Review

Transporters in cholelithiasis

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Abstract

Gallstones are a common and costly disease with a projected increase in prevalence due to the increasing ageing population. Numerous endogenous and environmental factors are aetiologically related to this multifactorial disease, and genetic studies continue to unravel the pathobiological mechanisms related to gallstone formation. In particular, variants of genes encoding hepatobiliary transporters have been implicated in gallstone disease and, given their ability to influence biliary lipid composition, have undergone considerable investigation. Here we summarize the role of enterohepatic transporters in cholelithogenesis with a particular focus on pertinent ATP-binding cassette transporters (ABCB4, ABCB11, ABCC7, and ABCG5/G8).

Keywords: ABC transporters; bile; gallstones; genome-wide association studies; lithogenic genes; multifactorial disease.

Introduction

The management of gallstones incurs one of the largest medical expenses among all gastroenterological disorders, secondary only to gastro-oesophageal reflux disease (Everhart and Ruhl, 2009). Patients with cholelithiasis frequently require hospital admission, and €1.9 billion and $6.5 billion are spent annually, primarily for performing more than 170 000 and 700 000 cholecystectomies in Germany and the US, respectively (Lammert et al., 2007; Everhart and Ruhl, 2009). The increasing prevalence of gallstones, which is currently reported at 15–20% of the population in Europe and America (Everhart et al., 1999; Völzke et al., 2005), will exacerbate these costs; hence, successful preventative measures are warranted.

The multifactorial nature of gallstone disease consists of a complex interaction between genetic and environmental risk factors. Genetic studies in experimental models and humans are continuously contributing pieces to the cholelithogenesis puzzle. Epidemiological studies analysing distinct human populations report a high prevalence of gallstones in specific ethnic groups, e.g., Amerindians (Carey and Paigen, 2002), thus reflective of a potential genetic component. The clustering of gallstones in families is also indicative of shared genetic and/or environmental risk factors. An analysis of 4394 Swedish twin pairs with symptomatic gallstones by Katsika et al. (2005) show that concordance rates are higher in monozygotic twins (12%) than in dizygotic twins (6%). Further, Katsika et al. (2005) explored phenotypic variation among twins through structural equation modelling and reported genetic factors to account for 25% of the variation. ‘Gallstone genes’ are continuously being corroborated in genome-wide association studies (GWAS), in case-control cohorts, and in family studies (Buch et al., 2007, 2010; Grünhage et al., 2007; Katsika et al., 2010).

Besides genetics, increasing age and female gender are also non-modifiable risk factors. Modifiable and other environmentally related risk factors include parity, hormonal therapy, obesity, rapid weight loss and weight cycling, as well as physical inactivity and chronic hypercaloric, high carbohydrate, low fiber intake – synonymous with ‘Westernized’ lifestyle habits (Stinton et al., 2010). The environmental influence on the increased prevalence of cholesterol gallstones is further reflected in areas where Westernized nutrition has infiltrated, such as in Native Americans, post-war European countries, and current urban dwellers in East Asia. Krawczyk et al. (2010) advocate gallstone risk to be amplified by higher-order interactions between genetic and environmental factors. A greater understanding of the role genetics play in gallstone disease affords the opportunity to identify high-risk individuals and take preventative measures. One such area includes variations in genes encoding hepatobiliary transporters that have, for example, been identified and explored for gallstone susceptibility (Figure 1). A discussion on the pathobiological role of transporters associated with cholelithogenesis follows.

Pathobiology

Cholesterol stones have a higher prevalence compared with black or brown pigment stones, and comprise >90% of cases. Cholesterol monohydrate crystals are the principal component in cholesterol stones, whereas calcium bilirubinate is the primary constituent of black and brown pigment stones (Schafmayer et al., 2006a). Gallstones primarily result from (i) supersaturation of bile, due to excess cholesterol and/or bilirubin, which cannot be solubilized by mixed micelles; (ii) gallbladder hypomotility; and (iii) destabilization of bile through proteins that offset the crystallization sequences (Maurer et al., 2009). Essentially, the following pathobiological process...
occurs: adenosine triphosphate (ATP)-dependent transport proteins, specifically known as ATP-binding cassette canalicul- 
transporters (ABC transporters), secrete biliary lipids into 
bile; unilamellar vesicles [composed of phosphatidylcholine (PC) and cholesterol] and simple micelles (composed of bile 
salts and cholesterol) form and are subsequently converted 
into mixed micelles while being channelled through the bili-
yary tract and into the gallbladder. If the bile is saturated with 
more cholesterol than can be solubilized by mixed micelles 
[indicated by a cholesterol saturation index (CSI) of >1], 
multilamellar vesicles (liquid crystals) occur and the aggrega-
tion of these cholesterol-rich multilamellar vesicles precedes 
the formation of solid cholesterol crystals.

Biliary cholesterol and bilirubin solubility in bile is there-
fore dependent on the intricate balance of biliary lipid concen-
trations (namely, bile salts, bilirubin, cholesterol, and 
phospholipids). Bile supersaturated with cholesterol is attrib-
utable to either cholesterol hypersecretion or the hyposecre-
tion of bile salts and/or phospholipids, hence an increased ratio 
of cholesterol and/or bilirubin to bile salts or phospholipids, 
or both. Consequently, alterations in the physical-chemical 
composition of bile with cholesterol or bilirubin saturation 
precipitate cholesterol or black pigment stones, respectively 
(Maurer et al., 2009). Intestinal hypomotility has also been 
linked to gallstones, partly by promoting lithogenic bile 
from the increased bacterial colonic formation of the second-
ary bile salt, deoxycholate, where increased levels due to slow 
intestinal transit are frequently reported in patients with cho-
lesterol gallstones (Portincasa et al., 1996).

ATP-dependent transport proteins (ABC transporters) play 
a significant role in the regulation of the physical chemistry of 
bile given that they control biliary lipid secretions across the 
canalicular hepatocyte membranes. Consequently, defects in 
ABC transporters have been associated with gallstone suscepti-
ubility. Specifically, the ATP-binding cassette trans-
porter, subfamily B, member 4 (ABCB4); ATP-binding cas-
tette transporter, subfamily B, member 11 (ABCB11); and 
ATP-binding cassette transporter, subfamily G, member 
5/ATP-binding cassette transporter, subfamily G, member 
8 [ABCG5/G8 (heterodimeric transporter of the two half trans-
porters ABCG5 and ABCG8)] transporters mediate the secre-
tion of PC, bile salts, and cholesterol, respectively (Figure 2). A 
deficiency of the ABCB4 transporter can alter the composition 
of primary bile due to insufficient PC, an essential component 
for mixed micelle formation. Further, a depressed concentra-
tion of bile salts from defects in the ABCB11 transporter dis-
enables the solubilization of biliary cholesterol, resulting in 
cholesterol supersaturation and the formation of cholesterol 
crystals, hence the increased risk of cholelithiasis (van Mil et 
al., 2004). The two cholesterol hemi-transporters ABCG5 and 
ABCG8 are active as a heterodimer that plays a critical role in 
cholesterol excretion, so much so that ABCG5 and/or ABCG8 
loss-of-function mutations have been shown to cause sitoster-
olemia, a rare genetic disorder of lipid metabolism, character-
ized by excess concentrations of cholesterol (and phytosterols) 
in serum (Fitzgerald et al., 2010). Finally, the ATP-binding 
cassette transporter, subfamily C, member 7 (ABCC7) trans-
porter that is responsible for facilitating chloride transport 
into bile ducts is also associated with changes to the physi-
chemical chemistry of bile and, hence, gallstones (Herrmann et al., 
2010). Therefore, mutations in genes encoding hepatocanal-
cular transporters have been implicated in cholelithogenesis 
owing to their ability to influence the process of bile formation 
by modifying bile composition and causing retention of sub-
stances normally secreted in bile.

Investigative techniques

The use of quantitative trait locus (QTL) analysis in experi-
mental crosses of inbred mouse lines (homozygous for all 
alleles) has enabled the identification of susceptibility factors 
in gallstone disease. QTL analysis is a powerful statistical 
technique that maps causative allele variations in complex 
traits. Essentially, QTL analysis links polymorphic genetic 
markers (DNA sequence variants) to disease phenotypes in 
large experimental cohorts (n>200). For this, two inbred 
mouse strains with homozygous alleles at all loci and dis-

tinct gallstone susceptibility are crossed (e.g., the gallstone-
susceptible strain C57L/J and the resistant strain AKR/J); the F1 progeny are crossed to generate a large number of intercross (F2) or backcross mice, which are all a mosaic of their grandparents’ genomes. Hence, these mice show variants in the risk of gallstones, i.e., some develop gallstones and others are more resistant when challenged with a lithogenic diet, which contains 15% fat, 1% cholesterol, and 0.5% cholic acid to ‘humanise’ bile salt pool composition and promote intestinal cholesterol absorption (Lyons and Wittenburg, 2006). After genotyping the second-generation progeny for a set of genetic markers covering the whole genome, the allele distributions are correlated with the individual gallstone phenotypes in a traditional genome-wide genetic linkage analysis. Thereby, inbred strains have set the stage for the identification of gene loci that confer gallstone risk, and QTL mapping in a set of different crosses has resulted in a susceptibility map of lithogenic gene (Lith gene) variants, such as those encoding the ABC transporters ABCB11, ABCC2, and ABCG5/G8. The latter have recently been corroborated in human GWAS, used to identify gene-disease associations. Although further investigation is required to identify the full spectrum of genetic susceptibility variants and to decipher their role in gallstone formation, to date major murine and human Lith genes have been discovered. For the most recent updated inventory of Lith genes in mice and humans, refer to Wang et al. (2010a) and Krawczyk et al. (2011), respectively.

The ABC canalicular transporters

ATP-binding cassette transporters belong to a large superfamily of proteins in membranes, which use energy from ATP hydrolysis to translocate a wide range of compounds across steep concentration gradients and through membranes, and hence are often referred to as ABC transporters (derived from their most characteristic feature). ABC transporters mostly transport lipids and toxic compounds, and thus play a crucial role in inborn or acquired diseases (e.g., gallstones), and in toxicology, particularly in the case of ABC gene point mutations. Within the ABC transporter superfamily, there are several subfamilies differentiated by the compounds transported.

The ABCB4/ABCB11 transporters

The ABCB4 and ABCB11 canalicular transporters (members of the ABCB subfamily) are expressed in the apical plasma membranes of hepatocytes and transport phospholipid and bile salts into bile, respectively. The ABCB11 was previously known as the bile salt export pump because of its inherent role transporting bile salts, and the ABCB4 gene, which encodes the transporter involved in PC translocation, is often referred to as the multiple drug resistance gene (MDR3 in humans and Mdr2 in mice). Both these candidate genes are purportedly associated with gallstone susceptibility. Specifically, an ABCB4 deficiency has been implicated in monogenic susceptibility to gallstones in human studies and in mouse models. Progressive familial intrahepatic cholestasis type 3 (PFIC3) is an autosomal-recessive disorder that causes liver cirrhosis in children. Mutations of the phospholipid transporter should result in the absence or very low levels of PC in bile, hence a high biliary CSI. Diseases related to ABCB4 mutations are also present in adults. For example, in Jacquemin et al. (1999), discovered an association between the linkage of heterozygous ABCB4 non-sense mutations and intrahepatic cholestasis of pregnancy (ICP) in family members of a child with PFIC3. Further, a family study by Schneider et al. (2007) found splicing mutations in the MDR3 gene to cause ICP and to be associated with gallstone disease.

The association between gallstones and the ABCB4 transporter is supported by Rosmorduc and Poupon (2007) who analysed the frequency of ABCB4 gene mutations in 60 patients with symptomatic or complicated cholelithiasis. Of these patients, ABCB4 point mutations were identified in 56% of the 32 patients with low phospholipid-associated cholelithiasis (LPAC), as defined by the following criteria: onset of symptoms <40 years, presence of intrahepatic microcholelithiasis and extrahepatic cholesterol stones, and recurrence of symptoms after cholecystectomy. However, no ABCB4 point mutations were identified in the 28 patients with classic gallstone disease, or in the 33 patients with no prior history of gallstones. Multivariate analysis showed ABCB4 mutations to correlate with three independent clinical features: (i) cholesterol gallbladder stones and intrahepatic sludge or microlithiasis [adjusted odds ratio (OR) 6.1, 95% confidence interval (95% CI) 1–46, p < 0.05]; (ii) recurrence of biliary symptoms after cholecystectomy (adjusted OR 8.5, 95% CI 2–79, p < 0.05); and (iii) age <40 years at symptom onset (adjusted OR 3.0, 95% CI 0.6–33, p < 0.05) (Rosmorduc et al., 2003; Rosmorduc and Poupon, 2007). This finding supports ABCB4 mutations as being a major risk factor for both symptomatic and recurrent gallstones in young adults. The association between LPAC and an ABCB4 deficiency has recently been corroborated by Denk et al. (2010) who identified a novel ABCB4 gene mutation.

Associations between gallstones and the ABCB4 gene are also reported in animal studies, where cholesterol gallstones spontaneously (e.g., without consuming a lithogenic diet) but consistently occur in Abcb4-deficient mice and alterations in the physical-chemical composition of bile are observed (Smit et al., 1993; Lammert et al., 2004). In Mdr2−/− (Abcb4−/−) knockout mice, cholesterol supersaturation in bile ensues owing to a lack of PC, resembling patients with LPAC. Lammert et al. (2004) found cholesterol crystals to precipitate in the gallbladder bile of Mdr2−/− female mice after 12 weeks of being fed a regular diet, and >50% developed gallbladder stones after 15 weeks. These findings were more prevalent in female mice displaying a higher susceptibility to gallstones with significantly larger gallstones at 18 weeks compared with male mice – a finding concomitant with the widespread reporting of the increased prevalence of gallstones in females from human studies. The monogenic type of ABCB4 deficiency appears to be relatively rare and encompasses Mendelian inheritance; however, its presence confers a high risk of disease (Krawczyk et al., 2010). Individuals with ABCB4 point mutations are particularly prone to cholesterol gallstones because they might lack the essential quantities of phospholipids required for
mixed micelle formation, which is needed to solubilize cholesterol (Rosmorduc and Poupon, 2007).

Mutations in the ABCB11 gene (located in the Lih1 locus of chromosome 2) have been causally linked to progressive familial intrahepatic cholestasis type 2 (PFIC2) (Jansen et al., 1999) and to benign recurrent intrahepatic cholestasis (BRIC) – a condition where intermittent cholestasis without progression to liver cirrhosis is observed (van Mil et al., 2004). Coincidently, gallstones are frequently observed in patients with PFIC2 (Strautnieks et al., 1998) and have also been reported in patients with BRIC (van Mil et al., 2004). Moreover, defects in the human ATPase, aminophospholipid transporter, class I, type 8B, member 1 (ATP8B1) genes are linked to BRIC (type 1); however, the presence of cholelithiasis was noted in 7 of 11 (65%) BRIC patients with the ABCB11 mutations but not in any patients with the ATP8B1 mutations. Hence, van Mil et al. (2004) proposed the differentiation of the disorder by naming the ABCB11-related variant as BRIC type 2. The high prevalence of cholelithiasis in BRIC patients with the ABCB11 mutation is consistent with monogenic cholelithiasis, which, despite its rare risk allele frequency, is characterized by a high susceptibility to gallstones. Abcb11 has been identified as one of the strongest candidate genes underlying the Lih1 locus in mice (e.g., Paigen et al., 2000). The evidence to date, however, is inconclusive with further studies failing to report and association between the ABCB11 candidate gene and gallstone disease. Schafmayer et al. (2006b) reported no association of the ABCB11 locus to gallstone susceptibility in a large German case control study. In a mouse model, Wang et al. (2010b) developed an Abcb11 bacterial artificial chromosome transgenic mouse strain (Abcb11.Tg) on the gallstone-resistant AKR genetic background to investigate the potential susceptibility to gallstones in the setting of hepatic Abcb11 overexpression. Nevertheless, 8 weeks on the lithogenic diet resulted in no differences in gallstone prevalence rates between Abcb11.Tg mice (25%) and wild-type mice (20%), and in both mice strains, 75% and 80% were gallstone free, respectively. Moreover, no difference in CSI between the two mouse strains was observed. Wang et al. (2010b), however, found the overexpression of Abcb11 to promote hepatic bile salt secretion, and to increase bile salt pool size as well as bile salt-dependent bile flow. Figge et al. (2004) also reported increased bile salt secretion rates in Abcb11 transgenic mice. Therefore, the observed changes noted in these two studies are synonymous with those expected for a bile salt transporter, although the findings are not in line with ABCB11 modifying susceptibility to gallstones, as noted in the above-mentioned human studies. On the whole, more research is required to elucidate the potential mechanistic effects of the ABCB11 transporter on gallstone formation, particularly as Abcb11 knockout mice also display residual bile salt secretion, suggesting the use of an alternate transport system (Wang et al., 2003).

The ABCG5/ABCG8 transporters

A recent GWAS related the genes encoding the hepatocanaliculc cholesterol transporters ABCG5/ABCG8 (belonging to the ABCG subfamily and expressed in the hepatocytes and enterocytes) to gallstone formation (Buch et al., 2007). Indeed, the ABCG8 gene is the most widely replicated human susceptibility gene for gallstone disease, with a two-fold increased risk reported in German, Sorb, Romanian, Swedish, Chilean, and Chinese patients (Krawczyk et al., 2011). Further, QTL mapping in inbred mice strains have related these cholesterol transporters to gallstone susceptibility (Lih9 locus). A study using crosses of inbred mouse strains reported a positive correlation of higher hepatic Abcg5/g8 expression levels with gallstone formation and biliary cholesterol concentrations (Wittenburg et al., 2005).

In humans, the ABCG8 p.D19H variant has consistently been associated with gallstone disease. Buch et al. (2007) reported an association between this amino acid substitution and gallstone susceptibility ($p$ = $7.7 \times 10^{-4}$, risk allele frequency 5%) when screening a cohort of 280 cases and 360 controls. The authors replicated the association scan in >2000 patients from Germany and Chile, and a combined analysis of all German individuals found an OR of 2.2 (95% CI 1.8–2.6) for carriers of the risk allele, corresponding to a population attributable risk of 11%.

Further, a combined linkage and association study in affected sibling pairs (ASP) from both Germany and Romania have also identified the ABCG8 p.D19H variant as being a susceptibility factor for gallstone disease. Compared to stone-free controls, carriers of the 19H allele in randomly selected cases from the ASP cohort displayed a significantly increased risk of gallstones (OR 3.0, 95% CI 1.2–7.7, $p$ = 0.017) (Grünhage et al., 2007). The susceptibility of ABCG8 p.D19H carriers has recently been corroborated by Katsika et al. (2010) where Swedish twins carrying either heterozygous or homozygous ABCG8 p.D19H genotypes had a significantly increased risk (OR 2.5, 95% CI 1.3–4.8, $p$ = 0.004) of gallstone disease.

The ABCG5/G8 heterodimer has also been implicated in cholesterol homeostasis in the intestine (the primary site for dietary and biliary cholesterol absorption and faecal cholesterol disposal). This heterodimer is located in the upper small intestine and is responsible for pumping cholesterol from the enterocyte into the intestinal lumen and into bile, and consequently, more faecal cholesterol is removed. The Niemann-Pick C1-like 1 protein is suggested to complement this process by regulating the hepatic reabsorption of cholesterol (Temel et al., 2007). Studies have shown significant associations between common polymorphisms in the ABCG5 and ABCG8 genes with plasma lipids. For example, Acalovschi et al. (2006) reported high triglyceride and low high-density lipoprotein cholesterol levels in siblings with gallstones compared with gallstone carriers with no positive family history. Previously, Gylling et al. (2004) studied the role of ABCG5/G8 transporter variants in the regulation of cholesterol metabolism and insulin sensitivity in 263 mildly hypercholesterolemic non-coronary subjects. The ABCG8 19H risk allele was strongly associated with low cholesterol absorption efficiency (of which phytosterols are the surrogate markers), but with a higher rate of cholesterol synthesis (where cholesterol precursors are the surrogate markers). In addition, low total cholesterol and low-density lipoprotein cholesterol
levels were observed in carriers of the risk allele (all p<0.05). The ABCG8 19H variant thus appears to inhibit cholesterol intestinal absorption and serum cholesterol levels, but trigger a compensatory increase in hepatic cholesterol synthesis and is associated with cholesterol gallstone susceptibility.

These findings have been reported in mouse models, whereby the overexpression of Abcg5 and Abcg8 in transgenic mice altered hepatobiliary cholesterol transport (Yu et al., 2002). Fractional absorption of dietary cholesterol was reduced by 50%; however, a striking five-fold increase in biliary cholesterol levels was reported. Moreover, hepatic cholesterol synthesis increased two- to four-fold, and faecal neutral sterol excretion increased three- to six-fold. No changes in bile salt pool size, composition, or faecal excretion were observed. Further, Yu et al. (2002) found disruption of the Abcg5/g8 gene in mice to lead to increased absorption of plant sterols and very low biliary cholesterol concentrations. In summary, the ABCG8 D19H variant may potentially represent a ‘gain-of-function’ mutation that increases the removal of sterols from the body. The Abcg5/g8 genes thus modulate plasma lipids and influence the cholesterol saturation of bile through the promotion of biliary cholesterol excretion, hence triggering a susceptibility to gallstone formation.

The ABCC7 transporter

The cystic fibrosis transmembrane conductance regulator (CFTR) gene (located on chromosome 7) encodes the ABCC7 transporter, which belongs to the ABC subfamily. The ABCC7 transports chloride ions (Cl⁻) across cell membranes and is located in the apical membrane of secretory and absorptive epithelial cells in the lungs, liver, pancreas, intestine, and sweat glands. Mutations in the CFTR gene are commonly present in patients with the autosomal-recessive disorder cystic fibrosis (CF). This is due to the critical loss of Cl⁻ transport, which upregulates the sodium/chloride ion balance and, consequently, a dense adhesive mucus layer in lungs and small bile ducts ensues, trapping bacteria and causing chronic infections. Interestingly, CF is a known risk factor for black pigment stones (Vitek and Carey, 2003; Wasmuth et al., 2006), with a prevalence of between 3% and 25% (Herrmann et al., 2010), thus CFTR gene mutations are associated with an increased risk of gallstones.

The pathomechanism was demonstrated in a study by Freundenberg et al. (2008) where increases in faecal bile salt loss resulted in hepatic bile containing more hydrophobic bile salts in conjunction with increased colonic absorption of bilirubin and consecutive hyperbilirubinemia in the DeltaF508 mouse model [a Cfr gene mutation where the deletion of three nucleotides causes the loss of phenylalanine (F) at position 508 of the protein]. Coincidently, the promoter mutation in the UDP-glucuronosyl transferase 1 (UGT1A1) gene causing Gilbert syndrome increases the enterohepatic cycling of unconjugated bilirubin and the risk of gallstones. Moreover, Wasmuth et al. (2006) showed that CF patients with gallstones were more likely to carry this variant UGT1A1 allele than controls; therefore, the gallstone-susceptible effects of CFTR mutations may be accentuated in these individuals or exert its effects in parallel to concomitant genetic mutations.

The SLC10A2 intestinal susceptibility variant

A novel common susceptibility factor for gallstone disease in humans, which is a variant of a transporter located in the intestine, has recently been identified. Specifically, the ileal apical sodium-dependent bile salt transporter (ASBT) belongs to the solute carrier family (SLC). A SLC transporter is assigned to a specific family when 20–25% of its amino acid sequence is identical to other family members. The ASBT is encoded by the solute carrier family 10 member 2 (SLC10A2) gene and represents the major intestinal bile salt uptake system, transporting both conjugated and unconjugated bile salts. Wong et al. (1995) first identified a dysfunctional SLC10A2 gene in a patient with Crohn’s disease, and Oelkers et al. (1997) subsequently reported an association between bile salt malabsorption and SLC10A2 mutations. ASBT therefore plays a central role in the enterohepatic circulation of bile salts and its reduced expression has been suggested as being cholelithogenic (Holzer et al., 2008; Renner et al., 2009). For example, a SLC10A2 gene variant was recently examined in a candidate gene study with a German-based cohort (Renner et al., 2009). A pooled analysis including 240 gallstone carriers and 255 controls found an association between the SLC10A2 rs9514089 single nucleotide polymorphism and gallstones (OR 2.0, 95% CI 1.2–3.6, p = 0.008). This finding was more prevalent in non-obese male gallstone carriers. Interestingly, reduced expression of this transporter was found to be weight specific, e.g., Bergheim et al. (2006) observed it in normal-weight individuals only. Furthermore, Renner et al. (2009) found the SLC10A2 risk allele to be associated with lower serum cholesterol levels, particularly in gallstone carriers. Hence, this transporter appears to govern the quantity of bile salts returning to the liver and is suggested as an important determinant of the primary bile salt pool size, which in turn influences cholesterol homeostasis. Further molecular mechanisms, however, remain to be determined.

Regulation of hepatobiliary transporters by the nuclear receptor network – implications for gallstones

Nuclear receptors (NR) are a class of proteins present in all eukaryotic cells and are often referred to as ligand-activated transcription factors regulating gene expression. A unique and characteristic feature of NRs is their direct interaction with DNA and control of promoter activity. As with ABC and SLC transporters, the amino acid sequence determines an NR’s subfamily. Although much still remains to be discovered about NRs, research continues to unravel the complex interactions of NRs with hepatobiliary transporters. A mouse study by Bookout et al. (2006) reported a higher-order transcriptional regulatory network of NRs through their ability to coordinate the transcriptional programs that affect distinct physiological pathways: (1) reproduction, growth, and development,
and (2) nutrient uptake, metabolism, and excretion. Bookout et al. (2006) clustered a variety of NRs based on the site of tissue expression. Of importance is the cluster containing NRs functioning at the top of a transcriptional cascade governing nutrient uptake and maintaining the protective barrier in the enterohepatic tract. Several NRs in this subcluster determine the metabolism of bile salts, such as the farnesoid X receptor (FXR). FXR controls bile salt homeostasis by tightly regulating the transport proteins for bile salts (ABCB11) and PC (ABCB4) (Gadaleta et al., 2010). In terms of physiological mechanisms, after bile salts are absorbed into the enterocyte, they activate FXR in the intestinal ileum, which induces fibroblast growth factor (FGF) 15/19 expression (FGF 15 in mice and FGF 19 in humans). Gadaleta et al. (2010) referred to this as ‘ileal brake’ since the activation of FXR 15/19 stops bile salt neosynthesis through a negative feedback loop. Moreover, this mechanistic reaction signals progression from the fed state back to the fasting state.

Currently, data on the effects of FXR on humans with regard to cholesterol gallstones are limited, with controversial findings in population studies examining FXR gene polymorphisms. For example, in a study of 1004 individuals from three distinct populations, Kovacs et al. (2008) reported an association between the most common haplotype NR1H4_1 and gallstone prevention in a Mexican population. However, this haplotype displayed no association with gallstone prevalence in the German population, which is in stark contrast to a Chilean population where a trend towards a protective effect was observed. This finding alludes to the possibility of complex interactions between NR1H4 alleles and other risk factors. In animal studies, however, Fxr knockout mice have an increased risk of cholesterol gallstone formation when fed the lithogenic diet (Moschetta et al., 2004). Moschetta et al. (2004) showed that FXR influences the physical chemistry of bile by reducing the expression of the transport proteins ABCB11 and ABCB4, hence decreasing bile salt and PC concentrations in bile. The influence of FXR on gallstones is further illustrated by the fact that a synthetic FXR agonist reduces the prevalence of gallstones in mice, where increased quantities of biliary bile salts and phospholipids are secreted, thus preventing the supersaturation of cholesterol in bile (Moschetta et al., 2004). A careful explanation of the nuclear regulation of ABC transporters with regards to gallstone disease has recently been published by Claudel et al. (2011).

Conclusions

The multifactorial nature of gallstone disease provides diverse preventative and therapeutic possibilities. Advances in the understanding of the genetic predisposition and pathobiological mechanisms underlying gallstone formation, such as the role of ABC or SLC transporters, enable the identification of high-risk groups where appropriate genotyping may be used to guide therapeutic options. Preventative measures and tailored treatment interventions are thus highly sought after, and a greater understanding of the molecular mechanisms in gallstone formation may help identify novel treatment and preventative targets. Examples of such targets include the use of FXR or G-protein coupled bile acid receptor 1 (GPBAR1) modulators. The GPBAR1 membrane receptor is involved in bile salt homeostasis, and hence influences the physical chemistry of bile. Moreover, the expression of cholesterol 7α-hydroxylase (Cyp7a1) – the rate-limiting enzyme in the elimination of cholesterol through its conversion to bile salts – is reportedly upregulated in Gpbar1-deficient mice, illustrating an altered feedback regulation of bile salt synthesis (Vassileva et al., 2006). The exact role of these receptors in gallstone disease remains to be elucidated; however, current research is portraying this, as well as the aetiological role of transporters, as exciting findings, which hold potential for guiding future therapeutic options. Most importantly, each time a piece of the cholelithogenesis puzzle unfolds, we come a step closer to reducing the burden imposed on individuals and society as a whole.

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