Regulation of bile acid homeostasis by the intestinal Diet1–FGF15/19 axis

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Abstract

Purpose of review—Hepatic bile acid synthesis is controlled, in part, by a complex enterohepatic feedback regulatory mechanism. In this review, we focus on the role of the intestinal FGF15/19 hormone in modulating bile acid levels, and additional metabolic effects on glucose metabolism, non-alcoholic liver disease (NAFLD), and liver regeneration. We also highlight the newly identified intestinal protein, Diet1, which is a modulator of FGF15/19 levels.

Recent findings—Low FGF19 levels are associated with bile acid diarrhea and NAFLD. In contrast, high FGF19 levels are associated with diabetes remission following Roux-en-Y gastric bypass surgery, suggesting new therapeutic approaches against type 2 diabetes. The effect of FGF15/19 on liver plasticity is a double-edged sword: whereas elevated FGF15/19 levels improve survival of mice after partial hepatectomy, FGF19 mitogenic activity is associated with liver carcinoma. Finally, a recent study has identified Diet1, an intestinal factor that influences FGF15/19 levels in mouse intestine and human enterocytes. Diet1 represents the first factor shown to influence FGF15/19 levels at a post-transcriptional level.

Summary—The biological effects of FGF15/19 make it an attractive target for treating metabolic dysregulation underlying conditions such as fatty liver and type 2 diabetes. Further elucidation of the role of Diet1 in FGF15/19 secretion may provide a control point for pharmacological modulation of FGF15/19 levels.

Keywords

enterohepatic circulation; fibroblast growth factor; NAFLD; gastric bypass

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Conflicts of Interest
None.

Introduction

Bile acids have been long known to be critical for efficient intestinal absorption of fats and lipid-soluble vitamins. The past dozen years have revealed a second life for bile acids as ligands for nuclear receptors that regulate bile acid synthesis, transport, and metabolism, and in metabolic processes such as glucose metabolism and energy expenditure. These roles necessitate precise regulation of bile acid levels. It is now established that bile acid homeostasis is achieved, in part, through communication from the small intestine to the liver to regulate hepatic bile acid synthesis. In response to bile acid reabsorption in the ileum, enterocytes secrete fibroblast growth factor 19 (FGF19; FGF15 in mouse) into the enterohepatic circulation. Uptake of FGF15/19 by hepatocyte receptors causes repression of bile acid synthesis, thereby coordinating bile acid synthesis in liver with reabsorption in intestine to maintain homeostasis. Herein, we describe recent findings related to the enterohepatic axis for bile acid regulation, with a focus on studies of FGF15/19 function in normal and pathophysiology, and the identification of Diet1, an intestinal protein that influences FGF15/19 production by enterocytes.

A very short history of bile

Bile has a prominent place in the history of Western medicine. In the ancient world, bile accounted for two of the mystical "four humours": blood, phlegm, choler (yellow bile), and melancholer (black bile) [1]. Hippocrates, the father of medicine, taught that the humours must be maintained in a delicate balance, and that imbalance resulted in disease. In the 1660's, the physician Jan Baptista van Helmont hypothesized that the emptying of choler (bile) into the intestine was important for digestion. In the 1800's, cholesterol and cholic acid were identified as components of bile [2], but it remained until the 1940's, for Konrad Bloch and colleagues to trace the incorporation of labeled cholesterol into newly synthesized bile acids in dogs [3]. The importance of bile acids in digestion in humans was established in the 1960's [4].

Bile acids and the enterohepatic circulation

Work throughout the last several decades established the itinerary of bile acids during digestion [5]. Bile acids are synthesized in the liver and conjugated with the amino acids glycine or taurine in order to pass from the hepatocyte into the bile pool. Upon feeding, conjugated bile acids are secreted into the bile ducts and enter into the gastrointestinal tract. In the proximal small intestine, bile acid-containing micelles allow solubilization and absorption of cholesterol and fat-soluble vitamins. When bile acids reach the ileum, > 95% of bile acids are reabsorbed and return to the liver through the enterohepatic circulation. The small proportion of intestinal bile acids that are not absorbed in the ileum are excreted in the feces.

Bile acids have several metabolic roles. Approximately half of the dietary cholesterol consumed daily is converted to bile acids, and loss of fecal bile acids serves as a route for the elimination of cholesterol from the body [5]. The maintenance of normal bile acid levels is important to prevent bacterial overgrowth and mucosal injury in the intestine [6] or cholestasis in the liver. Bile acids are ligands for the nuclear receptors farnesoid X receptor

(FXR), pregnane X receptor (PXR), vitamin D receptor, and the G protein-coupled receptor, TGR5 (reviewed in [7]). Bile acids activate these receptors to regulate gene expression in several metabolic tissues (including intestine, liver, brown adipose tissue, macrophages, and brain). Aberrations in bile acid metabolism are associated with conditions ranging from gallstones and bile acid diarrhea to common metabolic diseases such as type 2 diabetes and atherosclerosis (Fig. 1) [8–12]. Here, we will focus on recent developments related to the enterohepatic regulation of bile acid levels.

The enterohepatic regulation of bile acid homeostasis

Bile acid biosynthesis from cholesterol occurs primarily in hepatocytes via two pathways—the classic (or neutral) and alternate (or acidic) pathways [13]. The first and rate-limiting step in the classic pathway is the hydroxylation of cholesterol at the 7α-position, catalyzed by cholesterol 7α-hydroxylase (CYP7A1). *Cyp7a1* gene expression is stimulated by dietary cholesterol, and is repressed by bile acids. The alternate pathway utilizing oxysterols contributes 10% (mice) to 25% (humans) of total bile acid synthesis.

In liver, bile acid synthesis is partly under negative regulation by the FXR-SHP axis. Activation of FXR by bile acids induces expression of *Shp* (encoding the short heterodimeric partner, SHP), which represses expression of *Cyp8b1* (encoding the enzyme responsible for cholic acid synthesis) and to a lower extent, *Cyp7a1* [14, 15]. A fascinating aspect of bile acid regulation is the coordination between bile acid uptake in intestine and the control of bile acid synthesis in the liver (Fig. 2) [16–18]. In response to bile acid uptake at the apical surface of the enterocyte, FXR is activated and induces transcription of the gene encoding human FGF19 or its mouse ortholog, FGF15. FGF15/19 is secreted at the basolateral surface of the enterocyte into the enterohepatic circulation. In liver, FGF15/19 binds to the FGFR4 receptor and, through actions of the JNK and ERK signaling pathways, represses *Cyp7a1* transcription [15, 19]. In addition, FGF15/19 promotes gallbladder filling by acting on the relaxation of the gallbladder smooth muscle cells [6]. This ensures that hepatic bile acid synthetic levels and secretion to the gallbladder are coupled to bile acid reabsorption in the ileum. This prevents overproduction of bile acids, which is energetically undesirable and may result in deleterious conditions such as bile acid diarrhea.

Metabolic effects of FGF19

Recent studies in humans and non-human primates demonstrate the importance of FGF19 in the regulation of bile acid levels, and reveal additional metabolic effects of this hormone. In the first studies to examine bile acid and FGF19 levels in more than 400 individuals the general population, Gälman *et al.* reported 7-fold variations in serum FGF19 levels in healthy individuals, and demonstrated that FGF19 levels are inversely related to bile acid levels [20]. Bile acid levels were also positively correlated with serum triglyceride levels. Interestingly, women had lower bile acid and triglyceride levels than men. It will be interesting to determine if sex differences in FGF19 levels and/or bile acid production relate to the reduced prevalence of hypertriglyceridemia, and increased prevalence of gallstones, observed in women.

The presence of increased colonic bile acids causes primary bile acid diarrhea, which may affect approximately 1% of the European population [21]. It was previously shown that individuals with primary bile acid diarrhea have lower median levels of FGF19 than the general population [10]. This finding was extended in a recent prospective study in individuals assessed for 7-day retention of a radiolabeled bile acid (selenium homocholic acid taurine, SeHCAT). FGF19 levels were significantly lower in individuals classified with primary bile acid diarrhea compared to normal controls and to individuals with secondary bile acid diarrhea [22]. In agreement with these observations in humans, the inhibition of FGF19 action in cynomolgus monkeys with specific antibodies led to increased *CYP7A1* expression, enhanced bile acid synthesis and efflux, reduced bile acid uptake, and diarrhea [23]. Since the SeHCAT retention test is not available in the United States and many other countries, it is notable that serum FGF19 levels appear to be a viable marker for the diagnosis of bile acid diarrhea [24].

In recent studies, the FGF19 levels in serum have been associated with several metabolic conditions beyond bile acid diarrhea. For example, reduced FGF19 levels have been observed in children, adolescents, and adults with non-alcoholic fatty liver disease (NAFLD) [25–27]. In adults, the FGF19 levels did not independently predict liver histology findings [26]. However, in children with NAFLD, FGF19 levels were inversely correlated with the progression to steatohepatitis and fibrosis [25]. Additionally, in adolescents with NAFLD, those with insulin resistance had more pronounced decreases in FGF19 levels than the insulin-sensitive group [27]. No assessment of bile acid levels was performed in these studies, but may be valuable in the future to elucidate the mechanistic association between FGF19 and NAFLD.

A link between bile acid metabolism and type 2 diabetes mellitus has been noted by numerous investigators, although the results have not always been consistent [11]. Notably, in diabetic patients that do not respond to the typical arsenal of anti-diabetic therapeutics (including insulin), treatment with bile acid sequestrants tends to improve glucose levels and whole-body insulin sensitivity [28, 29]. Recently, FGF19 levels have also been implicated in diabetes remission after Roux-en-Y gastric bypass surgery to promote weight loss and glycemic control. Substantial evidence indicates that gastric bypass increases both bile acid and FGF19 levels [30–34], with the FGF19 increase likely occurring secondary to elevated bile acids and FXR activation [35, 36]. Among individuals undergoing gastric bypass surgery, there is inter-individual variation in the normalization of diabetic phenotypes despite similar weight loss, and the underlying mechanisms are mysterious. A recent study detected larger increases in FGF19 levels from pre-operative concentrations in patients that experienced diabetes remission for 12 months following surgery than those who did not [30].

A better understanding of the mechanisms involved in glycemic improvement in gastric bypass could suggest novel treatments for type 2 diabetes. One hypothesis is that changes in bile flow resulting from the altered anatomy following bypass could be involved. It is likely that additional factors also change as a result of bypass, such as intestinal microbiota, which play a role in bile acid modification and alter the bile acid pool composition. Effects of FGF15/19 on glucose metabolism could also occur by direct signaling in target tissues. For

example, FGF19 administration to mice stimulates hepatic glycogen synthesis (through activation of mitogen-activated protein kinase signaling) [37], and blocks gluconeogenesis (through inactivation of the cAMP regulatory element-binding protein transcription factor) [38]. These effects are insulin-independent. Studies in rodents also suggest that the central nervous system is a target for FGF19 action. Intracerebroventricular administration of FGF19 to rats caused acute reductions in food intake and body weight, and improved glucose tolerance [39]. Additionally, studies in the mouse characterized an insulin-independent effect of FGF19 on glucose disposal mediated through activity in the brain [40].

FGF19 may also be important during liver regeneration. After liver resection, hepatic bile acid levels must be controlled to prevent cholestasis. Studies in mice indicate that FGF15 deficiency leads to greater risk for liver injury and mortality following partial hepatectomy, and this was associated with elevated intrahepatic bile acid levels [41]. Importantly, FGF15 administration to mice after liver resection improved survival in both FGF15-deficient mice with partial hepatectomy and in wild-type mice after extensive liver resection [41]. These effects may be related to direct mitogenic activity of FGF15 on hepatocytes and biliary epithelial cells. However, alongside the apparent beneficial effects of FGF19 in liver regeneration, FGF19 mitogenic activity is associated with hepatic carcinoma. In recent studies, FGF19 mRNA and protein levels were positively correlated with larger tumor size and early recurrence of hepatocellular carcinomas [42], and negatively correlated with disease-free survival [43]. Furthermore, reduction of FGF19 or FGFR4 expression levels using siRNA in human hepatocellular carcinoma cell lines inhibited proliferation and enhanced apoptosis [43]. These findings suggest that FGF19 signaling is a potential therapeutic target in hepatocellular carcinoma.

The identification of Diet1 as a component of the enterohepatic signaling axis

As evidence builds for critical roles of FGF19/15 and bile acids in metabolism, there is impetus to identify the factors that regulate FGF15/19 levels and activity. Although substantial information exists regarding FGF15/19 transcriptional regulation and mechanism of action in liver, the factors governing the intracellular trafficking and secretion of FGF15/19 in enterocytes have not been addressed. The recent identification of Diet1 may shed some light on this process.

Several years ago, it was noted that the C57BL/6ByJ inbred mouse strain exhibits aberrant bile acid homeostasis. This year, the underlying genetic mutation was identified in a novel gene, *Diet1*. The C57BL/6ByJ (B6By) strain originated from the C57BL/6J strain, and the two substrains remain nearly genetically identical. However, a dramatic difference in lipid metabolism exists between the two strains: C57BL/6J mice are highly susceptible to diet-induced hyperlipidemia and atherosclerosis, whereas B6By mice are nearly resistant [44]. This difference could not be attributed to differences in food intake, dietary cholesterol absorption, or endogenous cholesterol synthesis [44, 45]. A clue to the underlying mechanism for maintenance of low cholesterol levels in B6By mice was the detection of enhanced bile acid excretion in the feces and urine [45]. Serum bile acid levels were also elevated, likely due to spillover of bile acids from the portal vein. Microarray analysis

revealed elevated expression of *Cyp7a1* and numerous other genes involved in bile acid synthesis and transport in liver, suggesting that low cholesterol levels result from enhanced conversion to bile acids followed by excretion [45]. However, the genetic lesion in B6By mice could not be attributed to known genes. The bile acid phenotype was therefore mapped to high resolution in a cross of nearly a thousand mice, leading to the identification of an underlying null mutation in the novel *Diet1* gene [46].

Diet1 encodes a 236 kD protein with a modular structure containing nine copies of the MAM (meprin-A5-tyrosine phosphatase μ) domain, which alternate with nine copies of the low density lipoprotein receptor (LDLR) class A domain (Fig. 3). At the C-terminus of Diet1 is a predicted transmembrane spanning sequence, raising the possibility that the protein is membrane associated. The Diet1 protein sequence is 70% identical between mouse and human, and is evolutionarily conserved in mammals, amphibians, and fish [46]. The only other protein with substantial similarity is endotubin (also known as apical early endosomal glycoprotein and MAM domain-containing 4), which is about half the size of Diet1 (Fig. 3). The physiological function of endotubin is unknown, but studies in cultured cells have implicated it in trafficking of tight junction proteins in polarized epithelial cells [47]. According to publicly available gene expression databases, endotubin is ubiquitously expressed, whereas *Diet1* is expressed almost exclusively in the small intestine in both mice and humans, with lower levels in kidney cortex [46].

Diet1 has a role in modulating the levels of FGF15/19 (Fig. 2). In B6By mice, the loss of Diet1 activity leads to reduced FGF15 mRNA and protein levels in intestine, and (presumably compensatory) increases in liver genes involved in FGF15 and bile acid uptake, bile acid synthesis, and bile acid export [46]. In human enterocyte cell lines, Diet1 levels modulate the amount of FGF19 that accumulates in the culture medium: Diet1 expression promotes, and Diet1 knockdown reduces, secreted FGF19 protein levels [46]. Furthermore, Diet1 and FGF15 co-localize in vesicle-like structures in cultured enterocytes, suggesting that the two proteins have a functional interaction [46]. Together, these data suggest that Diet1 is a determinant of FGF15/19 production by enterocytes, with downstream effects on hepatic bile acid synthesis and cholesterol levels.

A comparison of Diet1–deficient mice with other models with perturbations in bile acid metabolism is consistent with a role for Diet1 in the FGF15/19–FGFR4–Cyp7a1 axis. Although the information available in the literature is incomplete, the comparisons in Table 1 demonstrate strong similarities between Diet1–deficient mice and genetically engineered models with aberrant bile acid synthesis or signaling from the small intestine to liver [14, 15, 48–58]. Diet1–deficient mice resemble all models listed in having enhanced *Cyp7a1* mRNA expression, most of which also have increased bile acid pool size and enhanced fecal bile acid excretion. However, only a subset of models have reduced plasma cholesterol levels as observed in Diet1–deficient mice, raising the possibility that Diet1 has additional independent effects on cholesterol metabolism; it would be necessary to compare cholesterol levels across models under similar dietary conditions and genetic backgrounds to make firm conclusions.

Questions remain about the proportional contribution of the FGF15/19 signaling axis to determining bile acid levels [16], and multiple mechanisms may be at work in specific models. For example, it has been suggested that in CYP8B1–deficient mice, changes in bile acid composition (rather than simply bile acid levels) lead to diminished FXRα activation, and subsequent effects on bile acid homeostasis [49]. In addition, the role of FGF15/19 levels in portal blood in the regulation of hepatic Cyp7a1 expression remains unclear [16]. In a study performed in rats and rabbits, it was not possible to correlate FGF15/19 concentrations in the portal circulation with levels of ileal FGF15/19 mRNA or effects on hepatic gene expression [59]. With negative results, it is difficult to rule out that unknown parameters (such as the single time-point analyzed) did not allow optimal detection. Continued study is warranted, especially in light of strong evidence that the activation of FXRα–FGF15 in the intestine—but not liver—suppresses *Cyp7a1* [14], and that signaling through FGFR4 is the predominant mechanism for this regulation [15].

Association of *DIET1* locus with Alzheimer's Disease

It remains to be determined whether genetic polymorphisms or rare mutations in human *DIET1* influence bile acid levels and/or have a role in conditions such as bile acid diarrhea. Interestingly, a DNA polymorphism (D10S1423) that has been associated with late-onset Alzheimer's Disease (AD) in three independent case-control samples is located within what is now known to be an intron of the *DIET1* gene [60–62]. The association of D10S1423 with AD has further been confirmed in a prospective, longitudinal study performed over the course of 14 years [63]. These several studies indicate that a particular D10S1423 allele has a synergistic interaction with the well-characterized *APOE* E4 risk allele: carriers of the D10S1423 risk allele have an odds ratio for late-onset AD of 2.5; *APOE* E4 carriers have an odds ratio of 8.3; and carriers of both risk alleles have an odds ratio of 23.1 [64]. D10S1423 was originally identified in a predicted open reading frame, which is largely similar to *DIET1*, aside from errors in prediction of the intron/exon boundaries.

Future work is required to evaluate the potential role of *DIET1* in AD. It has been reported that the predicted open-reading frame containing D10S1423 is expressed in human brain [64]. However, the mRNA species detected was only 1.2 kb in length and was not detected in intestine, whereas *DIET1* mRNA is 7 kb in length and is prominently expressed in human small intestine, but not detectable in brain [46]. It is conceivable that a small, alternatively spliced form of *DIET1* is expressed is brain, or perhaps an additional gene resides within the *DIET1* locus and is expressed in brain. It is tempting to speculate that the role of Diet1 as a determinant of plasma cholesterol levels could be a link to the function of apolipoprotein E and lipid metabolism in the brain. Clearly, the elucidation of the relationship between *DIET1*, D10S1423, and late-onset AD merits further study.

Conclusion

Although bile acids have a long history in medicine and biomedical research, recent findings underscore new associations with prevalent diseases such as type 2 diabetes and non-alcoholic fatty liver disease. A critical area for understanding bile acid homeostasis is the further elucidation of the FGF15/19 enterohepatic signaling axis. Recent studies have shed

light on the myriad roles of FGF15/19, and have revealed a novel potential regulator of FGF15/19 levels, Diet1. It should be noted that although evidence in human, primate, and mouse studies points to a key role for FGF15/19 in the maintenance of enterohepatic feedback regulation of bile acid synthesis, it remains possible that additional mechanisms contribute.

Acknowledgments

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KEY POINTS

- Dysregulation of bile acid levels is associated with conditions ranging from gallstones and cholestasis to type 2 diabetes and metabolic syndrome.

- The intestinally-derived hormone FGF15/19 signals to liver to regulate bile acid synthesis and maintain bile acid homeostasis.

- FGF15/19 levels appear to influence non-alcoholic fatty liver disease, glycemic improvement following gastric bypass surgery, liver regeneration following partial hepatectomy, and liver carcinogenesis.

- A novel intestinal protein, Diet1, has been identified as a regulator of FGF15/19 production by enterocytes, with effects on bile acid and cholesterol levels.

- A genetic polymorphism in *DIET1* is associated with late-onset Alzheimer's disease, suggesting potential links between bile acid and cholesterol homeostasis and brain.
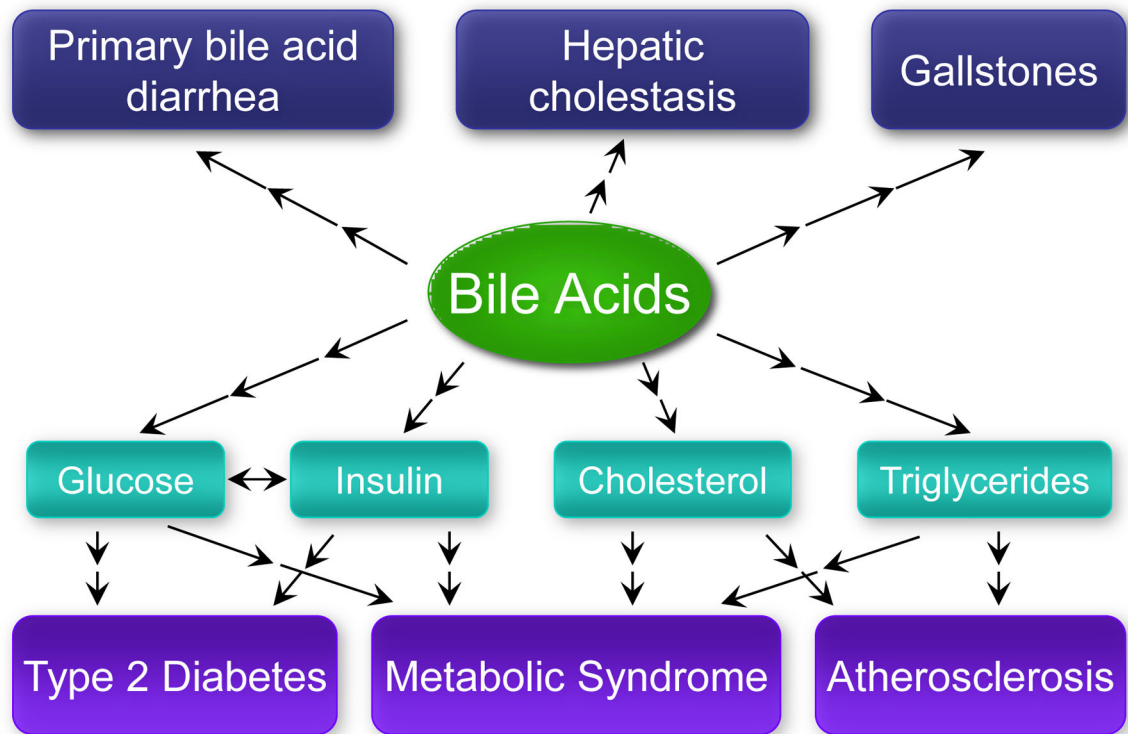
Figure 1.
Human diseases associated with altered bile acid metabolism include gallstones, hepatic cholestasis, and primary bile acid diarrhea, as well as common metabolic diseases such as type 2 diabetes and atherosclerosis.
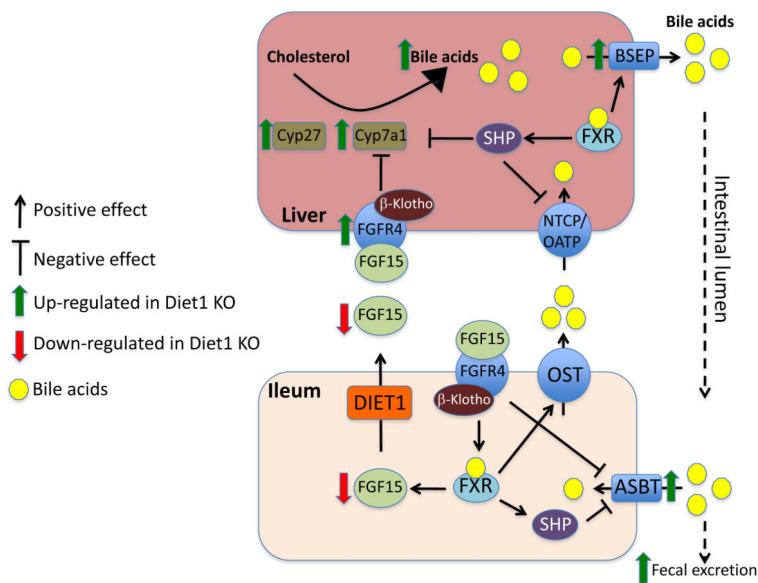
Figure 2.
Role of Diet1 and FGF15 in enterohepatic bile acid homeostasis. Bile acids are synthesized in the liver from cholesterol and are secreted into the gallbladder by BSEP (bile salt export pump). In liver, bile acids bind and activate the FXR (farnesoid X receptor) nuclear receptor, which induces expression of SHP (short heterodimer partner). In turn, SHP represses the expression of bile acid synthesis enzymes (including CYP7A1) and bile acid transporters NTCP (sodium-taurocholate cotransporting polypeptide) and OATP (organic anion-transporting polypeptide). In the distal small intestine, bile acids are actively reabsorbed by ASBT (apical sodium-dependent bile acid transporter), and can activate FXR. Bile acid excretion from enterocytes occurs through the organic solute transporter (OSTα/OSTβ heterodimer) at the basolateral membrane. FXR activation can induce expression of *FGF15* and *OST*, and inhibit expression of *ASBT* (via SHP). Diet1 promotes FGF15 secretion, which is thought to travel through the enterohepatic circulation and signals through the FGF receptor FGFR4 and β-Klotho to exert effects on hepatic gene expression, including *Cyp7a1* repression. In Diet1-deficient mice, enterohepatic regulation is altered, as indicated by red and green arrows: despite high bile acid levels in the intestine of Diet1-deficient mice, FGF15 expression and secretion are reduced, and hepatic *Cyp7a1* expression and bile acid pool is increased.
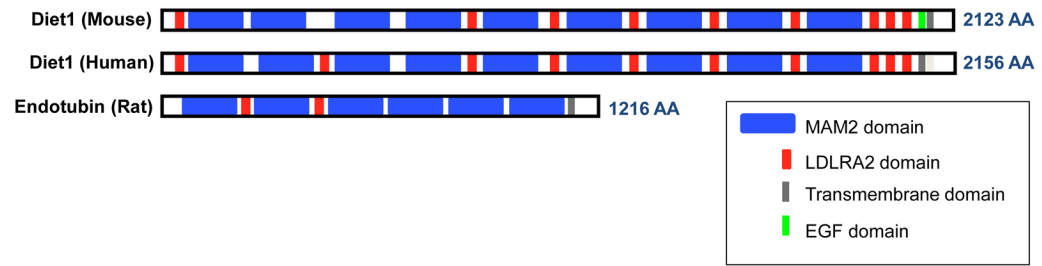
Figure 3.
Predicted domain structure of the Diet1 protein. Diet1 is characterized by alternating MAM (meprin-A5-tyrosine phosphatase μ) and LDL receptor class A2 domains, with C-terminal EGF (epidermal growth factor) and transmembrane motifs. Endotubin is the most closely related protein structurally.

Table 1

Mouse models with genetic defects in enterohepatic bile acid homeostasis.

Mouse model	BA pool	Fecal BA	Plasma BA	Plasma cholesterol	Ileal FGF15	Ileal SHP	Liver Cyp7a1	Liver SHP	Reference
Diet1-deficient	↑	↑	↑	↓	↓	↔	↑	↔	[46]
FXRα KO, whole body	↑	↔	↑	↑	↓	NA	↑	↓	[14, 15, 52, 56]
FXRα Liver KO	↑	↔	↔	↑	↔ or ↑	↔	↑	↔	[14, 15, 53]
FXRα Intestine KO	↑	↔	↔	↔ or ↓	↔ or ↓	↔	↔ or ↑	↔	[14, 15, 53]
SHP KO	↑	↑	↔	↔	NA	Absent	↑	Absent	[15, 48]
Cyp8b1 KO	↑	↑	NA	NA	↓	↓	↑	↓ or ↔	[49, 54]
Cyp7a1 Tg	↑	↑	↑	↓	↑	↑	↑	↑	[53, 65]
βKlotho KO	↑	↑	NA	↔	↑	NA	↑	↔	[51, 57]
FGF15 KO	NA	↑	NA	NA	Absent	NA	↑	↔	[50, 55]
FGFR4 KO	↑	↑	NA	↑	↔		↑	↓ or ↔	[15, 50, 55, 58]