Bile acid homeostasis in gastrointestinal and metabolic complications of cystic fibrosis

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Abstract

With the improved treatment of the pulmonary complications of cystic fibrosis (CF), gastrointestinal problems have become more important in the morbidity in CF. A hallmark of the gastrointestinal phenotype of CF, apart from pancreatic insufficiency, is a disruption of bile acid homeostasis. Bile acid homeostasis is important for many gastrointestinal processes including fat absorption, inflammation, microbial composition, as well as regulation of whole body energy metabolism. This review describes the impairment of bile acid homeostasis in CF, its possible consequences for gastrointestinal and metabolic complications and its potential as a target for therapy.
Introduction
Cystic fibrosis (CF), caused by a mutation in the gene encoding the CF transmembrane conductance regulator (CFTR), results in production of abnormally thick, viscous mucus in various organ systems (1). Aside from pulmonary problems, CF patients often suffer from GI disorders, hepatobiliary problems and cystic fibrosis related diabetes (CFRD). A specific GI feature common among CF patients is impaired bile acid (BA) homeostasis manifesting via BA malabsorption and subsequent increased fecal excretion (2). Recent studies show the interrelation of BA homeostasis with various other intestinal, hepatic and metabolic parameters. In this review we discuss the role of impaired BA homeostasis in CF, explaining its potential role in other GI and metabolic complications and as a therapeutic target.

Gastrointestinal complications of cystic fibrosis
A functional GI system is essential for maintaining adequate nutritional status and whole body homeostasis. Similar to pulmonary complications, viscous mucus, as a consequence of deficient surface fluid and bicarbonate flux is an important underlying factor in the GI phenotype of CF (3). Exocrine pancreatic insufficiency (EPI) is used as a marker for severity of the CF phenotype. However, other manifestations of the GI phenotype of CF are often highly variable and do not strongly correlated to allelic CFTR variation (4). Patients experience various symptoms including malabsorption, fatty stools (steatorrhea), abdominal pain, nausea, anorexia, bloating, gastro-esophageal reflux, constipation, distal intestinal obstruction syndrome (DIOS) and flatulence. Although most of the GI complications are interrelated, they can be subdivided in pancreatic, hepatobiliary and intestinal-luminal categories.

A severe CFTR gene mutation in both alleles results in little or no CFTR Cl⁻ channel activity and destruction of the exocrine pancreas (5). EPI is an early sign of CF and can present at birth or develop in the first months of life (6). Ultimately, around 85% of CF patients develop EPI and these patients are prone to nutritional deficiencies, severe malnutrition and growth retardation (7). Fortunately, EPI can be successfully treated with pancreatic enzyme replacement therapy (PERT). However, even with optimal PERT, fat malabsorption and GI complaints are often not fully corrected (8–10). Mice with targeted mutations in the Cfr gene do not display EPI but nevertheless have a lower bodyweight upon ad libitum feeding, possibly due to bacterial overgrowth or from impaired epithelial absorption of...
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nutrients (11). This suggests that, in addition to EPI, there are other changes in the intestinal tract in CF that have important effects on nutrient absorption and growth.

CF patients can suffer from a multitude of hepatobiliary problems including gallstones, hepatitis, steatosis and cirrhosis. Hepatobiliary problems are common in pediatric CF patients with reported prevalence rates up to 25% (12,13). Cystic fibrosis related liver disease (CFLD) was thought to develop mainly in early childhood. However, a recent follow-up study of a cohort of CF patients into adulthood incorporated novel markers into the CFLD diagnostic algorithm and suggests an additional wave of adult-onset CFLD with a median age of 37 years (14). Another recent study that assessed a large retrospective cohort of French CF patients found that CFLD incidence increased by approximately 1% every year reaching 32.2% by the age of 25 (13).

In the liver, CFTR is exclusively expressed at the apical membrane of cholangiocytes lining the bile ducts (15). CFLD is characterized by focal biliary cirrhosis which can lead to multilobular cirrhosis and portal hypertension in 1-10% of patients (16). The pathophysiology of biliary cirrhosis has been hypothesized to be secondary to occlusion of small bile ducts and/or to increased bile toxicity. In CF mouse models, however, evidence to support the hypothesis that increased bile toxicity contributes to CFLD has not been reported (17).

Luminal GI complications are highly prevalent in CF. Approximately 15-20% of CF infants present with meconium ileus, an obstruction of the distal small intestine by dehydrated mucofeculent material (18). After the neonatal phase, acute fecal obstruction of the ileocecum known as DIOS can occur and incidence increases with age (19,20). Nearly half of pediatric CF patients suffer from constipation and this is even more prevalent in adulthood (21). Another common luminal GI feature of CF is a change in intestinal microbiota characterized by small intestinal bacterial overgrowth (SIBO) and colonic dysbiosis (22). Important contributing factors include delayed intestinal transit time, luminal hyperacidity due to decreased bicarbonate secretion by pancreas and intestinal epithelium, frequent antibiotic use and inspissated mucus. Intestinal microbial composition is important for immune function and various metabolic processes in the body (23). Its disruption in CF is therefore likely to contribute to various aspects of the phenotype (24).

Along with the increased life expectancy the CF population has been shown to become exposed to an increased risk of malignant tumors especially of the small intestine and colon (22,25,26), possibly due to an increased proliferation rate of epithelial cells and disruption of anti-apoptotic pathways (27). Additionally, a recent study has shown a direct role of CFTR as a tumor suppressor gene in intestinal
cancer (28). After lung transplantation the risk for malignancies in CF patients is even further increased due to the use of immunosuppressant drugs (25,29).

**Impaired bile acid homeostasis and farnesoid X receptor signaling in cystic fibrosis**

One of the hallmarks of the GI complications in CF patients as well as in murine CF models is an up to 3-fold increase in fecal bile acid (BA) excretion (2,30–32). This increase is independent of exocrine pancreatic insufficiency and fat malabsorption. In the physiological situation the enterohepatic circulation of BAs is a tightly regulated system in which ~95% of total BAs are reabsorbed and the remaining ~5% is excreted via the feces (Fig. 1).
Bile acids (BAs) are synthesized and conjugated in the liver after which they are actively secreted via the bile duct into the duodenum. In the ileum BAs are reabsorbed by the enterocytes through the apical sodium-dependent bile acid transporter (ASBT). In the enterocyte BAs activate the nuclear farnesoid X receptor (FXR) which leads to subsequent release of fibroblast growth factor 19 (FGF19 or 15 in mice). FGF15/19 travels to the liver where it binds to fibroblast growth factor receptor 4 (FGFR4) β-Klotho complex and inhibits cholesterol 7α-hydroxylase (CYP7A1), the rate controlling enzyme of BA synthesis. Reabsorbed BAs can also travel directly to the liver and inhibit CYP7A1 by activating hepatic FXR. Microbiota in the intestine can further biotransform BAs to deconjugated and secondary BA species. After reabsorption, the remaining 5% of BAs (in the physiological situation) is excreted in feces.
Reabsorption mainly takes place by active transport of conjugated BAs into the ileal enterocyte by the apical sodium-dependent bile acid transporter (ASBT, SLC10A2) (33). In the ileal enterocyte BAs activate the farnesoid X receptor (FXR), a ligand-activated transcription factor of the family of nuclear receptors, which leads to increased expression and subsequent release of fibroblast growth factor 19 (FGF19, Fgf15 in mice) in the circulation (34). In the liver FGF19 can bind to and activate the FGF receptor 4 (FGFR4)/β-Klotho complex which in turn exerts negative feedback on the rate controlling enzyme of BA synthesis, cholesterol 7α-hydroxylase (CYP7A1). Reabsorbed BAs can also cause negative feedback by directly activating hepatic FXR. However, organ specific Fxr knockout studies in mice indicated a much more prominent role for the FXR-FGF15/19 axis in CYP7A1 repression (35).

The importance of FXR-FGF15/19 signaling in BA homeostasis is illustrated by various BA malabsorption syndromes. Clinically, BA malabsorption causes diarrhea due to high BA concentrations in the colon that lead to secretion of water and electrolytes and stimulation of propulsive contractions (36). Patients with primary BA diarrhea, a condition in which BA malabsorption occurs in the absence of ileal or other obvious GI disease, display lower levels of FGF19 and higher levels of 7α-hydroxy-4-cholesten-3-one (C4), a surrogate marker for BA synthesis (37,38). Additionally, in a murine model of BA malabsorption it has been shown that Fxr activation or Fgf15 administration reduces fecal BA excretion (39).

Even though CF patients display BA malabsorption, (BA) diarrhea is rare and constipation due to inspissated mucus and delayed intestinal transit is more common (further explained in section 6). However, BA malabsorption in CF patients does result in impaired FXR-FGF19 signaling. Our group recently demonstrated that plasma FGF19 levels are lower and C4 levels higher in CF patients with a G551D gating-mutation as compared to healthy controls (40). Conversely, improving CFTR function by treatment with the CFTR potentiator ivacaftor, altered the levels of these parameters towards normality, supporting a role for CFTR involvement in BA homeostasis. These results also indicate the possibility of using plasma C4 and FGF19 as surrogate biomarkers for CFTR function in the GI tract in CF. This is important as currently GI markers in CF are limited and/or difficult to obtain (41).

BA homeostasis has been more extensively studied in CF mouse models than in CF patients. Debray et al. showed that Cfr⁻/⁻ mice have lower ileal expression levels of Fgf15 compared to wild type controls, while having similar fecal BA excretion rates (42). Interestingly, most other studies in CF mouse models showed
an about 3-fold increase in fecal BA excretion, similar to what has been observed in CF patients, but did not directly assess FXR-FGF15/19 signaling (31,32). This discrepancy in fecal BA excretion might be explained by genotypic and dietary differences between studies and requires further investigation (43). Indeed, unpublished data from our group confirms lower ileal expression levels of Fgf15 in combination with increased hepatic Cyp7a1 levels in Cfr<sup>−/−</sup> mice, supporting the disruption of FXR-FGF15/19 signaling. The exact mechanism underlying the impaired FXR-FGF15/19 signaling in CF, however, remains unknown.

In wild type mice it has been shown that uptake of BAs by Asbt activates Cftr (31). Altered functionality or expression of ASBT might also be involved in BA malabsorption in CF. However, results on ileal Asbt expression in murine CF models have been conflicting, (31,42,44), likely due to differences in experimental setups. Additionally, gene expression might not adequately reflect protein expression or activity. When looking at protein abundance, Debray et al. (42) found decreased expression of Asbt by western blot in Cfr<sup>−/−</sup> mice, while Bijvelds et al. (31) showed a robust Asbt immunohistochemistry staining pattern and intensity in both Cfr<sup>−/−</sup> and F508del-Cftr mice indistinguishable from WT mice. The regulation of ASBT expression is complex and can be influenced by many factors including intestinal BA concentrations and microbial composition (33). ASBT expression is under a negative feedback regulation by intestinal BA concentrations in mice and humans (45,46). The proposed mechanism of this feedback regulation is that BAs activate FXR in the enterocyte which leads to subsequent small heterodimer partner (SHP) and liver receptor homologue-1 (LRH-1) activation which downregulate ASBT expression. This hypothesis is supported by the fact that BA depletion by feeding mice a BA binding resin increased Asbt expression (47). However, Debray et al. showed, besides decreased Asbt expression in Cfr<sup>−/−</sup> mice, a decrease in expression of ileal Fxr target genes, Fgf15 and Shp, arguing against suppression of ASBT by FXR (42). Conversely, while decreased intestinal BA concentrations seemed to induce ASBT expression, Stravitz et al. showed an induction of Asbt gene and protein expression in rats by feeding cholic acid or perfusing the intestine with taurocholic acid (48). Intestinal dysbiosis or SIBO could contribute to altered ASBT expression in CF. Germfree or antibiotic treated mice generally show higher expression levels of Asbt and a decrease in fecal BA excretion (49–51). Higher expression levels of Asbt were also observed more proximal in the intestine when mice were treated with antibiotics, regulated through reduced expression of the transcription factor Gata4 (50).
Next to their indirect effect on ASBT expression, microbiota are also directly involved in BA homeostasis and FXR-FGF15/19 signaling. Different species of intestinal microbiota have the ability to biotransform BAs mainly by deconjugation and subsequent de-hydroxylation, the latter resulting in more hydrophobic secondary BAs. The various BA species have different properties regarding absorption and receptor activation. Many species of microbiota express bile salt hydrolase (BSH) activity which results in deconjugation of part of the luminal BAs, thereby making them unable to be reabsorbed by ASBT but, due to decreased polarity (more hydrophobic), easier to be passively reabsorbed. Germ-free mice lack the microbial ability to deconjugate BAs, resulting in higher concentrations of tauro-β-muricholic acid, a conjugated BA that acts as an Fxr antagonist that can lower Fgf15 levels (49).

CF patients and murine CF models display intestinal dysbiosis and sometimes even bacterial overgrowth, potentially affecting BA handling. Cfr**−/−** mice generally show a decreased intestinal microbiota biodiversity with an increase in species associated with inflammation (52). Decreases within the *Bacteroides* and *Firmicutes* phyla were observed in Cfr**−/−** mice as well as CF patients (24). The *Firmicutes* phylum contains species known to be involved in de-hydroxylation of BAs (53). A decrease in one of the most important secondary BAs, deoxycholic acid (DCA), has been observed in feces of Cfr**−/−** mice (17,32). These observations suggest an important relation between intestinal dysbiosis in CF and BA handling. The exact role, however, of microbiota in BA homeostasis in CF remains to be elucidated.

**Bile acid homeostasis and its relation to metabolic function in cystic fibrosis**

BAs are primarily known as detergents with their main function to aid in digestion and absorption of fat and fat soluble vitamins. However, recent studies show that BAs also act as ligands for receptors resulting in the release of hormones that affect other metabolic processes in the body such as glucose metabolism (54). CF patients often display metabolic abnormalities, such as hyperglycemia, hypertriglyceridermia or steatosis, and these are more common with increasing age (55). Malnutrition is generally regarded as an important problem in CF management. However, due to better nutritional and supportive therapy, the prevalence of malnutrition in CF has declined and some CF patients even develop overweight or obesity (56). A recent study showed that some CF patients display “normal weight obesity”, defined by a normal body mass index (BMI) but increased fat percentage that was associated with decreased pulmonary function (57).
Table 1. Summary of the metabolic effects of bile acid homeostasis and the phenotype in CF as discussed in section 4. BA: bile acid; CF: cystic fibrosis; TGR5: G-protein coupled bile acid receptor 1; FGF19: fibroblast growth factor 19; GLP-1: glucagon-like peptide-1; CFRD: cystic fibrosis related diabetes.

<table>
<thead>
<tr>
<th>Lipid metabolism</th>
<th>Catabolism of cholesterol for BA synthesis</th>
<th>Lower plasma cholesterol</th>
<th>Often low plasma cholesterol (55,58) BA synthesis is elevated (40)</th>
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<tbody>
<tr>
<td>Glucose metabolism</td>
<td>Intestinal bile acids activate TGR5 signaling and GLP-1 release</td>
<td>GLP-1 improves post-prandial insulin secretion</td>
<td>Reduced GLP-1 release in CF patients (59–61)</td>
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<tr>
<td></td>
<td>Intestinal bile acids activate FXR to release FGF19</td>
<td>FGF19 inhibits bile acid synthesis and improves insulin sensitivity</td>
<td>Lower FGF19 due to reduced BA absorption (40)</td>
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BA homeostasis is also highly involved in lipid and cholesterol metabolism (54). Conversion of cholesterol to BAs and subsequent fecal excretion is one of the main routes of cholesterol disposal. Most CF patients display serum low-density lipoprotein cholesterol (LDL-C) levels that are lower compared to control subjects of similar age (55,58). However, some CF patients display elevated LDL-C and triglyceride levels which are associated with older age and other metabolic abnormalities such as a high BMI and lower insulin sensitivity (62–64). With increasing age the incidence of CF related diabetes (CFRD) is also rising. The pathophysiology of CFRD is not completely understood but its incidence is correlated with EPI and fibrosis (65). A combination of partial insulin deficiency and episodes of insulin resistance are often present. Interestingly, CF patients display lower levels of glucagon like peptide 1 (GLP-1), an incretin hormone regulating postprandial insulin secretion, that is improved by pancreatic enzyme replacement therapy (59–61). Intestinal activation of the G-protein coupled bile acid receptor 1 (GPBAR1, GPCR19 also known as TGR5) by BAs enhances the secretion of GLP-1 (66). Secondary BAs lithocholic acid (LCA) and DCA are the most potent naturally occurring TGR5 agonists. As these BA species are lower in murine CF models, it is tempting to speculate that TGR5 activation is reduced in CF due to impaired BA homeostasis. Unfortunately, however, no studies directly addressing this relationship have been performed.
In addition to the role of TGR5, the FXR-FGF15/19 axis has been implicated in other aspects of glucose metabolism and metabolic disorders. In humans, both obesity and type 2 diabetes mellitus (T2DM) are associated with lower plasma FGF19 levels (67,68). A derivative of the naturally occurring FXR agonist CDCA, 6α-ethyl-CDCA (obeti cholic acid, OCA), was recently approved by the Food and Drug Administration (FDA) for the treatment of primary biliary cholangitis (PBC), and is in clinical trials for treatment of non-alcoholic steatohepatitis. Administration of OCA to patients with non-alcoholic fatty liver disease and T2DM increased plasma FGF19 levels, decreased liver enzymes (alanine aminotransferase and γ-glutamyltransferase) and improved insulin sensitivity (69). In a phase 3 clinical trial, OCA ameliorated histology scores of non-alcoholic steatohepatitis patients (70). Animal studies, however, have not generated unambiguous results regarding the effects of the FXR-FGF15/19 axis on metabolism. Transgenic mice with hepatic overexpression of FGF19 display an increased metabolic rate and decreased adiposity (71). Kir et al. found that administration of FGF19 to WT mice improved glucose metabolism by inducing hepatic glycogen and protein synthesis (72). Conversely, Fgf15−/− mice showed glucose intolerance and a reduced hepatic glycogen content. Administration of FGF19 was even found to reverse diabetes mellitus in ob/ob mice (73). A direct involvement of Fxr-Fgf15 signaling was demonstrated by intestinal inactivation of Fxr or reducing intestinal Fxr signaling through remodeling the intestinal microbial profile by the anti-oxidant tempol, both of which reduced diet induced obesity and hepatic triglyceride accumulation in mice (74,75). The metabolic improvements by tempol were explained by reduced species of Lactobacillus and their BSH activity resulting in increased levels of tauro-β-muricholic acid, a naturally occurring FXR antagonist. This claim was further supported by a study in which administration of glyco-β-muricholic acid (Gly-MCA), a selective FXR inhibitor, reduced obesity and improved related metabolic abnormalities in mice (76). On the other hand, intestine-specific FXR activation using the intestinally restricted FXR agonist fexaramine, reduced diet-induced obesity, insulin resistance and steatosis in mice fed a HFD (77). However, a recent study explained the beneficial effects of fexaramine by an indirect effect on the microbiota and activation of TGR5 via pronounced alterations in BA composition (78).

As the pathology of CFRD is thought to be mainly due to (relative) insulin deficiency rather than insulin resistance as in T2DM, the effects of modulating the FXR-FGF15/19 axis in CFRD could be less pronounced. On the other hand, CF is associated with direct impairment of BA homeostasis and FXR-FGF15/19
signaling. It will therefore be interesting to evaluate strategies to improve FXR-FGF15/19 signaling in CF, especially in the prevention of metabolic complications such as CFRD and hepatic steatosis.

Bile acid homeostasis and its role in cystic fibrosis liver disease

Cystic fibrosis liver disease (CFLD) is a severe complication of CF and an independent risk factor for mortality (79,80). According to the 2016 CF patient registry report, CFLD accounted for 2.7% of mortality in CF patients (81). CFLD is an umbrella term used to describe various types of liver dysfunction in CF of which the pathophysiology is often not completely understood (82). In the liver, CFTR is expressed exclusively at the apical membrane of cholangiocytes lining the bile ducts (15,83). One proposed mechanism for the development of CFLD is that loss of CFTR function leads to obstruction of the bile ducts by thickening of the mucus, eventually resulting in obstructive biliary cirrhosis. Murine CF models generally do not display CFLD except upon ageing (84,85). The absence of CFLD in murine CF models might be partially explained by lower BA toxicity due to differences in biliary BA composition between mice and humans. Mice generally have a higher concentration of hydrophilic BAs which is regarded as less cytotoxic since hydrophilic BAs have a lower detergent capacity than hydrophobic BAs (86). High concentrations of hydrophobic BAs, mainly DCA, have been associated with increased risk of cholesterol gallstone disease, colon cancer and liver cancer (53,87).

FXR-FGF15/19 signaling and hepatic FXR activation have been consistently shown to affect liver regeneration and proliferation (88–91). This regenerative/proliferative response, measured by hepatic staining of the proliferation marker Ki67, is absent in Cfr−/− mice upon feeding cholic acid (CA), a strong FXR agonist (17). This suggests that a CF liver might have reduced regenerative ability due to BA malabsorption. Interestingly, in humans the presence of CFLD is associated with normalization of fecal BA excretion which could be due to impaired production as declining liver function occurs but this has not been further investigated (2).

Currently, ursodeoxycholic acid (UDCA) is the only recommended and widely used drug in the treatment of CFLD. However, the clinical efficacy of UDCA is controversial. The most recent Cochrane review only identified a small number of trials assessing the effectiveness of UDCA (92). The authors concluded that there is ‘currently insufficient evidence to justify its routine use in cystic fibrosis’. UDCA treatment is often started early in life to prevent severe CFLD and related
complications. This was also challenged by the results of a recent study, showing that treatment with UDCA started earlier in life had no effect on development of severe CFLD (13).

The effects of UDCA on BA homeostasis and FXR signaling have not been fully elucidated. UDCA increases hepatocellular and cholangiocellular secretion thereby increasing bile flow and reducing biliary toxicity (93). UDCA is also suggested to decrease hepatic steatosis in mice (94–96). In obese subjects UDCA was reported to lower FGF19 and subsequently increase hepatic bile acid synthesis (97). The authors explained this as UDCA having FXR antagonistic effects which was supported by showing decreased FXR activation in an avidin biotin complex DNA-assay. However, in vitro assays suggest UDCA has neither FXR agonistic nor antagonistic properties (96,98). UDCA is readily absorbed and constitutes a predominant part of the BA pool in UDCA treated patients (from 40% in PBC treated patients (99) to almost 90% in obese patients (97)), which is likely to contribute to the effects on the FXR-FGF15/19 axis. Fujita et al. showed a clear decrease in relative and absolute levels of muricholic acid levels in mouse livers after UDCA treatment, arguing the beneficial effects on hepatic steatosis (at least in mice) might be due to a reduction of the FXR antagonistic muricholic acid species (96).

FXR agonism has been shown to protect against hepatotoxicity in a rat model of intrahepatic cholestasis (100). In that study a systemic FXR agonist (i.e. GW4064) was used and effects could therefore be at least partly due to hepatic FXR activation. Direct evidence of the benefits of intestinal FXR-FGF15/19 signaling has been shown by Modica et al. (101) who demonstrated that transgenic overexpression of intestinal FXR or administration of FGF19 in mice protects against liver damage in three different models of cholestasis. The beneficial effects were attributed to a reduced BA pool size and more hydrophilic (i.e. less cytotoxic) biliary BA composition. However, FGF19 is a growth factor and is also associated with the induction of liver proliferation and growth of cancer cells (102). To overcome the potential tumorigenic effects of FGF19, a modified variant of FGF19 (M70) has been produced, with reduced tumorigenicity but retained benefits in cholestatic liver disease in mice (103,104).

These results make intestinal FXR an interesting target in developing treatment and prevention strategies for CFLD. Unfortunately, CF mice have not been an ideal model for CFLD. Recently, however, other potentially more useful CF animal models have been developed, including the CF pig which already shows signs of
CFLD at birth (105,106). CF pigs could therefore be interesting to study CFLD (107,108).

**Modulating bile acid homeostasis to improve gastrointestinal outcomes in cystic fibrosis**

Considering the close relation between BA homeostasis and GI and metabolic function, it is interesting to speculate about the effect of modulating the factors involved. In the previous section the effects of altering FXR-FGF15/19 and TGR5 signaling to improve metabolism were considered. Modulating the FXR-FGF15/19 axis might also ameliorate GI outcomes. In turn, modulating certain GI factors could improve BA homeostasis and metabolic outcomes.

CF patients often display SIBO or colonic dysbiosis. Not only do these conditions generate direct symptoms including abdominal discomfort, diarrhea and flatulence, they also increase the risk of developing metabolic abnormalities and liver disease (109). The relationship between BAs and intestinal microbiota is complex, with mutual interactions (87,110). In the CF intestine, inspissated mucus accumulates, making it easier for harmful bacteria to thrive (27). Other factors contributing to an altered microbial profile include a low intestinal pH due to reduced bicarbonate secretion, a longer intestinal transit time and exposure to antibiotics that CF patients frequently receive for (suspected) pulmonary infections. Interestingly, bacterial overgrowth itself has also been suggested to contribute to mucus secretion. Antibiotic treatment aimed at eradication of bacterial overgrowth in Cfrt-/- mice reduced mucus accumulation without a major effect on mucin gene expression, suggesting a more direct role for bacteria on mucus secretion by intestinal epithelium (111).

Treatment with probiotics can also be used to alter the microbiota profile of CF patients. One study reports that administration of the probiotic *Lactobacillus Reuteri* improved digestive health and inflammation (112). The fecal microbial profile changed, showing a decrease in *Proteobacteria* and an increase of the *Firmicutes* phylum. As numerous species of the *Firmicutes* phylum are involved in BA biotransformation, one could speculate that this change affects BA homeostasis. Treatment with another probiotic, *Lactobacillus GG*, decreased fecal calprotectin, a marker for intestinal inflammation, and changed microbial composition partially towards that of healthy controls (113,114). However, in a large trial in CF children, one year of treatment with *Lactobacillus GG* versus placebo did not affect hospitalization, pulmonary outcomes or BMI (115).
Nevertheless, as probiotic use does seem to improve the GI phenotype of CF, it will be interesting to evaluate its effects on BA homeostasis and metabolic function.

Another GI feature of CF patients is a delayed intestinal transit time, possibly leading to constipation or in severe cases distal intestinal obstruction syndrome (DIOS) (116–118). Interestingly, Bijvelds et al. showed that active BA absorption in the ileum triggered CFTR activation and subsequent local salt and water excretion (31). It is therefore tempting to speculate that the absence of this postprandial ileal water release contributes to specific distal localization of obstruction occurring in CF patients. The inability to sufficiently hydrate intestinal content and mucus likely explains the fact that BA malabsorption does not lead to diarrhea in CF.

As CF patients often suffer from constipation, laxatives are commonly prescribed. Laxative treatment shortens intestinal transit time and is able to alter microbiota and BA homeostasis. In rats, the commonly used laxative polyethylene glycol (PEG) decreased BA dehydroxylation, increasing the amount of primary BAs in the BA pool (119). Whole body Cftr knockout mice display a severe intestinal phenotype and need to be kept either on a liquid diet or a solid diet in combination with laxative. A study by De Lisle et al. compared the effects of either a solid diet with PEG or a liquid diet with or without n-acetylcysteine (NAC), a mucolytic agent, on various aspects of the intestinal phenotype (120). Laxative treatment had pronounced effects on the intestine and improved markers of intestinal inflammation and reduced bacterial overgrowth. In CF patients, laxative treatment was also found to be associated with a decrease in occurrence of SIBO (121). Considering these beneficial effects of laxative treatment on the CF intestine, it is tempting to speculate that laxative treatment could decrease fecal BA excretion.
Summary
The presently increased life span of the CF population changes the frequency and spectrum of symptoms regarding GI and metabolic function including intestinal dysbiosis, constipation, intestinal cancer, liver disease and diabetes. One part of the CF phenotype that recently received more attention is the GI tract, including impaired BA homeostasis, characterized by increased fecal BA excretion and reduced FXR-FGF15/19 signaling. Modulating BA homeostasis directly by altering FXR-FGF15/19 or TGR5 signaling or indirectly by improving intestinal transit or modifying intestinal microbiota are potential strategies to improve other GI and metabolic CF complications. Lastly, emerging research into BA homeostasis and the GI phenotype of CF could provide novel easily measurable surrogate biomarkers (e.g. C4, FGF19). Especially in the current era of new CFTR modulator therapies (e.g. ivacaftor and lumacaftor), the need for such biomarkers has increased.

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