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An update on the pathogenesis of cholesterol gallstone disease

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Abstract

Purpose of review—Gallstone disease is a major epidemiologic and economic burden worldwide, and the most frequent form is cholesterol gallstone disease.

Recent findings—Major pathogenetic factors for cholesterol gallstones include a genetic background, hepatic hypersecretion of cholesterol, and supersaturated bile which give life to precipitating cholesterol crystals that accumulate and grow in a sluggish gallbladder. Additional factors include mucin and inflammatory changes in the gallbladder, slow intestinal motility, increased intestinal absorption of cholesterol, and altered gut microbiota. Mechanisms of disease are linked with insulin resistance, obesity, the metabolic syndrome, and type 2 diabetes. The role of nuclear receptors, signaling pathways, gut microbiota, and epigenome are being actively investigated.

Summary—Ongoing research on cholesterol gallstone disease is intensively investigating several pathogenic mechanisms, associated metabolic disorders, new therapeutic approaches, and novel strategies for primary prevention, including lifestyles.

Keywords

cholesterol crystallization; gallbladder; mucin gel; supersaturated bile

INTRODUCTION

Gallstones have a prevalence of 10–15% in adults [1] in the United States and Europe. About 75% of adult patients are asymptomatic, but gallstone disease generates major economic and social burdens [1,2] if symptoms or complications occur.

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Conflicts of interest

There are no conflicts of interest.

Basic and clinical aspects of gallstone pathogenesis continue to receive attention worldwide [3,4▪,5,6,7▪,8▪]. Housset *et al.* [9▪] reviewed several functions of the gallbladder in health and disease. The European Society for the Study of the Liver has published exhaustive Clinical Practice Guidelines on prevention, diagnosis, and treatment of gallstones [10▪▪]. A study on 1 064 089 pregnant women [11], associated gallstone disease with adverse maternal and neonatal outcomes including preterm birth, a condition linked with risk of developmental problems [12]. The multivariable logistic regression models within the WHO Multinational mONItoring of trends and determinants in CArdiovascular disease (WHO MONICA) studies in Denmark confirmed a strong association between gallstone disease and insulin resistance, systemic inflammation, and genetic predisposition to obesity or type 2 diabetes [13▪].

Risk factors for gallstone disease include unmodifiable [i.e., aging, female gender, races, and lithogenic (*LITH*) genes] and modifiable conditions (Table 1). In Western countries, gallstones are comprised mainly of cholesterol in 75–80% of cases, and are often associated with systemic abnormalities [14] (Fig. 1). Primary prevention strategies in the general populations and in study participants at risk are conceivable [21] while studying metabolic pathways [22–26].

Five primary defects play a critical role in the pathogenesis of cholesterol gallstones: *LITH* genes and genetic factors; hepatic hypersecretion of cholesterol, resulting in supersaturated gallbladder bile; rapid phase transitions of cholesterol in bile, with the precipitation of solid cholesterol crystals; impaired gallbladder motility with hypersecretion and accumulation of mucin gel in the gallbladder lumen and immune-mediated gallbladder inflammation; and intestinal factors involving absorption of cholesterol, slow intestinal motility, and altered gut microbiota [7▪] (Fig. 2).

This complex scenario puts the studies on cholesterol gallstone disease at the frontline of ongoing research involving treatments and prevention strategies [4▪].

LITH GENE, GENE–ENVIRONMENT INTERACTIONS, AND EPIGENETIC FACTORS

The prevalence of gallstones is high in the case of family history [27,28] and in specific ethnic groups [15,29]. Predisposing genetic factors (*Lith* genes) identified in mouse models [30,31] are involved in the synthesis, transport, and metabolism of cholesterol and bile acids [32,33]. In humans, the genetic susceptibility to gallstones has been explored by genome-wide association study (GWAS) [34,35,36▪]. The ATP-binding cassette transporters G5 and G8 (*ABCG5/G8*) are responsible for hepatic cholesterol secretion. Two major variants (*ABCG5*-R50C and *ABCG8*-D19H) are associated with gallstones in German, Chilean, Chinese, and Indian populations [37–43]. Carriers of CG genotype of *ABCG8* rs11887534 are also at higher risk of gallstone disease, gallbladder and bile duct cancer, compared to carriers of the GG genotype [44]. The increased susceptibility to gallstone disease is also linked with three variants of the Farnesoid X receptor (*FXR*) gene (rs35724, rs11110385, rs11110386 [45]), as well as polymorphisms of apolipoprotein E4 allele [46], mucin genes

[47] and fibroblast grow factor receptor 4 (*FGFR4*) [48]. The polymorphism rs3758650 (mucin-like protocadherin gene) predicts the development of symptomatic gallstones [49].

A recent large-scale GWAS (8720 cases, 55 152 controls, European ancestry) searching for single-nucleotide polymorphisms associated with gallstone disease [36▪] identified four distinct loci (*SULT2A1, TM4SF4, GCKR*, and *CYP7A1*) encoding enzymes involved in cholesterol metabolism/transport, and in sulfonylation of bile acids or hydroxysteroids. The previously detected locus *ABCG8* [34], involved in cholesterol efflux [50], was also confirmed. Another contributing gene is the *ABCB4* in patients [51] and in mice [52] with gallstones because the ATP-binding cassette transporter B4 (ABCB4) is responsible for hepatic phospholipid secretion and its mutations or knockout lead to a lack of phospholipids in bile.

Another large study from Rodriguez *et al.* [53▪] on 15 241 women of European ancestry identified two new loci associated with gallbladder disease (*GCKR* rs1260326:T>C and *TTC39B* rs686030:C>A), and detected four independent single-nucleotide polymorphisms effects in *ABCG8* rs4953023:G>A, *ABCG8* rs4299376:G(>)T, *ABCG5* rs6544718:T>C, and *ABCG5* rs6720173:G>C in conditional analyses taking genotypes of rs4953023:G>A as a covariate.

However, studies on twin pairs show that genetic factors are responsible for no more than 25–30% of symptomatic gallstones [54,55]. Environmental factors and gene–environment interactions can affect gene expression through epigenetic mechanisms [16], which also involve fat storage and insulin resistance [56]. These factors primarily include microRNAs (a large class of tiny, noncoding RNAs) [57]: 114 miRNA target genes are identified and regulate gallstone-related pathways [58]. An inverse correlation has been shown between expression levels of miR-210 and its potential target gene, *ATP11A*, in human gallstones. The interaction involves the regulation of the ATP-binding cassette ABC transporters pathway of cholesterol [58]. At a cellular level, the miRNA miR-122 regulates cholesterol homeostasis [57]. High circulating levels of miR-122 (3.07-fold higher than in controls) were also detected in obese patients, where risk factors for cholesterol gallstones include insulin resistance [59]. Singh *et al.* [60▪] reported the epigenetic roles of mammary serine protease inhibitor and thrombospondin 11-methylated genes in gallbladder cancer, but not in gallstone disease, in Indian population.

ALTERED BILE LIPID COMPOSITION

Cholesterol gallstones originate from the precipitation of solid cholesterol crystals mainly from multilamellar vesicles in a concentrated bile supersaturated with cholesterol, in which cholesterol cannot be solubilized by micelles and vesicles [8▪].

Insulin resistance promotes the formation of cholesterol gallstone by stimulating activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase [61] (the rate-limiting step in cholesterol synthesis) and activating the genes involved in cholesterol secretion: *ABCG5* and *ABCG8* (in concert with dys-regulation of the liver transcription factor forkhead box protein O1) [4▪]. These molecular pathways [25], together with a condition of gallbladder stasis and

autonomic neuropathy [62], can account for the high gallstone prevalence in diabetic patients. Gallstone prevalence is markedly higher in women than in men and estrogen enhances cholesterol synthesis (while decreasing bile acid synthesis) by upregulation of estrogen receptor α and the G protein-coupled receptor 30 [63,64].

The nuclear receptors FXR and liver X receptor (LXR) act as bile acid sensors and govern important pathways of cholesterol and bile acid metabolism. FXR knockout mice fed a lithogenic diet show high susceptibility to cholesterol gallstones in parallel with decreased expression of the hepatocyte bile acid transporter Abcb11 and phospholipid transporter Abcb4 [65]. Hepatic insulin resistance influences expression of FXR [25,66,67], and activation of LXR promotes biliary cholesterol secretion because hepatic *ABCG5* and *ABCG8* are upregulated [68]. The liver-specific disruption of the insulin receptor in LIRKO mice model increases susceptibility to cholesterol gallstones, with mechanisms involving disinhibition of the forkhead box protein O1 signaling cascade, increased expression of the cholesterol transporters Abcg5/g8, and a consequent increase of biliary cholesterol secretion. Changes of *Abcg8* expression are also found in humans: the risk of gallstone disease is increased in twins with a heterozygous or homozygous *ABCG8* D19H genotype [69]. Furthermore, the characteristics of the insulin resistance syndrome in men were linked with the Q604E polymorphism of the *ABCG5* gene [70].

Recent studies have discussed factors modulating bile composition and cholesterol solubilization [4▪,71▪]. The multiligand class B scavenger receptor CD36 promotes cellular free fatty acid uptake and modulates both hepatic and intestinal cholesterol metabolism. Xie *et al.* [72▪] found that germline *Cd36* knockout mice are protected against diet-induced gallstones compared with wild-type mice. Also, *Cd36* knockout mice crossed into the susceptible phenotype of congenic gallstone-susceptible liver fatty acid binding protein knockout mice are protected against lithogenic diet-induced gallstones. CD36-modified gallstone susceptibility through a reduction in biliary cholesterol secretion and changes of the bile acid pool by shifting to more hydrophilic species. Notably, gallbladder contractility is also improved as tensiometric changes of gallbladder smooth muscle strips in response to methacholine and potassium chloride.

Biliary aquaporins (AQPs) also play a role in bile concentrating function [73]. Asai *et al.* [74▪] show that hepatic levels of the transcription factor hypoxia-inducible factor 1α subunit (HIF1A) promote cholesterol gallstone formation in the animal model. Suppression of hepatic AQP8 with decreased water secretion from hepatocytes are involved. In the same study, the activation of HIF1A in human gallstone patients with and nonalcoholic liver steatosis was also shown.

INTESTINAL ABSORPTION OF CHOLESTEROL

Gallstone patients display an imbalance between absorption and synthesis of cholesterol: increased biliary cholesterol secretion from high dietary cholesterol and decreased bile acid synthesis and pool, all driving bile supersaturation [75]. The small intestine absorbs dietary cholesterol and reabsorbs the secreted biliary cholesterol [76], with variable absorption efficiency [77,78]. Intestinal factors depend on expression of sterol transport proteins and on

dietary cholesterol [76,79]), which are regulated by multiple genes [76] and determined by the balance between influx and efflux of intraluminal cholesterol molecules crossing the brush border membrane of the enterocyte [76]. In mice, high-cholesterol diets and high intestinal cholesterol absorption efficiency are two independent risk factors for cholesterol gallstone formation [80]. Dysfunctional cholesterol transporters expressed on the enterocyte brush border membrane can account for such defects. In animal models, cholesterol uptake is altered with variants of the Niemann-Pick type C1-like protein (NPC1L1) transporter [81]. Ezetimibe, the potent NPC1L1 selective inhibitor, reduces the amount of cholesterol reaching the liver through the enterolymphatic circulation of cholesterol by inhibiting intestinal cholesterol absorption and the biliary cholesterol saturation. In this scenario, cholesterol gallstone formation is prevented in ezetimibe-treated gallstone-susceptible mice, even fed a lithogenic diet [82–84]. Furthermore, gallstone patients show significantly lower cholesterol absorption [85,86], and higher or unchanged [86] de novo synthesis of cholesterol [85]. This metabolic trait could precede gallstone formation in risk groups [85]. Insulin resistance also affects cholesterol homeostasis by reducing intestinal cholesterol absorption while increasing cholesterol synthesis, and the effect is independent on obesity [87,88].

Osteopontin (OPN) is a soluble cytokine and a matrix-associated protein expressed in several tissues and body fluids and is involved in cholesterol homeostasis [89]. Lin *et al.* [90▪] reported that OPN knockout mice are protected against lithogenic diet-induced gallstone formation because of reduced expression of intestinal NPC1L1 and intestinal cholesterol absorption.

GUT MICROBIOTA

Intestinal dysbiosis occurs in cholesterol gallstone patients and might play an important role in the pathogenesis of gallstone disease. Wu *et al.* [91] studied the composition of bacterial communities of gut, bile, and gallstones from 29 cholesterol gallstone patients and the gut of 38 healthy study participants, analyzing 299 217 bacterial 16S ribosomal RNA gene sequences. They found significant increment of intestinal bacterial phylum Proteobacteria and decrement of *Faecalibacterium* spp., *Lachnospira* spp., and *Roseburia* spp. Others reported increased amount of Gram-positive anaerobic bacteria with elevated 7α-dehydroxylation activity in the cecum of gallstone patients, a finding linked with increased concentrations of the hydrophobic and lithogenic secondary bile acid deoxycholate [92].

Gallstone patients undergoing cholecystectomy show microbial diversity of gut microbiota and the genus *Roseburia* spp. is reduced compared with that in controls. Moreover, the microbiota from gallstone patients is enriched with uncultivated genus *Oscillospira* spp. This last genus is positively correlated with the concentration of the secondary bile acids and negatively correlated with the primary bile acids, whereas the phylum Bacteroidetes shows an opposite trend [93].

Wang *et al.* [94▪] recently found that mice fed a lithogenic diet and forming gallstones display reduced microbiota richness and α diversity with lower levels of Firmicutes and decreased ratio of Firmicutes to Bacteroidetes.

The microbiota is also affected by environmental toxics introduced with food. This step might influence pathogenetic factors of gallstones. Liu *et al.* [95▪▪] found abnormal gut microbiota (as abundance and composition) after 8-week exposure to organochlorine pesticides such as dichlorodiphenyldichloroethylene (P,p'-DDE) and β-hexachlorocyclohexane. These changes include bile acid composition, enhanced hydrophobicity, decreased expression of genes regulating bile acid reabsorption in the terminal ileum, and a compensatory increase in expression of genes involved in the synthesis of hepatic bile acids.

GALLBLADDER MOTILITY

Several clinical conditions are associated with defective gallbladder motility (Table 2), which is another risk factor for cholesterol gallstones [15,96,97]. About one-third of cholesterol gallstone patients display enlarged fasting and postprandial residual gallbladder volumes with delayed emptying [98–100]. This defect antedates gallstone formation and is not affected by the presence of gallstones [98,101–103], unless chronic gallbladder inflammation and/or mechanical obstruction exist [98]. Sustained supersaturation of cholesterol in bile enhances the absorption of cholesterol into gallbladder muscularis propria, reduces back diffusion of cholesterol into bile, and inhibits action potentials and Ca^{2+}currents [104]. In animals on lithogenic diet, Tharp *et al.* [105] demonstrated that curbing the accumulation of triacylglycerol in the gallbladder wall increases its contractile strength and prevents gallstone formation.

The lipid-induced gallbladder lipotoxicity [106–108] is revealed to be associated with defective smooth muscle contractility and relaxation [109,110], whereas excessive cholesterol absorption may lead to cell proliferation and inflammation in the gallbladder mucosa and lamina propria [97,111]. Dysfunctional gallbladder motility provides sufficient time for cholesterol nucleation and gallstone growth [102,112] and predisposes to gallstone recurrence after successful extracorporeal shock-wave lithotripsy and/or oral bile acid dissolution therapy [113,114].

Endogenous CCK regulates postprandial gallbladder emptying [115–117] by activating CCK-1 receptors (CCK-1R) that are located on gallbladder myocytes [102,118]. Wang *et al.* [119▪] confirmed that CCK knockout mice fed a lithogenic diet have defective postprandial gallbladder emptying and show rapid cholesterol crystallization and gallstone formation. Mice also had enlarged fasting gallbladder volume, sluggish intestinal transit time, and increased intestinal cholesterol absorption with supersaturated bile. Devazepide, a CCK-1R antagonist produces similar outcomes [120▪].

Under lithogenic conditions, the signaling transduction decoupling of the CCK-1R deteriorates [112,121–123] as CCK binding to CCK-1R is not followed by G protein activation [121,124–126]. Indeed, tensiometric studies on isolated gallbladder smooth muscle strips show more severe dysfunction in patients with cholesterol stones than those with pigment stones [98]. Also, polymorphisms in the *CCK1-R* gene [127] and decreased density of CCK-1R [128] may be associated with cholesterol cholelithiasis in humans. Defective gallbladder motility is observed in lean, nondiabetic, gallstone-free study

participants with insulin resistance [129], whereas changes in CCK-1R density is evident in patients with gallstone and type 2 diabetes [130]. Impaired gallbladder motility is also found in women with polycystic ovary syndrome, a condition where insulin resistance often exists [131]. In these cases, gallbladder dysmotility is ameliorated by metformin treatment [132].

Villanacci *et al.* [133▪] recently explored by immunohistochemistry the main cell components of gallbladder intrinsic innervation in patients with cholesterol stones and in acalculous gallbladders. Neurons, enteric glial cells, mast cells, and interstitial cells of Cajal (ICC), were markedly decreased in gallstone patients. This study integrates the findings of Tan *et al.* [134] relating decreased stem cell factor/ckit signaling pathway with depletion of ICC and defective gallbladder motility in gallstone patients.

Impaired gallbladder motility during cholesterol gallstone formation also involves postprandial emptying and refilling phase and the interdigestive (i.e., fasting) rhythmic fluctuations of gallbladder volume. Postprandial refilling requires appropriate gallbladder relaxation promoted by the acid-stimulated duodenal release of vasoactive intestinal peptide and human fibroblast grow factor 19 protein (FGF19; FGF15 in mice) [135]. FGF19 works on the gallbladder epithelium, cholangiocytes [136], and the ileum [137], with concentrations about 23-fold higher in bile than in serum [136]. Increased FGF19 into the portal circulation depends on bile acids which reach the terminal ileum and activate FXR (rank order CDCA > LCA > DCA >> CA). FGF19, in turn, activates the gallbladder fibroblast growth factor receptor 4 (FGFR4) and its co-receptor β-klotho [9▪]. This pathway induces smooth muscle relaxation creating a feedback mechanism that leads to gallbladder refill before the next meal [97,135]. Intraluminal hydrophobic bile acids also act as signaling agents of the G protein-coupled bile acid receptor 1 (GPBAR-1) [138], located in the gallbladder epithelium and smooth muscle [9▪,139] and driving gallbladder relaxation independently on FGF19 [140]. Hydrophobic bile acids inhibit gallbladder smooth muscle contraction via stimulation of GPBAR-1 receptors and activation of ATP-sensitive potassium channel [141]. GPBAR-1 knockout mice display a decreased bile acid pool size, sluggish response to GPBAR-1, and dietary lithogenesis [140,142].

Whether impaired gallbladder refilling (mediated by FGF19 and/or GPBAR-1) contributes to the pathogenesis of gallstones needs to be further addressed. Zhou *et al.* [143▪] modulated bile acid metabolism by FGF19 in 12-week old Abcb4 knockout mice, which resemble biochemical, histological, and clinical features of human cholangiopathies and cholelithiasis. FGF19 reverses liver injury, decreases hepatic inflammation, attenuates biliary fibrosis, and reduces cholecystolithiasis in Abcb4 knockout mice by inhibiting the hepatic expression of *Cyp7a1* and *Cyp27a1,* encoding enzymes responsible for the rate-limiting steps in the classic and alternate bile acid synthetic pathways, and reducing the bile acid hepatic pool and blood levels.

During fasting, the gallbladder regulates the enterohepatic circulation of bile acids through coordinated neurohormonal mechanisms involving the liver and gut [97,144,145]. Small phasic contractions decrease the gallbladder volume by 20–30% of the fasting volume through vagal-motilin-mediated stimuli at the end of phase II of the migrating myoelectric complexes [146,147]. Cholesterol gallstone patients may have an altered interprandial

gallbladder motility [102,148] mainly secondary to less frequent migrating myoelectric complexes cycles and abnormal motilin release compared with healthy control subjects [97–99,148]. The fasting motility defect could increase the direct liver secretion of lithogenic bile to the small intestine with faster recycling of bile acids and increased hydrophobicity of the bile acid pool [149]. This mechanism is another predisposing factor for cholesterol crystallization and stone growth [150].

DIETARY FACTORS AND LIFESTYLES

Lifestyle and dietary factors (Table 3) influence the pathogenesis of gallstone disease. Bertola-Compagnucci *et al.* [151▪] estimated by specific questionnaire that mean energy intake may be higher in gallstone patients than in control subjects. Thus, diet and lifestyle have a potential role in primary prevention of cholesterol gallstones. The European Society for the Study of the Liver panel concludes that healthy lifestyle and food, regular physical activity, and maintenance of an ideal body weight might prevent cholesterol stones and symptomatic gallstones [10▪▪].

CONCLUSION AND FUTURE RESEARCH

Risk factors of cholesterol gallstone share some common pathogenic pathways across major metabolic abnormalities, including insulin resistance, with those in obesity, the metabolic syndrome, and type 2 diabetes. Current research points to some key mechanisms involving the role of *LITH* genes, nuclear receptors, signaling pathways, gut microbiota, epigenetic factors, and lifestyles in the pathogenesis of cholesterol gallstone disease. Finding modifiable pathogenic factors for cholesterol cholelithiasis will pave the way to primary prevention of cholesterol gallstone disease, a very prevalent hepatobiliary disease worldwide.

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KEY POINTS

- Five primary defects determine the pathogenesis of cholesterol gallstones: genetic background and *LITH* genes, hepatic hypersecretion of biliary cholesterol (with supersaturated gallbladder bile), rapid phase transitions of cholesterol in bile (with the precipitation of solid cholesterol crystals), gallbladder dysmotility (with the accumulation of mucin gel in the gallbladder lumen and immune-mediated gallbladder inflammation), and intestinal factors (with increased absorption of cholesterol, slow intestinal motility, and dysbiosis).
- Pathogenetic pathways link cholesterol gallstones with widely diffused metabolic conditions which include insulin resistance, obesity, the metabolic syndrome, and type 2 diabetes.
- The burden of cholesterol gallstones depends on potentially modifiable mechanisms.
- Research on cholesterol gallstones should ameliorate the efficiency of current therapies, test novel therapies, and employ appropriate lifestyles for primary prevention.

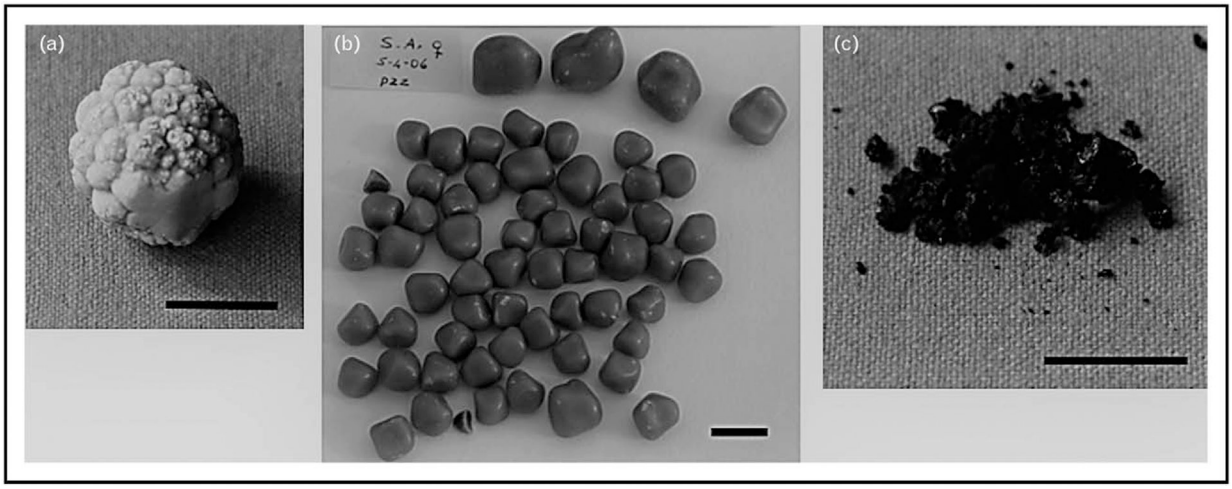

FIGURE 1.

(a) Solitary cholesterol gallstone showing a spheroidal modular surface. (b) Multiple cholesterol gallstones showing a multifaceted surface. Cholesterol content in both cases is more that 75%. (c) Pigment gallstones. Black, soft, friable, and easily pulverized material contains mainly calcium bilirubinate, calcium carbonate, and phosphate. A tiny amount of cholesterol (less than 10% cholesterol) can be found. The black horizontal lines at the bottom are equal to 1 cm.

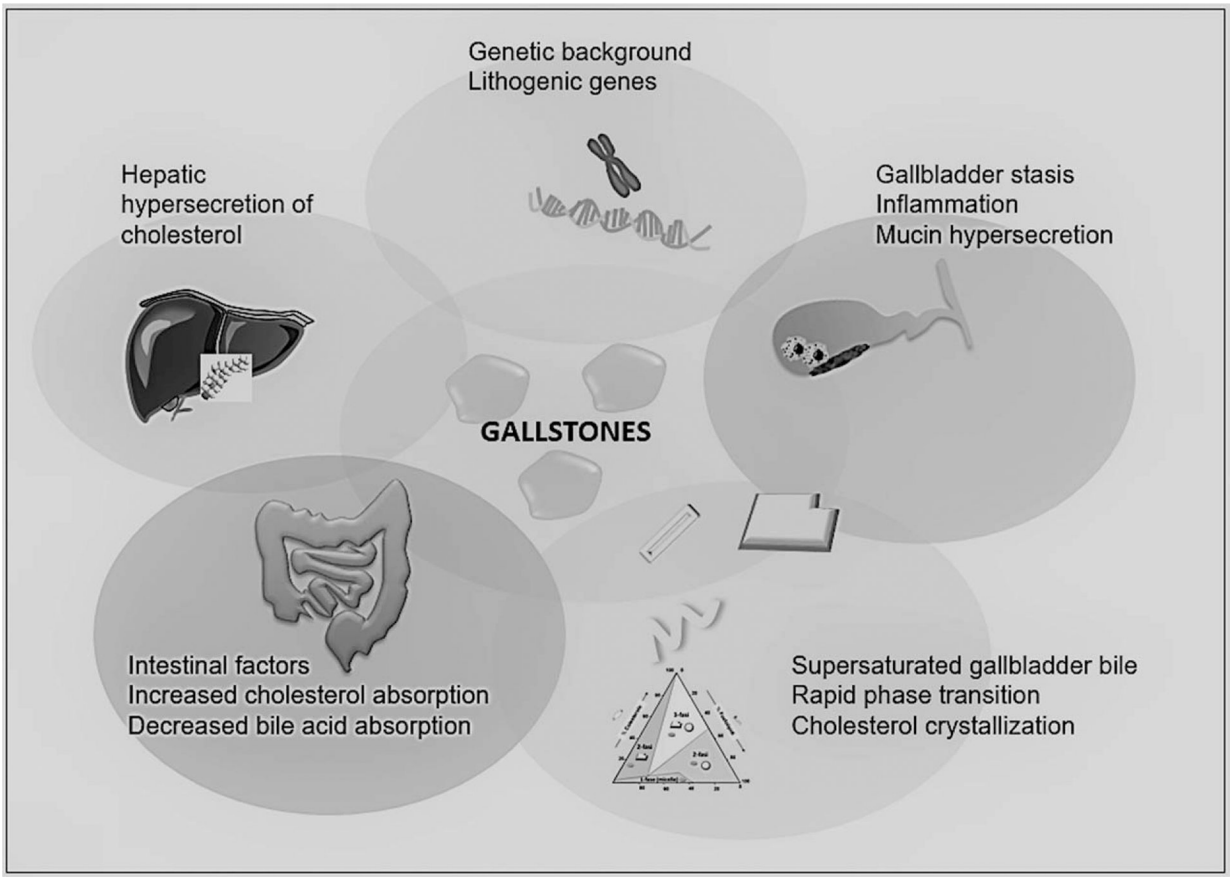

FIGURE 2.

Pathogenetic factors involved in the formation of cholesterol gallstones. Five primary defects are involved. The primary cause of cholesterol gallstone formation originates from increased hepatic hypersecretion of cholesterol. The genetic predisposition is largely involved in this process.

Table 1.

Exogenous risk factors associated with any type cholelithiasis

Metabolic syndrome (Chol)
Physical inactivity
Insulin resistance
Diabetes mellitus
Obesity (visceral)
Nonalcoholic fatty liver disease
Dietary factors (Chol)
High carbohydrate intake
High calorie intake
High glycemic load
Low fiber intake
High heme iron intake
Increased enterohepatic circulation of bilirubin
Liver cirrhosis (Chol, Pigm)
Ileal resections (Pigm)
Crohn’s disease (Chol, Pigm)
Medications (Chol)
Hormone replacement therapy
Octreotide
Fibrates
Calcineurin inhibitors
Defective gallbladder motility
See Table 2

Chol, cholesterol stones; Pigm, black pigment stones.

Adapted with permission [4▪,14–20].

Table 2.

Conditions associated with defective gallbladder motility

Physiological, dietary, and metabolic factors
Pregnancy
Obesity
Insulin resistance, diabetes mellitus
Rapid body weight loss (bariatric surgery for morbid obesity, and very low calorie diet)
Increased biliary cholesterol secretion
Physical inactivity (men>women)
Westernized diet: high calorie, low fiber, high-refined carbohydrate, and high lipids
Total parenteral nutrition
Gastrointestinal diseases
Irritable bowel syndrome
Primary sclerosing cholangitis
Acute hepatitis A
Chronic pancreatitis
Liver cirrhosis
Neural factors
Neural damage after total gastrectomy, and spinal cord injury
Drugs and hormones
Inhibition of CCK release by somatostatin, somatostatinoma, therapy with somatostatin analogues (octreotide), celiac disease
Estrogens and oral contraceptives
Oral bile acid therapy
Use of 5-hydroxytryptamine inhibitors

Table 3.

Dietary factors influencing the pathogenesis of cholesterol gallstone disease

Factors increasing the risk	Factors decreasing the risk
Increased energy intake	High consumption of monounsaturated fats and fiber
Highly refined sugars and sweet foods	
High fructose intake	Olive oil
Low fiber consumption	Fish (ω-3 fatty acids)
High fat content	Vitamin C supplementation
Consumption of fast food	Vegetable proteins
Consumption of meat	Fruit consumption
Low vitamin C intake	

Adapted with permission [10■■].