Gallstone disease is very common in the Western world with an estimated prevalence of 10% to 15% in white adults, leading to significant morbidity, mortality, and considerable health care costs. In the Western world, approximately 70% of gallstone carriers exhibit cholesterol gallbladder stones (cholesterol content >50%), and 30% exhibit black pigment gallbladder stones. In East Asia, there is a high prevalence of brown pigment stones residing in the bile ducts, and causing potentially devastating cholangitis. Nevertheless, also in these countries, prevalence of cholesterol gallstones increases, supposedly caused by the introduction of Western diet. Because of increased prevalence of overweight or a higher proportion of elderly subjects in the population, the prevalence of gallstone disease may further increase in the near future. This article focuses on the pathogenesis of cholesterol gallstones. Cholesterol crystal nucleation is considered the earliest step in cholesterol gallstone formation. Various conditions affecting the crystallization process are discussed, such as biliary cholesterol supersaturation, excess pronucleating proteins, or shortage of nucleation-inhibiting proteins, and factors related to the gallbladder, such as hypomotility. Pigment gallstone pathogenesis is briefly discussed.

**PHYSICAL-CHEMICAL ASPECTS OF BILIARY CHOLESTEROL SOLUBILIZATION AND CHOLESTEROL CRYSTALLIZATION**

Although solubility of cholesterol in aqueous solutions is extremely limited, in gallbladder bile a relatively large amount (approximately $20 \times 10^{-3} \text{ M}$) of the sterol can be kept in solution. This significant increase in solubility is explained by incorporation of cholesterol in mixed micelles, together with bile salts and phospholipids (mainly phosphatidylcholine). Supersaturation occurs when either too much cholesterol or not enough solubilizing bile salt and phosphatidylcholine molecules are secreted to allow complete micellar solubilization of all cholesterol. Excessive cholesterol may
be kept in vesicles (ie, spherical bilayers of cholesterol and phospholipids, without bile salts) or in cholesterol crystals. Cholesterol crystal nucleation is thought to occur in general from vesicles supersaturated with cholesterol (ie, vesicular cholesterol/phospholipid ratio >1).¹ First, small unilamellar supersaturated vesicles aggregate or fuse into larger multilamellar vesicles (“liquid crystals”), with subsequent phase-separation of cholesterol crystals.² Pivotal information on the process of cholesterol crystal nucleation, the earliest step in cholesterol gallstone formation,³ has been obtained from in vitro studies in model bile systems. Wang and Carey⁴ found that cholesterol crystallization pathways and sequences in human gallbladder biles are identical to model biles matched for all relevant physical-chemical conditions, underlining the relevance of the model bile data. Based on these model bile data, the equilibrium bile salt–phospholipid–cholesterol ternary phase diagram was constructed, which allows one to predict behavior of mixtures of the three biliary lipids when present in various proportions.⁵ As shown in Fig. 1, the phase diagram contains a bottom one-phase zone (only micelles);

![Equilibrium bile salt–phospholipid–cholesterol phase diagram](image)

**Fig. 1.** Equilibrium bile salt–phospholipid–cholesterol phase diagram. The components are expressed in mol percent. Depicted are a one-phase (micellar) zone at the bottom; a left two-phase zone (containing micelles and crystals); a central three-phase zone (containing micelles, vesicles and crystals); and a right two-phase zone (containing micelles and vesicles). Phospholipid/(bile salt + phospholipid) ratios are given at the bottom axis, and going from left to right indicates increased relative amounts of phospholipids compared with bile salts, with increased vesicular cholesterol solubilization. Any line connecting the bottom axis with the top of the triangle (100% cholesterol) represents identical phospholipid/(bile salt + phospholipid) ratio, with increased relative amounts of cholesterol when moving from bottom to top. For example, in the Figure, all biles plotting on the coarse interrupted line exhibit identical phospholipid/(bile salt + phospholipid) ratio of 0.8. In the Figure, fine lines indicate zones in case of diluted bile, hydrophilic bile salts or saturated phospholipid acyl chains. Thick lines indicate zones in case of concentrated bile, hydrophobic bile salts, or unsaturated phospholipid acyl chains. Under the latter circumstances, cholesterol crystallization is promoted by an expansion of the crystal-containing zones to the right. The bile sample initially plotting in the right two-phase zone (indicated by the dot) now plots in the central three-phase zone (cholesterol crystal-containing zone), despite identical relative lipid composition.
a left two-phase (micelles and cholesterol crystals containing) zone; a central three-phase (micelles, vesicles, and cholesterol crystals containing) zone; and a right two-phase (micelles and vesicles containing) zone. Going from baseline to top in the phase diagram the relative percentage of cholesterol increases, with progressive tendency of cholesterol crystallization as a result. Second, a shift from left to right in the phase diagram increases relative amounts of phospholipids compared with bile salts, allowing more solubilization of cholesterol in vesicles, lower vesicular cholesterol/phospholipid ratios, and less cholesterol crystallization as a result. If gallstones are present in supersaturated bile, competition may occur between the gallstone surface (gallstone growth) and the surrounding bile for available cholesterol molecules. Three factors strongly affect the ternary equilibrium bile salt–phospholipid–cholesterol ternary phase diagram, with potential consequences for in vivo cholesterol crystallization: (1) increased bile concentration, (2) increased bile salt hydrophobicity, and (3) phospholipids containing unsaturated acyl chains all strongly promote cholesterol crystallization. Corresponding effects on the ternary equilibrium bile salt–phospholipid–cholesterol ternary phase diagram are in all three cases an increase of the bottom one-phase (micellar) zone, an expansion of the cholesterol crystal–containing zones to the right, and a decrease of the vesicles-containing zones (see Fig. 1).

**BILE CONCENTRATION**

Water is a major component of bile. Significant net water absorption occurs during bile transfer through the bile ducts and during prolonged storage in the gallbladder. As a result, bile water content decreases from 97% weight in the bile ducts to 90% weight in the gallbladder. This threefold to fourfold concentration of bile enhances cholesterol crystallization and gallstone formation considerably. During the process of bile formation, detergent bile salt monomers first induce formation of nascent cholesterol-phospholipid vesicles in the bile canalicular space. These vesicles are stable because they are relatively cholesterol-poor (cholesterol/phospholipid ratio <1), and cholesterol crystallization does not occur. During bile concentration in the bile ducts and gallbladder, mixed cholesterol–phospholipid–bile salt micelles are increasingly formed, because bile salt concentrations now progressively exceed critical micellar concentrations required for micelle formation. Cholesterol and phospholipid transfer then occurs from vesicles to these mixed micelles. Because solubilizing capacity of micelles for phospholipids is much higher than for cholesterol, however, there is preferential phospholipid transfer. Although fewer vesicles remain, they are now cholesterol supersaturated (ie, cholesterol/phospholipid ratio >1) and may nucleate cholesterol crystals. This sequence explains why gallstones are generally formed in the gallbladder rather than in the bile ducts.

**BILE SALT HYDROPHOBICITY**

More hydrophobic bile salts strongly promote cholesterol crystallization, by affecting the ternary phase diagram in a similar way as bile concentration (increased micellar cholesterol solubilization, shift of crystal-containing zones to the right, cholesterol supersaturated vesicles, promotion of cholesterol crystallization). In the animal kingdom, human bile exhibits the most hydrophobic bile salt composition, with strong propensity to gallstone formation. Nevertheless, there is considerable variation in hydrophobicity of human bile salt composition (especially amounts of deoxycholate). In gallbladder bile of cholesterol gallstone patients, increased amounts of the hydrophobic bile salt deoxycholate are associated with fast crystallization. The primary bile salts cholate and chenodeoxycholate are synthesized from cholesterol in the liver,
and secondary bile salts (mainly deoxycholate) are formed from primary bile salts in the intestine by bacterial 7α-dehydroxylase activity. Interestingly, gallstone patients exhibit larger amounts of bacteria and more 7α-dehydroxylase activity in cecal aspirates in conjunction with higher colonic pH values and prolonged small and large bowel transit times, all favoring solubilization and absorption of deoxycholate into the enterohepatic circulation. Because of the presence of \textit{Lith} genes, male C57L inbred mice are highly susceptible to cholesterol gallstone formation during lithogenic diet, provided that a hydrophobic biliary bile salt composition (quite hydrophilic at baseline) is obtained by dietary measures (15% fat, 1% cholesterol, 0.5% cholic acid), supporting a role for bile salt composition in gallstone formation. Modulating biliary bile salt composition may have therapeutic consequences. In selected patients with cholesterol gallstones, treatment with the hydrophilic bile salt ursodeoxycholate may dissolve their stones. Under these circumstances, 30% to 60% of the total bile salt pool consists of ursodeoxycholate, with the result that cholesterol crystallization is inhibited.

\section*{BILIARY PHOSPHOLIPID COMPOSITION}

In in vitro studies with model biles, phospholipid class and phospholipid acyl chain composition exert profound effects on cholesterol crystallization. Similar to increased bile concentration and increased bile salt hydrophobicity, phospholipids with more unsaturated acyl chains affect the ternary equilibrium bile salt–phospholipid–cholesterol ternary phase diagram by increasing the bottom one-phase (micellar) zone, expanding the cholesterol crystal–containing zones to the right and decreasing the vesicles-containing zones, with the result that cholesterol supersaturated vesicles and cholesterol crystallization occur (see \textit{Fig. 1}). The underlying physical-chemical explanation for these findings is that phospholipids with saturated acyl chains by their \textit{“trans”} configuration fit easily in the vesicular cholesterol-phospholipid bilayer, which is not the case for phospholipids with \textit{cis}-unsaturated acyl chains with a bend in the molecule (leading to preferential micellar containment). Human biliary phospholipid composition is tightly regulated, however, and almost exclusively composed of phosphatidylcholine with unsaturated acyl chains (mainly 16:0 acyl chains on \textit{sn}-1 position, 18:2 > 18:1 > 20:4 acyl chains on \textit{sn}-2 position), contributing to human vulnerability for gallstone formation. Although modification of biliary phospholipids toward a more saturated acyl chain composition is in theory attractive, dietary modifications to accomplish this have not been successful.

\section*{BILIARY NUCLEATION PROMOTING AND INHIBITING PROTEINS}

During the last decades, numerous biliary proteins have been suggested to enhance or inhibit cholesterol crystallization in gallbladder bile, based on their in vitro or ex vivo effects. Immunoglobulins M and G, haptoglobin, \(\alpha_1\)-acid glycoprotein, aminopeptidase-N, \(\alpha_1\)-antichymotrypsin, and mucin are regarded as pronucleating proteins. By contrast, human apolipoprotein A-I and IgA have been postulated to exert antinucleating activity. Cholesterol crystallization often occurs much faster in bile of patients with (especially multiple) cholesterol stones than in bile of patients with pigment stones or subjects without stones, or in model biles, even in case of comparable relative lipid composition. Excess biliary pronucleating compared with crystallization-inhibiting proteins could contribute to this phenomenon. Nevertheless, in more recent years, a growing number of publications have marshaled experimental evidence arguing against a role of most of these biliary proteins in cholesterol gallstone formation. In a recent study on a large number of gallstone patients, cholesterol
saturation was an independent predictor of speed of crystallization, which was not the case for biliary protein, immunoglobulins, α1-acid glycoprotein, or aminopeptidase-N content. Also, Wang and coworkers showed that after the extraction of biliary lipids from human bile and their reconstitution in buffer solution, the resulting model system displayed the same speed and pattern of crystallization as the original bile sample. Of note, whereas subsequent addition of purified concanavalin A–binding glycoprotein fraction did not affect speed of cholesterol crystallization, the nucleation process was markedly enhanced by adding purified mucin. Furthermore, in the inbred mouse model, most pronucleating proteins in bile (again with the exception of mucin) were found to decrease during the earliest stages of gallstone formation, arguing against an appreciable role of these biliary proteins in gallstone pathogenesis. Mucin remains one of the few candidate proteins with a potential role in human gallstone formation. Marked hypersecretion of mucin occurs in the earliest stages of human and experimental gallstone formation. Several MUC genes are expressed in human gallbladder mucosa, including MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, and MUC6. Upregulation of these MUC genes could lead to the observed increased gallbladder mucin concentrations. Mucin may increase bile viscosity leading to the formation of a gel matrix that can entrap cholesterol crystals in the gallbladder. Mucin may also enhance cholesterol crystallization by offering low-affinity binding sites for cholesterol. Indeed, Lith genes have been identified that control mucin accumulation, cholesterol crystallization, and gallstone formation in the mouse model. Also, decreasing biliary mucin content with aspirin decreases risk of gallstones in the prairie dog model and risk of gallstone recurrence after nonsurgical treatment in humans. Ursodeoxycholic acid also decreases biliary mucin contents.

**GALLBLADDER AND INTESTINAL MOTILITY**

Meal ingestion induces considerable gallbladder emptying (up to 70%–80% of fasting gallbladder volumes) by releasing the hormone cholecystokinin from the upper intestine. Impaired gallbladder emptying may prolong residence of bile in the gallbladder, allowing more time for nucleation of cholesterol crystals from supersaturated bile. Furthermore, in case of adequate emptying, cholesterol crystals that have nucleated may be ejected to the duodenum, whereas in case of impaired gallbladder emptying, these crystals may aggregate into macroscopic gallstones. Several studies have shown that gallstone patients may be divided into a group with severely impaired or even absent postprandial emptying (“bad contractors”) and a group with good postprandial gallbladder emptying (“good contractors”). Patients with good postprandial contraction often have increased fasting and residual gallbladder volumes compared with normal controls. Prospective studies also indicate that impaired postprandial gallbladder motility is an independent risk factor for gallstone recurrence after successful treatment with extracorporeal shockwave lithotripsy. It is less well appreciated that significant periodic gallbladder emptying also occurs during the fasting state (20%–30% emptying in the fasting state vs 70%–80% emptying after a meal) at 1- to 2-hour intervals, associated with the cycle of the intestinal migrating motor complex and with a rise of plasma motilin levels. It has been found that gallstone patients show a pattern of less frequent migrating motor complex cycles, with absent interdigestive gallbladder emptying and altered motilin release compared with controls. A similarly prolonged migrating motor complex cycle has been found in the ground squirrel model of gallstone formation. The fasting state (ie, the night) seems to be the most vulnerable period for gallstone formation. During this period, biliary cholesterol saturation is highest, because of relatively low bile salt secretion.
and relatively high cholesterol secretion. There is also a progressive concentration of gallbladder bile during this period, which is partially counteracted by periodic interdigestive gallbladder contraction in association with antral phase 3 of the migrating motor complex of the intestine.

There is increasing insight into pathogenesis of impaired gallbladder motility. Significant absorption of cholesterol seems to occur from supersaturated bile in the gallbladder.\textsuperscript{37,38} Excess cholesterol is then incorporated within the sarcolemmal plasma membrane of the gallbladder smooth muscle cell, with decreased membrane fluidity, impaired contractility, and impaired relaxation as a result.\textsuperscript{39} In addition, the gallbladder wall is exposed to detergent bile salts, unesterified cholesterol, and bacteria.\textsuperscript{40,41} As a result, a proinflammatory Th1 immune response may occur, which contributes to hypomotility. Although impaired motility could be in many cases secondary to biliary cholesterol supersaturation, it may still facilitate the process of gallstone formation. Gallbladder motility is often impaired in high-risk situations for gallstone formation, such as pregnancy, obesity, diabetes mellitus, gastric surgery, treatment with the somatostatin analogue octreotide, very low calorie dieting, and total parenteral nutrition.

**PATHOPHYSIOLOGY OF CHOLESTEROL GALLSTONE FORMATION**

It is not surprising, that in a polygenetic disorder as cholesterol gallstone disease, several underlying mechanisms may be involved in its pathogenesis. Nevertheless, the common theme remains excess biliary cholesterol compared with solubilizing bile salts or phospholipids. In Chilean patients (especially of Amerindian descent), increased bile salt and cholesterol synthesis have been reported.\textsuperscript{42} The defect was supposed to be secondary to increased intestinal loss of bile salts, and preceded gallstone formation. Interestingly, decreased expression of ileal bile salt transport proteins apical sodium-dependent bile acid transporter, cytosolic ileal lipid binding protein, and basolateral organic solute transporter $\alpha$ and $\beta$ were recently described in female nonobese patients as a possible explanation of these findings.\textsuperscript{43,44} It has also been reported that high dietary cholesterol increases biliary cholesterol secretion and decreases bile acid synthesis and pool in cholesterol gallstone subjects but not in controls.\textsuperscript{45} These findings point to the importance of intestinal cholesterol absorption in gallstone pathogenesis. Interestingly, increased expression of the intestinal cholesterol uptake protein NPC1L1 (Niemann-Pick C1–like protein 1) was recently reported in gallstone patients.\textsuperscript{46} Also, inhibiting cholesterol absorption with etezimibe prevents gallstone formation in the mouse model and decreases biliary cholesterol saturation in gallstone patients with slower crystallization as a result.\textsuperscript{47} Nevertheless, current evidence points to hepatic hypersecretion of cholesterol as the primary defect in most Western patients with cholesterol gallstones.\textsuperscript{48} In vivo, biliary lipid composition is determined to a large extent at the level of the hepatocyte canalicular membrane. The process of nascent bile formation is maintained by an elaborate network of adenosine triphosphate–binding cassette (ABC) transporters in the hepatocyte canalicular membrane that regulate biliary secretion of cholesterol, bile salts, and phospholipids (Fig. 2). The ABCG5/G8 genes encode protein half-transporters that heterodimerize to form the functional transporter localized in the canalicular membrane of hepatocytes and facilitating cholesterol secretion into bile.\textsuperscript{49} Recently, in genome-wide association studies, the ABCG8 19H allele was found to be associated with gallstone formation.\textsuperscript{50,51} ABCG5/G8 is also present in the intestine, and decreases net cholesterol absorption by transfer of cholesterol molecules taken up by the intestinal cell back to the lumen.\textsuperscript{52} ABCG5/G8 polymorphisms associated with increased gallstone risk
could also affect intestinal cholesterol absorption. The bile salt export pump (current nomenclature ABCB11) pumps bile salts over the membrane into bile.\(^{53}\) Severe mutations in ABCB11 lead to progressive intrahepatic cholestasis in the first decade of life that rapidly leads to liver failure (PFIC2), whereas less severe mutations may lead to benign recurrent cholestasis (BRIC2) and intermittent intractable pruritus. A considerable number of patients with BRIC2 exhibit associated gallstones, supposedly caused by insufficient amounts of biliary bile salts.\(^{54}\) The human MDR3 (multidrug resistance 3) P-glycoprotein (current nomenclature ABCB4) functions as a "floppase," translocating phosphatidylcholine molecules from the inner to the outer leaflet of the canalicular membrane, enabling their secretion into bile.\(^{55}\) Recently, a subset of gallstone patients has been identified with intrahepatic and bile duct stones at young age (<40 years) and high risk of recurrent biliary symptoms after cholecystectomy. The underlying pathogenetic mechanism of this so-called "low phospholipid-associated cholelithiasis" is thought to be relative biliary phospholipid deficiency caused by a missense mutation in the MDR3 gene.\(^{56}\)

**NUCLEAR RECEPTORS AND CHOLESTEROL GALLSTONE FORMATION**

In their turn, the lipid transport proteins in the hepatocytic canalicular membrane are regulated by nuclear receptors (see Fig. 2). Farnesoid X receptor ([FXR] NR1H4) is a member of the nuclear receptor superfamily\(^{57}\) and functions as a bile salt receptor that regulates transcription of numerous genes involved in maintaining cholesterol

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**Fig. 2.** Nascent bile formation at the hepatocytic canalicular membrane. ABCG5-G8 transports cholesterol into bile, and is regulated by nuclear receptor LXR. ABCB11 and ABCB4 transport bile salts and phosphatidylcholine into bile, and are regulated by nuclear receptor FXR. Excess hepatic cholesterol secretion or insufficient bile salt–phosphatidylcholine secretion lead to biliary cholesterol supersaturation. Subsequently, cholesterol supersaturated vesicles may form, which is promoted by bile concentration, hydrophobic bile composition, or unsaturated phospholipid acyl chains. Nucleation of cholesterol crystals may occur from aggregated or fused supersaturated vesicles.
and bile salt homeostasis. The primary bile salt chenodeoxycholic acid is the highest affinity endogenous ligand characterized for FXR in the enterohepatic system. In the liver, the activation of FXR by endogenous bile salts inhibits through intermediate small heterodimer partner the transcription of the gene encoding cholesterol 7α-hydroxylase, the rate-limiting enzyme in the major synthetic pathway of bile salts. The FXR–small heterodimer partner signaling pathway is an important molecular basis for the feedback repression of bile salt synthesis. FXR has also been shown to regulate expression of ABCB11 and ABCB4, affecting amounts of solubilizing bile salts and phospholipids in bile. As expected, FXR “knockout” mice are highly susceptible to gallstone formation on a lithogenic diet, because of low relative amounts of biliary bile salts and phospholipids. Also, gallstone formation can be prevented in wild-type mice by the synthetic FXR agonist GW4064, because increased amounts of solubilizing bile salts and phospholipids prevent cholesterol supersaturation. Data on role of FXR in human cholesterol gallstone formation are limited. FXR is also expressed in the ileal cell, and regulates activity of transport proteins involved in bile salt reabsorption into the enterohepatic circulation: apical sodium-dependent bile acid transporter, cytosolic ileal lipid binding protein, and basolateral organic solute transporter α and β. Decreased expression of these ileal transport proteins occurs in female nonobese gallstone patients, with an associated decreased expression of FXR. Data on gene polymorphisms for FXR have revealed controversial results. In a Mexican population, the most common haplotype NR1H4_1 was associated with gallstone prevalence. In contrast, NR1H4_1 displayed no association with gallstone prevalence in a German population, whereas in a Chilean population a trend toward a protective effect of NR1H4_1 was observed.

Another subfamily of nuclear receptors, liver X receptor (LXR), regulates expression of ABCG5/G8 cholesterol transport protein. In the murine model, activation of LXR increases risk of gallstone formation. In a limited number of human gallstone patients, hepatic mRNA levels of ABCG5, ABCG8, and LXRα were increased by 51%, 59%, and 102%, respectively, and significantly correlated with cholesterol saturation index. Further research is needed on the role of nuclear receptors in gallstone pathogenesis and the therapeutic feasibility of nuclear receptor agonists.

PATHOGENESIS OF PIGMENT GALLSTONES

In the Western world, approximately 30% of gallstone carriers exhibit black pigment gallbladder stones (<20% cholesterol content). Whereas black gallbladder pigment stones are extremely rare below age 50 years, there is a progressive relative contribution of this stone type at older age. In East Asia, there is a relatively high prevalence of brown pigment stones residing in the bile ducts, and causing potentially devastating cholangitis.

Black Pigment Stones

Black pigment stones are formed in sterile bile in the gallbladder. In contrast to cholesterol gallstones, impaired gallbladder motility does not contribute to pathogenesis. Black pigment stones are primarily composed of calcium bilirubinate. Other important components are calcium carbonate and calcium phosphate in polymer-like complexes with mucin glycoproteins. Normally most bilirubin, the breakdown product of hemoglobin, is conjugated in the liver to bilirubin monoglucuronide and subsequently to water-soluble bilirubin diglucuronide. Unconjugated bilirubin is poorly soluble in water. In case of hemolysis, biliary excretion of bilirubin may increase 10-fold with increased risk of calcium bilirubinate precipitation. This
phenomenon explains the high prevalence of black pigment stones in chronic hemolytic disorders, such as sickle cell anemia, hereditary spherocytosis, and Gilbert syndrome. Concomitant presence of Gilbert syndrome is associated with increased gallstone prevalence in sickle cell disease. Evidence from experimental animal models indicates that enterohepatic cycling of bilirubin may contribute to high frequency of pigment stones in patients with ileal Crohn disease, especially in case of ileal resection. The proposed mechanism is that increased amounts of bile salts reach the cecum and solubilize unconjugated bilirubin, allowing their reabsorption with subsequent hyperbilirubinobilia. A similar mechanism could contribute to increased incidence of pigment stones in patients with cystic fibrosis. Interestingly, prevalence of Gilbert syndrome is increased in patients with cystic fibrosis and gallstones, suggesting that hemolysis could also contribute to pigment stone formation in this patient category. Last, insufficient acidification of bile in the gallbladder and reduced buffering capacity of mucin gel also promote biliary calcium supersaturation and pigment stone formation.

**Brown Pigment Stones**

In contrast to black pigment stones, their brown pigment counterparts are formed in the bile ducts. They are primarily composed of calcium salts of unconjugated bilirubin and varying amounts of cholesterol and protein. Brown pigment stones are associated with chronic bacterial infection of the bile ducts by *Escherichia coli*, *Bacteroides* spp, and *Clostridium* spp, and parasites *Opisthorchis veverrini*, *Clonorchis sinensis*, and *Ascaris lumbricoides*. Bacteria in the bile ducts produce β-glucoronidase, phospholipase A, and bile acid hydrolase leading to increased amounts of unconjugated bilirubin, palmitic and stearic acids, and unconjugated bile acids, which can complex with calcium, resulting in stone formation. Parasites in the bile ducts may stimulate stone formation by the calcified overcoat of the parasitis egg, which may serve as a nidus and enhance precipitation of calcium bilirubinate.

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