Cystic fibrosis (CF) is a monogenic disease caused by mutation of Cfr. CF-associated liver disease (CFLD) is a common nonpulmonary cause of mortality in CF and accounts for approximately 2.5%-5% of overall CF mortality. The peak of the disease is in the pediatric population, but a second wave of liver disease in CF adults has been reported in the past decade in association with an increase in the life expectancy of these patients. New drugs are available to correct the basic defect in CF but their efficacy in CFLD is not known. The cystic fibrosis transmembrane conductance regulator, expressed in the apical membrane of cholangiocytes, is a major determinant for bile secretion and CFLD classically has been considered a channelopathy. However, the recent findings of the cystic fibrosis transmembrane conductance regulator as a regulator of epithelial innate immunity and the possible influence of the intestinal disease with an altered microbiota on the liver complication have opened new mechanistic insights on the pathogenesis of CFLD. This review provides an overview of the current understanding of the pathophysiology of the disease and discusses a potential target for intervention. (Cell Mol Gastroenterol Hepatol 2019;8:197–207; https://doi.org/10.1016/j.jcmgh.2019.04.013)

Keywords: CFTR; Toll-Like Receptor 4; Inflammation; cholangiocyte; gut; Modulators.

Cystic fibrosis (CF) is a life-threatening autosomal-recessive disease that affects almost 30,000 individuals in the United States and more than 70,000 worldwide.1 The cystic fibrosis transmembrane conductance regulator (CFTR) was identified as the gene causing CF in 1989.2 CFTR encodes for a protein of the family of the adenosine triphosphate (ATP) binding cassette transporter superfamily and functions as a protein kinase A/cyclic adenosine monophosphate (cAMP)-activated chloride ion channel primarily in secretory epithelia.3 After the gene discovery, approximately 2000 mutations have been identified, but their functional importance is known for fewer than 300 of them.4

Based on the defect caused to the protein function, most common mutations can be grouped into 6 different classes.5 Class I mutations include premature stop codons, canonical splice mutations, and chromosomal deletions that result in the lack of the CFTR protein synthesis. Class II is represented by missense mutations and amino acid deletions that produce a misfolded protein unable to traffic at the membrane and results in a minimum or absent functional CFTR at the apical membrane. Both classes III and IV include missense mutations that cause amino acid changes that allow the protein to be produced and to reach the membrane but affect its function at different levels. Class III mutations affect the gating of the channel and reduce its open probability while class IV mutations cause decreased conductance of chloride. Finally, classes V and VI include missense mutations that reduce the amount of CFTR at the membrane either by reducing the synthesis of the protein or by decreasing its stability at the membrane.5–6

Among all of these mutations, Phe508del, a class II missense mutation that causes the deletion of a phenylalanine at position 508, accounts for approximately 75% of mutated alleles in European and North American populations. Interestingly, no other single mutation occurs in more than 5% of the population, suggesting that there is a wide spectrum of compound mutations and an even more variable range of manifestations of the disease, also considering the contribution of other genetic or environmental factors.

CF is a complex systemic disease and involves the epithelia of the respiratory tract, exocrine pancreas,
intestine, hepatobiliary system, and sweat glands, that is, tissues in which CFTR is expressed and has a critical function. Recently, CFTR expression was found in myeloid cells (ie, neutrophils, macrophages, and dendritic cells), mainly localized in the membrane of secretory vesicles. Studies in conditional mice carrying a M lysozyme Cre-driven Cfr deletion that specifically deletes Cfr in myeloid cells and whose lungs were infected with Pseudomonas aeruginosa, have confirmed that lack of CFTR in these cells contributes to the inflammatory response and inefficient resolution of the infection.

Since the discovery of CFTR, many clinical and scientific progresses have contributed remarkably to increase the life expectancy of CF patients from age 20 in the 1980s to almost age 45 in 2017. The changes in the CF demographic population and the improvements in the treatment of pulmonary symptoms have increased the attention toward other organs affected by CF. Among these, CF-associated liver disease (CFLD) accounts for the 3.4% of deaths among CF patients after pulmonary and transplant-related complications.

This review provides an overview of the current knowledge about CFLD and how new insight into its pathogenesis will open the door to possible novel treatments in the future.

**CFTR and Biliary Secretion**

In the liver, CFTR is expressed specifically on the luminal membrane of the cells that line the biliary tree (ie, cholangiocytes). The biliary epithelium forms a 3-dimensional arborized tubular structure inside the liver that collects the primary bile from the hepatic canaliculi at the level of the canal of Hering and carries it into the duodenum. Along its passage through the biliary system and before being delivered into the intestine, the bile is modified to meet digestive needs. Cholangiocytes express an array of transporters that finely control the fluidity and alkalinity of the bile. CFTR is located on the apical membrane of cholangiocytes and plays a leading function in this process. In a cascade of events, the hormone secretin, secreted by the duodenum in response to a meal, binds to the secretin receptor on the basolateral membrane of cholangiocytes and triggers an increase in intracellular cAMP that in turn activates a PKA-mediated efflux of chloride through CFTR into the biliary lumen. In response to the electrolytic gradient generated, chloride is transported back into the cell and bicarbonate is secreted into the lumen by a Cl/HCO$_3$ exchanger (anion exchange protein 2). At the same time, this gradient also favors the luminal movement of water via specialized water channels (ie, aquaporins). These processes are a major determinant of the ductular-dependent modification of composition and volume of the bile in response to physiological needs.

Hepatobiliary secretion can be regulated in part by purinergic signaling in a CFTR-independent way. Both hepatocytes and cholangiocytes secrete ATP in the bile, which stimulates apical purinergic receptors on cholangiocytes and leads to the activation of calcium-activated chloride channels. These channels recently were identified as transmembrane member 16A. However, different studies have shown that CFTR itself may directly or indirectly regulate the extracellular transport of bicarbonate and ATP.

Beside its channel activity, CFTR has been shown to be able to regulate the function of other proteins by virtue of its ability to physically associate in macromolecular complexes at the membrane. The CFTR interactome is mediated by scaffold proteins rich in PDZ domains and contains other transporters, receptors, kinases, signaling molecules, and cytoskeletal proteins. A clear understanding of these interactions and of the cellular signaling involved has a critical role to unveil the functional significance of CFTR mutations and how it could influence their phenotypic manifestation. Interestingly, we recently showed that the interaction of CFTR with proteins regulating the function of nonreceptorial tyrosine kinase Rous sarcoma oncogene cellular homolog (Src) can modulate innate immune responses in cholangiocytes, as discussed later.

**CFLD Clinical Manifestations and Current Treatment**

Liver disease represents a severe complication in the management of CF patients. Clinical manifestations are quite heterogeneous and range from asymptomatic abnormal liver biochemical values that unexpectedly can aggravate advanced liver disease, characterized by biliary cirrhosis with or without portal hypertension or portal hypertension in the absence of cirrhosis (ie, nodular regenerative hyperplasia). Common manifestations related to liver disease are the presence of a microgallbladder and steatosis. Although severe CFLD occurs only in a small percentage of patients, it is considered life-threatening because, in association with the development of portal hypertension, it has a deleterious effect on the respiratory function of these patients and represents an indication for liver transplantation. A recent study in a large European cohort showed that severe complications are not common early in life (before age 5), but reach more than 10% at the age of 30 years. This number is subject to change because of the increase in the number of adult CF liver patients and the increased life expectancy of these patients.

A major challenge for the clinical management of these patients is the early diagnosis of their liver disease. In fact, in most cases, liver-related symptoms are absent until the development of cirrhosis and portal hypertension. In addition, among the patients with biochemical test abnormalities it is difficult to predict the ones that might progress to severe liver disease. There is no clear correlation between specific mutations and the risk of liver complications, but among the recognized risk factors, there is the presence of more severe mutations (ie, classes I–III) and the association with pancreatic insufficiency, followed by male sex and meconium ileum at birth, even though the latter is controversial.

Patients with CFLD are treated routinely with ursodeoxycholic acid. However, Cochrane Reviews repeatedly have stated that its efficacy has not been proven clearly.
Patients with CFLD usually are monitored closely to evaluate the eventual progression to cirrhosis, while supportive treatments (ie, β-blockers or transjugular intrahepatic portosystemic shunts) are applied to cirrhotic patients to treat portal hypertension and its complications. In cases of severe liver dysfunction or when the liver disease compromises the function of other organs (ie, lungs), CF patients may undergo liver transplantation.42

Recently, CF research efforts have been concentrated on the development of a new class of drugs that aim to repair the basic CFTR defect.43,44 These are small molecules called CFTR modulators, and are designed to improve the function of CFTR mutated proteins. CFTR modulators act at different levels and include potentiators (ie, increase open channel probability), correctors (ie, improve membrane trafficking), stabilizers (ie, increase the half-life of the protein), amplifiers (increase the availability of a protein variant), and read-through agents (ie, bypass premature termination codons).43,45 Interestingly, different CFTR variants can benefit from the same type of modulator and this is the base of “theraturing,” a new system recently introduced to classify and group common and rare variants based on their response to modulators.46 This novel strategy has been used together with preclinical model systems to support the drug discovery pipeline and has brought attention to rare mutations that otherwise would not be considered.47,48 The Food and Drug Administration has approved different modulators and their combinations (ie, ivacaftor or combinations of ivacaftor/lumacaftor and ivacaftor/tezacaftor) for a discrete number of CFTR variants that correspond to approximately 60% of CF patients (either homozygous for the Phe508 allele or carrying a gating or residual-function CFTR mutation).49,50 However, a large percentage of patients (approximately 30%) who are heterozygous for the Phe508 allele and for a minimal function mutation (ie, mutations that produce no protein or defective proteins not responding to the approved modulators) still are lacking a treatment. These patients likely will benefit from the next generation correctors (ie, VX-659 and VX-445), which currently are in phase 3 trials in a triple combination with tezacaftor and ivacaftor. The main advantage of these new correctors is that they have different mechanisms of action compared with previously approved tezacaftor, and when combined together they show an additive effect on the processing and trafficking of the Phe508del CFTR at the membrane and a significant improvement of outcomes (ie, lung function, sweat chloride concentration, Cystic Fibrosis Questionnaire Revised respiratory domain score) as compared with the combination tezacaftor/ivacaftor.51,52

However, most of the functional studies take into consideration the lung function as the main end point and no data have been reported on the benefit of these molecules in patients with CFLD.

Altered Innate Immunity and Microbiota, a Unifying Hypothesis

A pathogenetic mechanism that explains the development of liver disease in CF patients is still not well defined and the classic view of CFLD pathophysiology has failed its experimental testing.53 In the classic view of the disease, CFTR channel dysfunction on biliary cells causes changes in the amount and composition of the bile with loss of the protective effect of biliary bicarbonate and mucus and an accumulation of toxic bile acids that then would damage the epithelium.54,55 The damaged biliary cells would react by secreting inflammatory molecules (ie, chemokines and cytokines) that attract inflammatory cells and in a chronic setting create the milieu for the development of fibrosis around the portal space. This peribiliary fibrosis eventually would progress into focal and multifocal fibrosis.56 However, this explanation is not fully supported by clinical data because bile inspissation is a rare finding and jaundice is uncommon or a late sign in these patients. Moreover, although CFTR dysfunction affects all CF patients, severe liver disease progresses only in a percentage of them, independently of their genotype, suggesting that other factors play a role in the pathogenesis of CFLD.35

The search for gene modifiers has identified a few candidates (ie, the SERPINA-1 Z allele, angiotensin 1-converting enzyme, glutathione S-transferase P1, mannose binding lectin Z, and transforming growth factor B1) that are associated with an increased risk of developing liver disease.57 but only SERPINA 1 was confirmed by a second study.58 Despite a strong association with CFLD (odds ratio, 5.04), the Z allele is relatively uncommon and only 2.2% of patients with CF are carriers.59

The lack of valuable experimental models that recapitulate the key aspects of liver disease in CF has prevented complete understanding of the pathologic process. Animal models have been used to model CF.60–62 More representative of the human CF disease are larger mammal models (ie, ferret and pig), as reported by recent studies.63 Both of these models present the spontaneous development of lung, liver, and pancreas diseases together with intestinal complications (ie, meconium ileus), but studies have been limited by the demanding costs and resources needed to maintain these animals and by limitations in available technologies and reagents.54–67 Instead, most of the knowledge acquired in the past 2 decades has derived from the use of transgenic mice with specific CFTR mutations.68 Although mice are relatively easy to work with, a critical limitation is that most of the models available reproduce the intestinal phenotype (ie, inflammation and intestinal obstructions at weaning) but lack a spontaneous development of multiorgan diseases (ie, lung, liver, pancreas), as seen in human beings and in larger animal models.69

Liver histology has shown the presence of steatosis in some of these mice that somehow was attributed to the use of a high-calorie liquid diet necessary to prevent the intestinal phenotype, but no other abnormalities (such as portal inflammation, focal biliary fibrosis) were evident.69,70 On the other hand, studies in isolated perfused livers have shown similar biliary volumes when comparing CFTR knockout (KO) and wild-type littermates at baseline, whereas bicarbonate output in CF mice is reduced significantly.71–73 In these studies, bile produced by
hepatocytes (that cannot be differentiated from the ductular component. Therefore, in vitro studies were performed in cholangiocytes isolated from CFTR KO and ΔF508-mutated mice and clearly showed that after stimulation of cAMP signaling (ie, by using forskolin or secretin), cholangiocyte secretion was decreased significantly. 74 Interestingly, as a result of 2 separated studies, the composition of the bile acid pool in CFTR KO is significantly different from that of normal mice. 75,76 This finding has been attributed to changes in the biotransformation of bile acids caused by a different intestinal microflora in CF mice, 75 an aspect that will be discussed further later; or, as in the second study, by defective gallbladder emptying in CF mice that results in reduced enterohepatic circulation and increased colecysto-hepatic shunt. 76 Regardless of the mechanism, both studies convey the observation that CF mice contain more hydrophilic primary bile acid in their bile, which is known to be less cytotoxic, 75,76 an observation that complicates the hypothesis of a bile acid–dependent liver toxicity.

A study from Blanco et al, 77 and later from our group, 78 showed that it is possible to induce liver disease in CFTR KO mice by treating them with dextran sodium sulfate (DSS), a chemical used to generate experimental colitis. As a consequence of the intestinal inflammation induced by colitis, the permeability of the intestinal epithelium increases and allows the translocation of bacterial products into the portal blood stream and up to the liver. The liver histology of CFTR KO mice exposed to DSS show focal foci of periportal inflammation and an associated expansion of reactive ductular structures, consistent with the occurrence of biliary damage. 77,78 However, such changes are not detected in wild-type and heterozygous mice, suggesting that the lack of CFTR predisposes the biliary epithelium to react when exposed to gut endotoxins. 77,78

Interestingly, in vitro experiments on isolated cholangiocytes exposed to lipopolysaccharide showed an unknown role of CFTR as a regulator of epithelial innate immunity. 25,76 As mentioned earlier, CFTR can interact with other proteins, and by stabilizing them at the apical membrane can regulate their function. 25 In cholangiocytes, CFTR was found to bind proteins involved in the regulation of the nonreceptor Src kinases (ie, Cbp and Csk), which keep these kinases in an inactive state. 25 If these proteins are not bound to CFTR, their ability to negatively regulate Src kinases is lost and the kinase is turned on. Among other targets, the Scr kinase family is able to phosphorylate Toll-like receptor (TLR)4 at the Y674 residue. 25,76 It has been shown previously that tyrosine phosphorylation of TLR4 is important for the signaling function of the receptor and normally is absent or impaired in endotoxin-tolerant cells. 79

The biliary epithelium continuously is exposed to pathogen-associated molecular patterns. Some of them come from the bile and some from the gut through the portal blood side. 80 It is well established that hepatocytes can take up bacterial endotoxins form portal blood and secrete them unmodified into the bile. 81 In the apical membrane, biliary cells express several receptors of the TLR family and, in particular, TLR4. 80,82 To avoid unnecessary and harmful reactions, biliary epithelia develop a certain degree of “endotoxin tolerance.” 83 This in general is achieved via a number of mechanisms, such as expression or activation of negative regulators of TLR signaling (ie, Interleukin 1 receptor-associated kinase M and peroxisome proliferator-activated receptor-γ), 83 production of antimicrobial peptides (ie, defensins), 84 secretion of IgA, expression of programmed death ligands whose receptors are expressed by leukocytes, 85,86 and, as reported by our study, post-translational regulation of TLR signaling. 78 Indeed, TLR4 phosphorylation was almost undetectable in normal cholangiocytes before exposure to lipopolysaccharide, but already was increased in unstimulated CFTR KO cholangiocytes. 76 Notably, the administration of inhibitors of the Src family of kinases to CFTR KO mice treated with DSS significantly diminished the extent of liver damage. 25

The finding that CFTR is a regulator of cholangiocyte innate immune responses and that, when defective, the level of tolerance of the biliary epithelium to endotoxins is altered, changes the classic view of CFLD as a disease of bile secretion and proposes a more complex scenario. 87

A strong correlation between the gut and the liver in the establishment and progression of several liver diseases (ie, nonalcoholic steatohepatitis, alcoholic liver disease) has been well validated 88 and recently extended to biliary diseases with an immune-mediated component (ie, primary biliary cholangitis [PBC], primary sclerosing cholangitis [PSC], and biliary atresia). In fact, specific alterations of both the biliary and intestinal microbiota have been reported in PBC patients as well as in PSC patients, together with a strong association with inflammatory bowel disease in the latest. 89,90 Moreover, antibodies against the mitochondrial E2 subunit of the pyruvate dehydrogenase that are found in the sera of PBC patients have been shown to also cross-react with bacterial conserved proteins. 91 More recently, a study in a Rhesus Rotavirus-infected mouse model of biliary atresia showed that post-natal changes in the gut microbiota can influence the susceptibility to experimental biliary atresia. 92

Human studies evaluating the gut microbiota in CF have reported similar findings: the presence of gut dysbiosis with a decrease in number and richness in bacteria species but an overall increase in more pathogenic bacteria strains. 93–97 Changes in gut microbiota in these patients often are associated with inflammation as also confirmed by a significant increase in fecal calprotectin, a protein produced by neutrophils that migrate to the inflamed intestinal mucosa, and with altered permeability of the intestinal epithelia. 97–99 It still is not clear if intestinal inflammation is caused by the altered microbiota in CF or is the consequence of an altered environment. In fact, CF patients are exposed to proton pump inhibitors that alter the pH of the mucosa or to antibiotics and pancreatic enzymes that might modulate the intestinal microflora. 94 A recent study has linked the intestinal dysbiosis in CF children with the presence of bacterial species that preferentially degrade short-chain fatty acids that otherwise are known to have anti-inflammatory and protective properties for the gastrointestinal mucosa. 100

Altered ratios of arachidonic acid/linoleic acid and...
arachidonic acid/docosahexaenoic acid have been reported previously in the blood and tissues of CF patients and Cftr KO mice and are considered to play a role in the pathogenesis of CF inflammation. Interestingly, a study from our group showed that the lower levels of polyunsaturated fatty acids (linoleic acid and docosahexaenoic acid) in biliary cells of CF mice might be responsible for the decreased activity of peroxisome proliferator-activated receptor-γ, a nuclear receptor with anti-inflammatory properties. Notably, activation of the receptor with a synthetic agonist decreased inflammation in CF cholangiocytes. Moreover, another study has reported a strong association between these alterations of the fatty acid metabolism and the development of cirrhosis in CF patients.

The presence of dysbiosis has been confirmed further in studies using CF animal models, which showed increased bacterial load in the small intestine and overgrowth of species from Enterobacteriaceae. As indirect evidence, our group has shown that if CFTR-KO mice exposed to DSS are treated with polymyxin B and neomycin, a specific cocktail of antibiotics that target gram-negative bacteria of the gastrointestinal tract, liver/biliary inflammation and damage are decreased significantly. Moreover, a recent study has shown that changes in the diet can modulate the gut microbiota of CF mice. All of these observations make a circumstantial case for the involvement of the gut microbiota in CFLD, but they do not show a mechanism. Therefore, well-designed studies that take into consideration all the relevant variables (ie, diet, geography, handler characteristics, circadian rhythms, and maternal effect) are necessary to better delineate whether these changes in the microbiota play a causal role in the development and progression of CFLD.

Figure 1. Proposed pathogenetic mechanism of CFLD and potential treatment approaches. In normal cholangiocytes, CFTR is expressed on the apical membrane of cholangiocytes where it regulates alkalinity and fluidity of the bile and is associated in a multiprotein complex with Src tyrosine kinase that controls TLR4 signaling and regulates innate immune responses of the epithelium. Mutations in the gene encoding for CFTR lead to CFLD. Lack of CFTR in the apical membrane of cholangiocytes causes impaired bile secretion (ie, decreased bile alkalinity and fluidity, accumulation of toxic bile acids) and disrupts the protein complex with Src, which results in increased TLR4 signaling in response to gut-derived endotoxins and affects F-actin and tight junction integrity, altering the epithelial barrier function. Lack of the CFTR in the gut alters the normal composition of the microbiota, causing dysbiosis and inflammation of the mucosa accompanied by increased intestinal epithelial permeability that favors the translocations of bacterial products into the portal circulation. The combination of a leak of pathologic endotoxins from the gut to the liver together with the altered innate immune response of cholangiocytes might explain the development and progression of CFLD. Potential targets for interventions and druggable pathways are outlined.

LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; PPAR, peroxisome proliferator-activated receptor.
Future Prospects for Therapy and Conclusions

To date, the lack of a precise pathogenetic mechanism to explain the development and progression of liver disease in CF patients has hampered the search for a cure. The current treatment with ursodeoxycholic acid has been considered safe based largely on its use in several different cholestatic disorders, but proof that it actually is preventing the progression of liver disease in CF is missing.

In principle, the mechanisms described earlier suggest potential novel therapeutic targets for CFLD. The next challenge is to validate these findings in experimental models that more closely reproduce human CF disease. This has become possible thanks to the novel technology of induced pluripotent stem cells (iPSCs). iPSCs are generated by inducing the expression of factors of pluripotency in somatic cells isolated from a patient sample (ie, blood, skin biopsy, urine). iPSCs then can be differentiated in the adult cell of the tissue of interest but would maintain the same genetic background of the patient, offering an innovative platform to model genetic disease and test new treatments.

Our group, among others, has used iPSCs to generate human cholangiocytes. We have obtained iPSCs from a patient carrying the most common CFTR mutation, ΔF508, and we have tested the effect of the first combination of modulators approved to rescue the ΔF508 mutation (ie, VX-809 and VX-770). When both modulators were administered together with inhibitors of Src kinase, the secretion was significantly potentiated and endotoxin-induced inflammation was decreased. The significant observation is that inhibition of Src kinase itself induces a rearrangement of the defective F-actin cytoskeleton in ΔF508 cholangiocytes that favors the stabilization of CFTR at the apical membrane of cholangiocytes and facilitates the action of the modulators.

This strongly indicates that treatment of CFLD requires the synergic action of modulators to repair the basic defect together with agents that specifically decrease inflammation and stabilize the CFTR protein.

In the near future, iPSC-derived cholangiocytes might offer a system to study altered innate immune pathways and potential treatments (ie, next-generation modulators in the pipeline) using a more personalized approach, thus including rare CFTR variants for which animal models are not available. A similar approach has been validated using rectal-derived patient organoids to preclinically select subjects that would benefit from the use of approved CFTR modulators independent of their CFTR mutation.

Another important mechanism that has emerged from these studies is the potential role of the gut microbiota. In CF the gut microbiota has been implicated in several inflammatory conditions. Earlier studies, for example, have reported a correspondence between the respiratory and the gut microbiota in CF infants, suggesting a potential influence between the 2 anatomic compartments, and treatment with probiotics indeed has been reported to decrease pulmonary exacerbations. Although confirmatory studies still are ongoing for the liver counterpart, the resulting message is that manipulation of the gut microbiota is possible and might be of therapeutic value in CFLD.

In conclusion, this review has summarized the current understanding of CFLD, highlighting the change in the interpretation of the nature of CFLD that for a long time has been considered and treated as a consequence of the lack of CFTR-mediated chloride secretion. Overall, these new findings can be combined in a unifying pathogenic mechanism caused by the genetic CFTR defect (Figure 1). On the intestinal side, lack of CFTR promotes changes in the gut microbiota along with an increase of the epithelial permeability permissive for the translocation of bacterial products up to the liver; on the cholangiocyte side, lack of CFTR leads to a persistent activation of the Src family of tyrosine kinases that triggers an aberrant production of TLR4-mediated proinflammatory cytokines in response to gut-derived endotoxins and pathogen-associated molecular patterns. Moreover, this inflammatory milieu affects the organization of the cytoskeleton and the function of tight junctions, therefore impairing barrier function of the biliary epithelium and increasing the back-diffusion of bile acid that damages the epithelium (Figure 1).

A few questions still remain unresolved. Liver disease is present only in a small percentage of CF patients and manifests more frequently in pediatric CF patients and less in adults. We could speculate that as suggested by our unifying hypothesis, liver disease results from the combination of multiple hits (ie, severe CFTR mutations, alteration of epithelial innate immunity, increased intestinal permeability); the majority of patients most likely escape this “perfect storm,” and, as suggested by Flass et al, in a homogeneous CF population (ie, similar mutations, age, presence of pancreatic insufficiency), intestinal and microbiota alterations are present only in subjects with confirmed liver disease (ie, cirrhotic). This novel scenario opens the way for new treatments and with the rapid development of reliable human in vitro models we predict a brighter future for the care of these patients.

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