olivier et al. 2012). Fifteen percent of ferrets are pancreatic sufficient and have normal pancreas, suggesting a possible role of genetic modifiers. The CF ferret model is emerging as an important tool to investigate the early pathogenesis of CF-related diabetes.

2.1.5 Zebrafish

Recently a zebrafish model of CF has been described (Navis and Bagnat 2015). The development of the pancreas is normal initially, followed by rapid destruction during larval life via a process that involves neutrophil infiltration. The zebrafish model along with pig and ferret models underlines the importance of inflammation in pancreatic damage.

Phenotypic differences among the various CF models may be due to species-specific CFTR processing, genetic and environmental influences, and effects of

other ion channels. These animal models offer a unique opportunity to study the pathogenesis of CF pancreatic disease and various therapeutic approaches.

2.2 Clinical Characterization of Pancreatic Function in CF

The consequences of mutations in the *CFTR* gene have been demonstrated by pancreatic function studies which show that CF patients have low-flow secretions with a high protein concentration, which can precipitate in the duct lumina causing obstruction and damage. These changes begin in utero, and after delivery the process of small duct obstruction leading to large duct obstruction continues. At birth and for several months afterward, there is a release into the blood stream of proteins originating in the pancreas. An example of this is immunoreactive trypsinogen (IRT) which serves as the basis for the neonatal screening test for CF. Interestingly, despite the wholesale destruction of the exocrine pancreas occurring, the infant is asymptomatic; the reason for this is yet to be determined. Eventually, this process results in severe inflammatory changes, obstruction of ducts by mucus and calcium-containing debris, destruction of acini, and generalized fibrosis. Contrary to popular belief that the pancreas is entirely nonfunctioning at birth, the high IRT does show that some exocrine pancreatic tissue is still present and this may have a bearing on possible small molecule therapy targeted at the remainder of the pancreas which may rescue enough tissue to cause viability of the remaining pancreas.

One of the most remarkable observations is that genetic factors exquisitely influence the degree of pancreatic disease and its rate of progression. Large studies of CF patients resulted in their classification as pancreatic insufficient (PI) or pancreatic sufficient (PS). PI patients comprise of 85% of all CF patients and have maldigestion as defined by evidence of steatorrhea following 72-h fat balance studies; these patients require pancreatic enzyme replacement therapy with meals. In contrast, PS patients have evidence of pancreatic damage (as shown by the high neonatal IRT test) but retain sufficient endogenous exocrine pancreatic function to sustain normal digestion.

Exocrine pancreatic status is directly linked to genotype (Kristidis et al. 1992). Analysis of particular CFTR mutations in patients with these pancreatic phenotypes (PI vs. PS) revealed two categories of alleles: “severe” and “mild.” Patients who are homozygous or compound heterozygous for severe alleles belonging to classes I, II, III, or VI exhibit pancreatic insufficiency, whereas a mild class IV or V allele sustains pancreatic function in a dominant fashion, even if the second mutation is severe. This observation finds plausible explanation since all known mild alleles belong to class IV or class V, all of which are (or predicted to be) associated with some residual channel activity at the epithelial apical membranes. However, this classification system is not entirely consistent as there are some class I mutations with a stop codon at the end of the gene which are in fact PS. A small proportion (2–3%) of patients carrying severe mutations on both alleles are PS at diagnosis, but

most experience gradual transition from PS to PI. A few missense mutations (e.g., p.G85E) confer a variable pancreatic phenotype.

While mild mutations confer sufficient CFTR function to prevent complete pancreas destruction, many PS patients have reduced exocrine pancreatic capacity and are at an increased risk of pancreatitis. Recurrent acute and chronic pancreatitis is a relatively infrequent complication of CF first reported by Shwachman in 1975 (Shwachman et al. 1975). In this retrospective study, only 0.5% of CF patients had pancreatitis. More recently Durno et al. reported that in a cohort of over 1000 patients, followed over a period of 30 years, the incidence was 1.7% (Durno et al. 2002). All the patients with pancreatitis were PS. In fact, this subgroup of PS patients appears to be highly susceptible to pancreatitis, since almost one in five was affected by this complication. In the largest study to date of CF PS patients, Ooi et al. in a seminal paper determined the association between severity of CFTR genotype and the risk of pancreatitis (Ooi et al. 2011). They examined a large cohort of 277 PS patients from two CF centers of which 62 had well-documented pancreatitis. Using a novel pancreatic insufficiency prevalence score, the mutations were divided into three main groups, severe, moderate-severe, and mild. They found that the proportion of patients who developed pancreatitis was significantly greater for genotypes in the mild group than the moderate-severe group. Thus, the more mild mutations are associated with increased risk of pancreatitis.

The sweat Cl^- and NPD results in patients with pancreatitis ranged from the values for healthy controls and obligate heterozygotes to the values for CF patients with PS and PI. Median sweat Cl^- and NPD results in patients with no mutation or one mutation were clustered with values obtained in controls and obligate heterozygotes. In contrast, in patients with pancreatitis carrying *CFTR* mutations on both alleles, median ion transport values were intermediate between those of the controls and obligate heterozygotes and those of PS CF patients. Some individual values overlapped with the CF patients, and in 21% of patients, the diagnosis of CF could be confirmed by abnormal ion channel measurements. Thus, CFTR-mediated ion channel abnormalities are influenced by the number or severity of the *CFTR* mutations and show a range of abnormalities similar to those in patients with mild or severe classic CF at one extreme and controls and obligate CF heterozygotes on the other. This continuum of electrophysiological abnormalities is not surprising as PS patients have a 17% risk of developing pancreatitis and many of these presentations are in adulthood.

Similar observations have been made in individuals with other CF-like phenotypes, such as men with infertility due to congenital bilateral absence of the vas deferens, who are known to carry a high frequency of *CFTR* mutations (Wilschanski et al. 2006). A relatively large population was examined, and similar to the patients with idiopathic pancreatitis, a wide range of electrophysiological abnormalities was observed. Abnormalities of CFTR function correlated closely with the number and severity of *CFTR* mutations.

3 Effects of Pancreatitis-Inducing Factors on CFTR

The two most common causes of pancreatitis are alcohol abuse and biliary disease. Since only a small fraction of alcoholics and patients with biliary disease ever develops pancreatitis, it is evident that there is individual susceptibility. Other intrinsic (genetic) and extrinsic factors (e.g., smoking, diet) must also be responsible for the induction of the disease. Furthermore, the importance of nonoxidative ethanol metabolites such as fatty acid ethyl esters as well as cytotoxic fatty acids in acute pancreatitis is now being increasingly recognized (Huang et al. 2014).

3.1 Ethanol and Fatty Acids

The potential role of CFTR in pancreatitis is highlighted by the fact that genetic deletion of the apical Cl^- channel increased the severity of alcohol-induced acute pancreatitis in the mouse (Maléth et al. 2015). Furthermore, patients with alcoholic acute pancreatitis had lower levels of CFTR in their pancreatic ducts than control subjects (Maléth et al. 2015). Using numerous complimentary techniques, we demonstrated that ethanol and fatty acids dose-dependently reduced CFTR expression and activity in pancreatic ductal epithelial cells and inhibited secretion of fluid and HCO_3^- (Maléth et al. 2015; Judák et al. 2014). The oxidative ethanol metabolite acetaldehyde had no such effects. The decrease in CFTR expression and PM density in response to ethanol, palmitoleic acid, or palmitoleic acid ethyl ester administration was caused by accelerated channel turnover at the apical membrane and by aberrant protein folding (Maléth et al. 2015). The inhibition of CFTR by ethanol and fatty acids was associated with a sustained increase in concentrations of intracellular Ca^{2+} and cAMP, depolarization of mitochondrial membranes, and depletion of ATP. Supplementation with ATP almost completely prevented inhibition of CFTR activity by ethanol and fatty acids (Judák et al. 2014). These findings are in accord with those of Yamamoto et al. (Yamamoto et al. 2003), who found that 0.3–30-mM ethanol augmented, whereas 100-mM ethanol inhibited secretin-stimulated pancreatic ductal fluid secretion in the guinea pig. Notably, ethanol and fatty acids also evoke a rise in the cytosolic Ca^{2+} concentration and cause depolarization of the mitochondrial membrane and ATP depletion in pancreatic acinar cells, which served as the basis for the above described studies (Criddle et al. 2006; Criddle et al. 2004).

3.2 Bile Acids

Bile acids are probably the most important components of the bile which are predominantly synthesized in the liver. In humans, the most abundant primary

bile acids are chenodeoxycholate and cholate that are conjugated with taurine or glycine.

Pancreatic ductal HCO_3^- secretion is likely to be markedly reduced in patients with biliary acute pancreatitis (Takács et al. 2013). Furthermore, under experimental conditions, luminal administration of a low dose of chenodeoxycholate (0.1 mM) stimulated ductal HCO_3^- secretion (Venglovecz et al. 2008) which was caused by significantly elevated apical Cl^-/HCO_3^- exchange activity in guinea pig ducts (Venglovecz et al. 2008) and human CFPAC-1 pancreatic duct cells (Ignáth et al. 2009). This stimulatory effect was dependent on Ca^{2+} signaling and CFTR expression; however, it is unlikely to be the result of increased Cl^- conductance, since chenodeoxycholate administration did not activate CFTR Cl^- channel activity in guinea pig pancreatic duct cells. High concentrations (1 mM) of chenodeoxycholate administered from the basolateral or luminal membranes strongly inhibited pancreatic ductal HCO_3^- secretion by causing severe mitochondrial damage (Venglovecz et al. 2008; Maléth et al. 2011). The consequent ATP depletion will affect ATP-dependent transporters like the Na^+/K^+ pump, K^+ channels, and CFTR.

3.3 Smoking

Data on the effects of smoking on CFTR in the pancreas are scarce and contradictory. Pancreatic CFTR mRNA expression in rats exposed to chronic smoke inhalation was increased in animals that developed inflammatory signs upon cigarette smoke exposure compared with animals not showing morphological pancreatic damage (Wittel et al. 2006). In another study, it has been shown in human subjects that cigarette smoking impairs secretin-stimulated pancreatic ductal secretion which implicates the role of CFTR in the process (Kadiyala et al. 2013). Our preliminary data also support this notion since cigarette smoke extract dose-dependently inhibited pancreatic ductal CFTR activity and HCO_3^- secretion in guinea pigs. In fact, the latter results are in accord with those found in other organs such as the airway (Bagheri-Hanson et al. 2014) or intestinal epithelial cells (Raju et al. 2013). It seems that CFTR dysfunction due to smoking is primarily an acquired phenomenon and is not affected by the presence of heterozygous *CFTR* mutations (Raju et al. 2014). The interpretation of these results, at least with respect to the compounds responsible for the inhibitory effect of CFTR, is difficult as cigarette smoke is estimated to contain more than 7000 chemical components. Nevertheless, several constituents of cigarette smoke have been implicated in the inhibitory effects on CFTR such as acrolein (Raju et al. 2013), cadmium, and manganese (Hassan et al. 2014). Moreover, these effects were ameliorated by the use of various antioxidants like *N*-acetylcysteine (a known scavenger of acrolein) or alpha-tocopherol, suggesting that free radical-induced damage may be responsible for the inhibition of CFTR by cigarette smoke (Rab et al. 2013).

Interestingly, CFTR is also prominently involved in maintaining the antioxidant glutathione levels in the basal epithelial lining fluid which is also decreased by cigarette smoke (Gould et al. 2012). Thus, cigarette smoke may further reduce the antioxidant defense system in a vicious circle. Cigarette smoke exposure has also been reported to induce CFTR internalization and insolubility (Clunes et al. 2012). Taken together, these data strongly suggest that CFTR could be a therapeutic target in smoking-related diseases. In fact, drugs which increase CFTR function (the CFTR potentiator ivacaftor and the phosphodiesterase 4 inhibitor roflumilast) were found to have beneficial effects on cigarette smoke-induced Cl^- channel inhibition (Lambert et al. 2014; Sloane et al. 2012).

4 CFTR Dysfunction in Pancreatitis

4.1 Acute Pancreatitis

An association between CFTR and the pathogenesis of acute pancreatitis has been presumed for a long time. Impaired CFTR expression in *Cftr*$^{-/-}$ and p.F508del *Cftr* mice resulted in continuous overexpression of proinflammatory cytokine genes and more severe acute pancreatitis upon cerulein hyperstimulation (DiMagno et al. 2005, 2010). The *Cftr*$^{-/-}$ and p.F508del *Cftr* mice displayed elevated pancreatic edema, neutrophil infiltration, and increased mRNA expression of multiple inflammatory mediators. However, induction of acute pancreatitis in *Cftr*$^{-/-}$ mice did not cause acinar cell injury but rather decreased acinar cell apoptosis with mild exocrine pancreatic insufficiency (DiMagno et al. 2005; DiMagno et al. 2010). Although the authors focused on the alterations in acinar cells function, CFTR is expressed in the pancreatic duct, and thus, the increased severity of pancreatitis is most likely due to compromised ductal fluid and HCO_3^- secretion. Recently, we investigated the role of CFTR in the pathogenesis of acute alcohol-induced pancreatitis and found markedly reduced in vivo pancreatic fluid secretion in control and *Cftr*$^{-/-}$ mice treated with ethanol and fatty acids (Maléth et al. 2015). The *Cftr*$^{-/-}$ mice also displayed more severe acute pancreatitis induced by i.p. injection of ethanol and palmitic acid, including more extensive necrosis (Maléth et al. 2015). The role of CFTR in acute pancreatitis was further demonstrated in a study examining the scaffolding protein Na^+/H^+ exchanger regulatory factor-1 (NHERF-1) in ductal function (Pallagi et al. 2014). Deletion of *Nherf1* reduced expression of CFTR at the apical membrane of the duct and consequently reduced fluid and HCO_3^- secretion. Furthermore, it resulted in more severe acute pancreatitis induced by cerulein hyperstimulation or sodium taurocholate in mice (Pallagi et al. 2014). These findings have potential clinical relevance, since we detected markedly decreased CFTR protein and mRNA expression in small pancreatic ducts in tissue samples from patients diagnosed with alcohol-induced acute pancreatitis (Maléth et al. 2015).

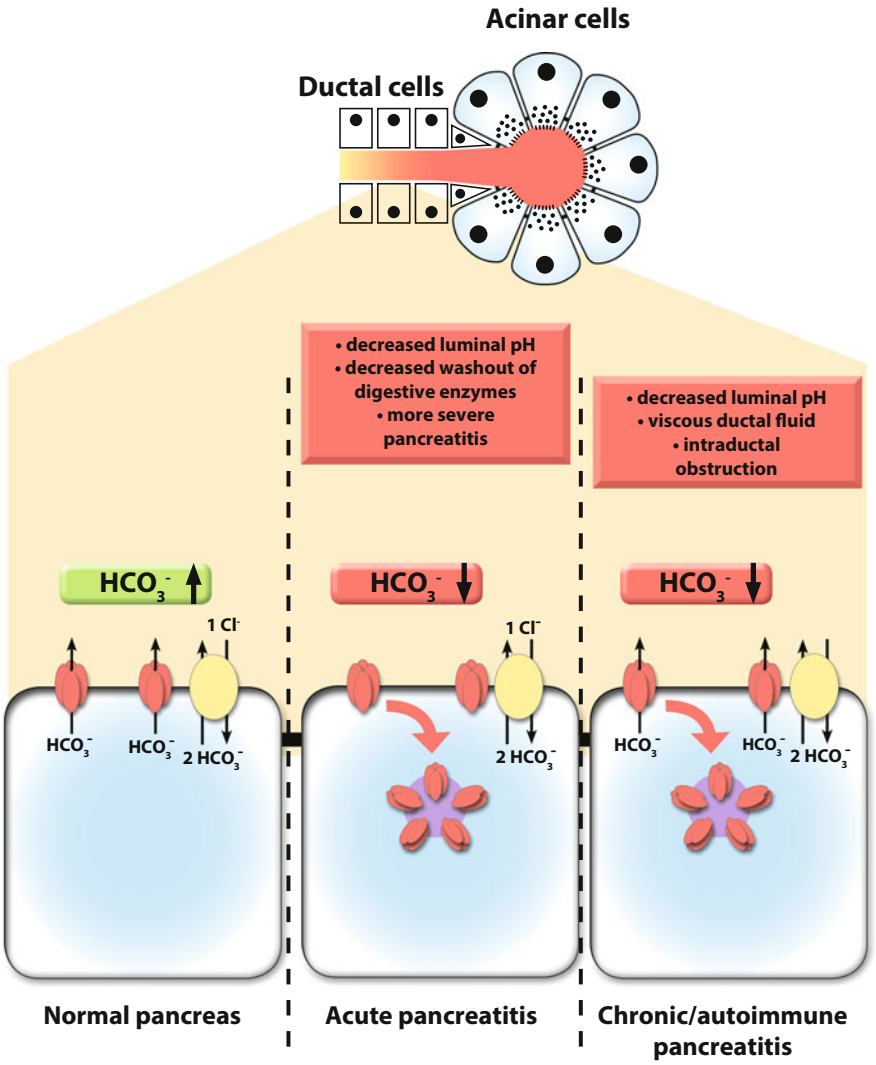

Fig. 5 CFTR dysfunction in pancreatitis. Under physiological conditions (*left*), CFTR (*red*) is expressed on the luminal membrane of small inter/intralobular pancreatic ducts with the SLC26A6 Cl^-/HCO_3^- exchanger (*yellow*). This expression and the close connection of the two proteins are required for the maintenance of the alkaline luminal pH. During acute pancreatitis (*middle*) the function of CFTR is inhibited, and its expression is decreased leading to impaired bicarbonate and fluid secretion and consequently decreased luminal pH. The washout of the activated digestive enzymes is insufficient. Overall, these changes together will increase the severity of acute pancreatitis. In chronic or autoimmune pancreatitis (*right*), CFTR expression is markedly decreased leading to the described consequences. The more viscous, protein-rich ductal fluid promotes the formation of intraluminal protein plugs. The intraductal obstruction will result in pancreatic atrophy and exocrine pancreatic insufficiency

Together, these observations suggest that diminished CFTR function, due to either reduced expression or activity, may have crucial importance in the pathogenesis of pancreatitis (Fig. 5).

4.2 Chronic Pancreatitis

CFTR dysfunction due to mislocalized protein expression in pancreatic ductal cells has been observed in alcoholic, obstructive, autoimmune, and idiopathic chronic pancreatitis (Maléth et al. 2015; Ko et al. 2010). In addition to the direct effects of toxic factors (see section III), indirect mechanisms such as inflammation and necrosis likely contribute to impaired CFTR expression. Importantly, the decreased expression of CFTR observed in chronic pancreatitis is likely the cause of impaired ductal function during pancreatitis (Ko et al. 2010). Aberrant expression of CFTR leads to diminished fluid and HCO_3^- secretion due to a reduction in the interrelated activity of both the electrogenic $1Cl^-/2HCO_3^-$ exchanger SLC26A6 and CFTR. This leads to decreased intraluminal pH, decreased washout of the digestive enzymes, and more viscous protein-rich ductal fluid (Ko et al. 2012) (Fig. 5). These changes promote the formation of intraluminal protein gel or plugs, which are early histological features of chronic pancreatitis (Sarles et al. 1965). Intraductal obstruction can lead to pancreatic atrophy, ductal mucinous hyperplasia (Allen-Mersh 1985; Hegyi and Petersen 2013), goblet cell metaplasia, and perhaps pancreatic stone formation (Ko et al. 2012).

5 Future Perspectives

5.1 Current Treatment Options

In the 26 years since the discovery of the gene that causes CF, our understanding of how mutations in *CFTR* cause the varied pathophysiological manifestations of this disease has increased substantially. This knowledge has led to the possibility of new therapeutic approaches aimed at the basic defect. This section will summarize the current state of mutation-specific therapy and will focus on orally bioavailable potentiators, correctors, and suppressors of *CFTR* gene mutations.

5.1.1 Gene Therapy

After the *CFTR* gene was discovered, there was considerable enthusiasm that gene therapy could be rapidly developed. Indeed, CF has had among the highest number of gene therapy trials. Several vector systems have been tested in human trials

including adenoviruses, adeno-associated viruses, and cationic lipids. Despite optimism from in vitro and initial in vivo studies, the trials have hitherto been unsuccessful. The most ambitious gene therapy study in the United Kingdom which randomized 140 patients using a cationic lipid-based vector showed a modest improvement in lung function (Alton et al. 2015).

5.1.2 Mutation Class-Specific Therapy

An alternative to gene therapy are compounds that affect synthesis, trafficking, and channel function of CFTR. The classification of *CFTR* mutations shown in Fig. 2 enabled researchers to consider targeting distinct groups of patients.

5.1.3 Class I Mutations

This class of mutations includes premature termination codons (PTCs) or nonsense codons. Nonsense mutations are responsible for about 10% of CF cases worldwide. However, in Israel nonsense mutations are the cause of CF in most patients (Kerem et al. 1997). Because such mutations produce little functional CFTR, these patients usually have a severe CF phenotype. The increased understanding of ribosomal function, the process of translation, and small molecules that change the interaction between the ribosome and mRNA have led to the identification of several agents that are capable of suppressing PTCs. This has resulted in a novel strategy to treat CF and other genetic disorders caused by PTCs by restoring full-length protein expression. Aminoglycoside antibiotics were the first drugs demonstrated to suppress PTCs in disease-causing mutations, allowing the translation of full-length proteins (Hermann 2007). In 1996, Howard et al. (Howard et al. 1996) described PTC suppression by the synthetic aminoglycoside Geneticin (G418) to restore function in HeLa cells expressing nonsense codons. This pivotal work was extended to four nonsense mutations of the *CFTR* gene that were expressed in the human airway cell line IB3-1. In this study the commonly used aminoglycoside, gentamicin, was incubated with these cells and full-length protein was produced (Bedwell et al. 1997). The preclinical studies mentioned above have led to a number of clinical trials designed to test both proof of principle and efficacy in patients with genetic diseases caused by PTCs (Table 3).

As stated earlier, about 60% of CF patients in Israel carry PTCs or class I mutations. Either nasal or intravenous administration of gentamicin improved NPD (Wilschanski et al. 2000, 2003; Clancy et al. 2001; Sermet-Gaudelus et al. 2007). In all of these studies, there was a variability of response with some patients not responding to gentamicin. Linde et al. (Linde et al. 2007) showed that this variability may be related to nonsense-mediated mRNA decay (NMD) – the major machinery evolved to protect against harmful products of nonsense mutations. This is a posttranscriptional translation-dependent surveillance mechanism that prevents the synthesis of proteins carrying PTCs. NMD has been shown to

Table 3 Clinical trials in CF (class I mutations)

	Type of administration	Type of study	Results	References
Gentamicin	Nose drops	Pilot	Significant improvement of NPD	Kerem et al. (1997)
		Double-blind, placebo-controlled	Significant improvements of NPD and chloride secretion compared with placebo. Positive immunofluorescent staining in the treatment group	Hermann (2007)
			Specific for patients with class I mutations with no effect in the control group of patients homozygous for the p.F508del mutation	
			Vast majority of patients with PTCs expressed at least one copy of the p.W1282X CFTR mutation	
	Intravenous	Pilot	Significant improvement of NPD	Howard et al. (1996)
		Pilot	Significant improvement of NPD in CF patients carrying the p.Y122X mutation	Bedwell et al. (1997)
Ataluren	Oral	Phase II clinical trial	Two consecutive 28-day cycles, each of 14 days of treatment followed by 14 days of washout.	Sermet-Gaudelus et al. (2007)
			Significant improvement of NPD	
			Modest but statistically significant improvements in lung function and bodyweight	
		Open-label extension study	NPD improvements were reported over time in both the higher- and lower-dose treatment groups including 4 patients who did not respond to ataluren in the 2-week study (see above)	Linde et al. (2007)
			Modest improvements in pulmonary function and a significant reduction in quantitative cough assessment	
		Phase II clinical trial	Significant improvement in NPD and nasal epithelial CFTR protein by immunofluorescence	Welch et al. (2007)
		Phase III clinical trial	Disappointing results, no difference in FEV_1 between the ataluren and placebo groups	Du et al. (2008)
			In patients not receiving chronic inhaled tobramycin: The mean pulmonary exacerbation rate was 40% lower in the ataluren arm	

(continued)

Table 3 (continued)

	Type of administration	Type of study	Results	References
			Similar difference in mean relative change from baseline in % predicted FEV_1 at week 48 was 5.7% favoring ataluren with a mean change from baseline of −0.7% in the ataluren arm, and 6.4% in the placebo arm was observed	
			In patients who received chronic inhaled tobramycin: No significant difference in mean relative change from baseline in % predicted FEV_1 at week 48 between ataluren and placebo	
			In patients who received other antibiotics (e.g., colistin and aztreonam): No modification in the treatment effect of ataluren	

degrade transcripts carrying disease-causing nonsense or frameshift mutations. It is the efficiency of NMD which affects the level of transcripts carrying PTCs, which govern the response to read-through treatment. Response to gentamicin was found only in patients with a higher level of transcripts (Linde et al. 2007). Downregulation of NMD in cells carrying the p.W1282X mutation increased the level of CFTR nonsense transcripts and enhanced the CFTR Cl^- channel activity in response to gentamicin. This may have a critical clinical correlation in the read-through of PTCs in various diseases. However, the inconvenience of parenteral administration and the potential for serious toxic effects preclude long-term systemic use of gentamicin for suppression of nonsense mutations.

Another PTC suppressor ataluren was developed through an extensive high-throughput screening program using a luciferase-based system (Welch et al. 2007). The molecule is a 1,2,4 oxadiazole benzoic acid and is reported to interact with mammalian ribosomes in a manner distinct from aminoglycosides. Ataluren does not have antibiotic activity and is orally bioavailable. Studies in myocytes isolated from the *mdx* mouse defined target doses and exposures to rescue dystrophin function. After treatment with ataluren, full-length dystrophin was localized in skeletal and cardiac tissue. In the p.G542X-hCFTR mouse oral and intraperitoneal administration led to detectable full-length CFTR localization at the apical cell membrane of intestinal glandular cells by immunofluorescent staining together with improved Cl^- conductance as assayed by transepithelial ion transport (Du et al. 2008). Correction of CFTR Cl^- transport was incomplete. Less than 30% of the short-circuit current that was observed in wild-type mice occurred in the

treated CF mice. This suggests that potential clinical benefit would only need partial restoration of protein function. Phase 2 clinical trials show clear improvement of NPDs (Kerem et al. 2008; Sermet-Gaudelus et al. 2010; Wilschanski et al. 2011); however, a phase 3 clinical trial showed no difference in FEV_1 between the ataluren and placebo group (Kerem et al. 2014) (Table 3). Although the primary outcome was not achieved, the findings are encouraging since it demonstrates a positive effect of disease-modifying therapy using a corrector of CFTR. A confirmatory phase 3 efficacy and safety trial of ataluren in CF patients not receiving chronic inhaled tobramycin (ACT CF trial) is ongoing (Kerem et al. 2014).

5.1.4 Class II Mutations

Class II mutations include the most common mutation, p.F508del. High-throughput screening assays have been used to screen drug libraries, and some compounds called correctors have shown promise in improving CFTR processing (Pedemonte et al. 2005; Varga et al. 2008). One drug, VX-809 (lumacaftor), an orally bioavailable p.F508del corrector, showed moderate improvement in lung function in a phase 2 trial in adults homozygous for p.F508del. This breakthrough may be enhanced by using these compounds with a potentiator for class II mutations like VX-809, the logic being that the CFTR will be trafficked to the apical surface of the cell and then activated by a potentiator.

The results of a combination therapy have recently been published in the *New England Journal of Medicine* (Wainwright et al. 2015). 1108 patients with CF who were homozygous p.F508del were randomized to lumacaftor, which corrects cellular misprocessing to increase the amount of functional mutated CFTR, with ivacaftor, which increases opening of the channel protein, or to placebo. Results at 24 weeks showed that patients treated with the combination therapy had a mean improvement of 2.6–4.0% in FEV_1, when compared with participants randomized to placebo ($P < 0.001$).

The rate of pulmonary exacerbations in the two trials was 30–39% lower among the patients given the combination treatment. This combination therapy has now been approved for CF patients homozygous for p.F508del.

5.1.5 Class III Mutations

Class III mutations require drugs that activate the protein, which is inactive but properly targeted. These compounds are termed potentiators. VX-770 or ivacaftor is a very promising potentiator and is the first FDA-approved drug for patients carrying the p.G551D *CFTR* mutation. It was discovered by high-throughput screening. In vitro studies of the effect of VX-770 on CFTR-mediated Cl^- secretion were performed on both recombinant cell lines and primary cultures of human bronchial epithelial cells. Van Goor et al. demonstrated that VX-770 increases Cl^- transport by increasing the open probability of the CFTR channel and increases

apical fluid height and ciliary beat frequency (Van Goor et al. 2009). In an important clinical study, orally administered ivacaftor improved CFTR function in patients carrying the p.G55D mutation as measured by sweat Cl^- concentration and NPD measurements. A phase 3 trial demonstrated significant improvements in FEV_1 from baseline, average weight gain, concentration in sweat Cl^-, and reductions in pulmonary exacerbations (Ramsey et al. 2011). Ivacaftor has shown similar results in patients carrying other class III mutations (De Boeck et al. 2014).

5.1.6 Class IV and V Mutations

Class IV defects may be susceptible to augmentation of channel function. Possible compounds of potential treatments are flavonoid, compounds like genistein which acts directly on the channel to increase open probability.

Class V mutations often include splicing mutations which produce a variable phenotype. The disease expression is inversely related to the level of correctly spliced transcripts. The effect of overexpression of splicing factors on the level of correctly spliced CFTR transcripts was studied. It has been shown that increasing the level of correctly spliced RNA activated the CFTR channel and restored function. Class IV and V mutations may respond to potentiators like ivacaftor and these studies are ongoing.

These new developments provide hope that a treatment strategy could be applied to the basic defect rather than downstream manifestations of the disease.

5.2 Possible Treatments in Pancreatitis

The protective role of CFTR against alcohol-induced pancreatitis and pancreatic damage has been demonstrated in the *Cftr* knockout mouse (Maléth et al. 2015). As a corollary, compelling evidence indicates that CF-causing mutations are associated with an increased prevalence of pancreatitis (De Boeck et al. 2005). Furthermore, the loss-of-function CFTR phenotype, caused by ethanol, palmitic acid, and palmitoleic acid, at least partly, can be attributed to attenuated biogenesis, accelerated PM turnover, and channel inhibition of CFTR (Maléth et al. 2015). Thus, restoring the functional cell surface expression of CFTR may partly alleviate the ethanol-induced exocrine pancreas damage.

While the precise molecular mechanism of CFTR downregulation has not been fully elucidated, based on sustained cytosolic $[Ca^{2+}]$ elevation and mitochondrial Ca^{2+}-overload in ethanol- or palmitoleic acid-exposed cells (Maléth et al. 2015), it is plausible to assume that the folding capacity of molecular chaperone network, confined to the ER and cytosol, is reduced. Depletion of cytosolic [ATP] due to mitochondrial dysfunction upon cellular Ca^{2+}-overload could also contribute to impaired CFTR domain assembly. These events, jointly, elicit the conformational destabilization of newly synthesized CFTR, which leads to the channel accelerated

metabolic turnover at the ER and PM (Maléth et al. 2015). Accordingly, we envision multifaceted therapeutic approaches to alleviate the exocrine pancreas damage in pancreatitis.

Cytosolic Ca^{2+}-overload and the associated mitochondrial dysfunction during ethanol-induced pancreatitis could be attenuated by inhibiting the PM Ca^{2+} entry channels (such as the store operated Ca^{2+} channel Orai1 (Gerasimenko et al. 2013)). Intriguingly, inhibiting PM Ca^{2+}-channels also upregulates several molecular chaperones, which could be an added advantage in stabilizing CFTR. This treatment had some success in rescuing mutant β-glucocerebrosidase in Gaucher patient-derived fibroblasts (Mu et al. 2008). The cytosolic folding capacity of the chaperone systems could be increased also by inducing the unfolded protein response (UPR) or inhibiting the histone deacetylase 7 (HDAC7). Inhibiting the ER-associated degradation and PM mistargeting may help to increase the number of functional channels at the cell surface (Balch et al. 2011). Finally, the recently FDA-approved pharmacological chaperone, lumacaftor (VX-809) (Van Goor et al. 2011), that partially corrects the p.F508del CFTR folding/processing defect and the CFTR gating activator ivacaftor (VX-770) (Van Goor et al. 2009) can be considered as potential therapeutics. While both drugs are assumed to directly bind to mutant and wild-type CFTR, lumacaftor has been shown to stabilize the NBD1/MSDs interface in CFTR variants both in the ER and the PM (Ren et al. 2013; Okiyoneda et al. 2013). In contrast, ivacaftor can stimulate the gating of several missense mutations with impaired channel activation upon PKA-mediated phosphorylation (Van Goor et al. 2014). Thus, lumacaftor and ivacaftor may have the potential to revert the conformational and functional defects, respectively, of the ethanol-induced dysfunctional CFTR, a scenario that has to be evaluated experimentally in the future.

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