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Mouse models of gallstone disease

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Abstract

Purpose of review—The establishment of mouse models of gallstones, and the contribution of mouse models to genetic studies of gallstone disease, as well as the latest advances in the pathophysiology of gallstones from mouse experiments are summarized.

Recent findings—The combined uses of genomic strategies and phenotypic studies in mice have successfully led to the identification of many *Lith* genes, which pave the way for the discovery of human *LITH* genes. The physical–chemical, genetic, and molecular biological studies of gallstone disease in mice with knockout or transgene of specific target genes have provided many novel insights into the complex pathophysiological mechanisms of this very common hepatobiliary disease worldwide, showing that interactions of five primary defects play a critical role in the pathogenesis of cholesterol gallstones. Based on mouse studies, a new concept has been proposed that hepatic hypersecretion of biliary cholesterol is induced by multiple *Lith* genes, with insulin resistance as part of the metabolic syndrome interacting with cholelithogenic environmental factors to cause the phenotype.

Summary—The mouse model of gallstones is crucial for elucidating the physical–chemical and genetic mechanisms of cholesterol crystallization and gallstone formation, which greatly increase our understanding of the pathogenesis of this disease in humans.

Keywords

bile acid; cholesterol crystallization; gallbladder motility; insulin resistance; lith genes; lithogenic bile; nonalcoholic fatty liver disease

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Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

Cholesterol gallstone disease is one of the most prevalent digestive diseases and affects ~12% of American adults, leading to a considerable financial and social burden in the USA [1[■],2[■],3–5]. To reduce the morbidity, mortality, and costs of healthcare associated with this disease, it is important to understand the pathophysiology of gallstones and this will facilitate the development of a novel, effective, and noninvasive therapy for patients with gallstones. From 1950s to 1970s, to establish animal models of cholesterol gallstones for studying the pathogenesis of this disease, many animal species, including hamsters, mice, guinea pigs, ground squirrels, prairie dogs, rabbits, and monkeys, have been extensively investigated by feeding different types of lithogenic diets from weeks to months. Although cholesterol gallstones were successfully induced in mice after feeding a lithogenic diet in 1964 [6], mouse gallstones were largely studied until the early 1990s because the first gallstone gene, *Lith1* was identified in the inbred C57L/J strain by genetic methods [7]. In this review, we summarize the establishment of mouse models of gallstones, and the contribution of mouse models to genetic studies of gallstone disease, as well as the latest advances in the pathophysiology of gallstones from mouse experiments.

ESTABLISHMENT OF MOUSE MODELS OF GALLSTONES

Tepperman *et al.* [6] were the first to find that the combination of cholesterol and cholic acid in the diet is required for the formation of cholesterol gallstones in mice. Under lithogenic diet feeding conditions, the extracted sterols from mouse gallstones are predominantly cholesterol, which constitutes ~94% of the pooled stone weight [6]. Because mouse bile contains large amounts of hydrophilic bile acid, tauro- β -muricholic acid [8–10], feeding 1% cholesterol alone for 2–8 months cannot induce gallstones [6,8,10]. Thus, cholic acid is essential in the mouse gallstone model because after hepatic conjugation with taurine, taurocholate replaces most tauro- β -muricholate in mouse bile [8], enhancing cholelithogenesis. In 1978, Fujihira *et al.* [11] observed that at 8 weeks on the lithogenic diet, the prevalence of gallstones varies from 0 to 100% in seven strains of inbred mice. Alexander and Portman [12] further found that even feeding the lithogenic diet for a longer time, from 8 to 12 weeks, strain differences in gallstone prevalence still exist in mice. These studies paved the way for the genetic analysis of gallstone disease in mice. More importantly, Wang *et al.* [13] found that the evolutionary sequences of cholesterol crystallization and gallstone formation in mice during the 8-week period of feeding the lithogenic diet are characterized by the initial accumulation of mucin gel, followed by appearances of liquid crystals and/or anhydrous cholesterol crystals and cholesterol monohydrate crystals, and then agglomerated cholesterol crystals, sandy stones, and true gallstones (Fig. 1). These sequences are in agreement with the results from other animal models of cholesterol gallstone formation and in humans.

CONTRIBUTION OF MOUSE MODELS TO GENETIC STUDIES OF GALLSTONE DISEASE

Epidemiological and clinical investigations, as well as family and twin studies have clearly demonstrated that a complex genetic basis plays a key role in determining individual

predisposition to develop gallstones in response to environmental factors [14]. However, the genetic contribution to gallstones in humans is not completely understood. Because gallstone disease could be caused by complex interactions of many environmental factors and the effects of multiple but as yet unknown genes [14–16], methods for studying genetics of gallstones, a complex genetic trait, are completely different from those that are used to discover genes for simple Mendelian defects. Furthermore, to study quantitative polygenic traits, conventional genetic mapping methods are inappropriate because they are designed for single gene traits. The inbred mouse, with its superior genetic resources [17,18], is an excellent animal model for investigating genetic determinants of gallstone disease. By using mouse backcross strategies and a powerful genetic technique, quantitative trait locus (QTL) analysis [19–24], Khanuja *et al.* [7] were the first to find that differences in gallstone susceptibility between gallstone-susceptible C57L/J and gallstone-resistant AKR/J strains, after fed the lithogenic diet for 12 weeks, were determined by at least two *Lith* genes, with *Lith1* and *Lith2* mapping on mouse chromosomes 2 and 19, respectively. Subsequently, the physical–chemical phenotypes of these *Lith* genes in gallbladder and hepatic bile, as well as in the liver, gallbladder, and small intestine of C57L/J vs. AKR/J mice were systematically investigated [13,25–27]. These studies found that gallstone disease is determined by multiple genes, which is a dominant trait. To confirm whether *Lith1* or *Lith2* alone could independently induce gallstones, congenic strains carrying the C57L/J alleles only in the *Lith1* or the *Lith2* QTL regions on an AKR/J genetic background were created [28]. Both *Lith1* and *Lith2* congenic strains showed gallstone formation comparable to the C57L/J parent, confirming that *Lith1* and *Lith2* could cause gallstones mostly through different lithogenic mechanisms [28]. Furthermore, *Lith1* plays a major role in determining hepatic cholesterol hypersecretion as the biliary phenotypes in the *Lith1* congenic strain are basically the same as those in the C57L/J parent. *Lith2* could influence bile acid-independent bile flow. After many additional strains of inbred mice fed the lithogenic diet for 8–12 weeks were investigated for the prevalence and pathophysiology of gallstones, new gallstone QTL regions containing *Lith* genes were identified. As a result, a mouse gallstone gene map, containing 25 *Lith* genes from *Lith1* to *Lith25* and other candidate genes, was established, which are distributed from chromosome 1 to X in mice, as shown in Fig. 2 and Table 1 [14–16,29–31].

These results from mouse genetic studies have led to the discovery of human *LITH* genes because of homologues between human and mouse chromosomes. Such a successful study is the confirmation of *ABCG5/G8* as a human *LITH* gene based on mouse studies. The *Abcg5/g8* is first identified as the mouse *Lith9* by the QTL mapping methods [29,32,33], and subsequently, two major gallstone-associated variants in *ABCG5/G8* (i.e., *ABCG5*-R50C and *ABCG8*-D19H) are found not only in Germans and Chileans, but also in Chinese and Indians [34–41]. Therefore, based on the mouse *Lith* gene map, more human *LITH* genes will be identified and their pathogenic mechanisms will be elucidated in the near future.

Based on extensive mouse and human studies, it was first proposed in 2010 [31] that interactions of five primary defects play a critical role in the pathogenesis of cholesterol gallstone disease (Fig. 3): genetic factors and *Lith* genes; hepatic cholesterol hypersecretion, leading to cholesterol-supersaturated gallbladder bile (i.e., high cholesterol saturation index);

rapid phase transitions by accelerated cholesterol crystallization and solid cholesterol crystal growth; impaired gallbladder motility; and intestinal factors, including increased amounts of the absorbed cholesterol delivered from the small intestine to the liver for biliary hypersecretion, as well as changes in intestinal microbiota, warrant a systematical study to provide novel insight into gallstone pathogenesis. Obviously, rapid growth and agglomeration of solid cholesterol crystals to form microlithiasis and macroscopic stones is a consequence of cholesterol-supersaturated gallbladder bile, followed by gallbladder mucin hypersecretion and gel formation with impaired gallbladder emptying and refilling, eventually leading to the formation of biliary sludge, that is, the precursor of gallstones.

THE LATEST ADVANCES IN THE PATHOPHYSIOLOGY OF GALLSTONES FROM MOUSE EXPERIMENTS

Gallstone prevalence is markedly higher in women than in men at all ages, suggesting that estrogen plays a critical role in the pathogenesis of cholesterol cholelithiasis [42]. Although the classical estrogen receptor α (ER α), but not ER β , in the liver plays a critical role in the formation of 17 β -estradiol (E₂)-induced gallstones in female mice [43–45], the metabolic abnormalities underlying the lithogenic effect of E₂ on gallstone formation is still unclear. Gallstones are still found in ~30% of ovariectomized ER α knockout mice treated with high doses of E₂ [46]. Moreover, genetic analysis in mice reveals that the G-protein-coupled receptor 30 (GPR30), a novel estrogen receptor, is a gallstone gene named *Lith18* [16,31,47,48]. This discovery led to a new question of how E₂, through GPR30, ER α , or both, influences the biliary and gallstone phenotypes because it can efficiently bind to and activate both GPR30 and ER α [49]. To distinguish the lithogenic effect of ER α from that of GPR30, the entire spectrum of cholesterol crystallization pathways and sequences during the early stage of gallstone formation are investigated in gallbladder bile of ovariectomized female wild-type, GPR30 knockout, ER α knockout, and GPR30/ER α double knockout (DKO) mice. E₂ activates GPR30 and ER α to produce liquid crystalline vs. anhydrous crystalline metastable intermediates, respectively, evolving to cholesterol monohydrate crystals from supersaturated bile [50[■]]. Moreover, cholesterol crystallization is drastically retarded in GPR30/ER α DKO mice. This indicates that GPR30 exerts a synergistic lithogenic action with ER α to enhance E₂-induced gallstone formation. Because GPR30 is localized predominantly in the endoplasmic reticulum, but not in the nucleus, of hepatocytes, GPR30 activation by E₂, likely through the epidermal growth factor receptor signaling cascade, could inhibit hepatic cholesterol 7 α -hydroxylase and the classical pathway of bile acid synthesis, leading to the availability of excess cholesterol for hepatic hypersecretion and bile lithogenesis [50[■]].

Although nonalcoholic fatty liver disease (NAFLD) is an important risk factor for gallstone formation [51–53], the mechanisms of linking NAFLD with gallstone disease are still unknown. Hypoxia-inducible factor 1 (HIF1) is an important transcription factor for regulating expression of the genes involved in oxygen delivery, cellular growth, and redox homeostasis, facilitating adaptive responses to hypoxic conditions [54–57]. HIF1A is found mainly in the perivenous area of the liver, where it is physiologically hypoxic [58]. During the development of liver steatosis, lipid accumulation markedly enlarges the size of

hepatocytes, thereby reducing hepatic sinusoidal perfusion and microcirculation and eventually leading to hepatic hypoxia [59]. Asai *et al.* [60] found that expression of aquaporin-8 (AQP8), a water channel that is responsible for hepatic water secretion into the bile canaliculi [61–63], is markedly increased in the inducible hepatocyte-selective HIF1A knockout mice fed the lithogenic diet for 2 weeks. These changes dramatically increase bile flow by 35%, dilute biliary lipid concentrations by 36% in both gallbladder and hepatic bile, and alleviate gallbladder inflammation. As a result, cholesterol crystallization is inhibited and gallstone formation is prevented in liver-specific HIF1A knockout mice. In contrast, activation of the HIF1A pathway in diet-induced steatotic liver accelerates gallstone formation in wild-type mice. Moreover, expression of HIF1A and its downstream targets in the liver is increased in NAFLD patients with gallstones, suggesting that hepatic HIF1A may play a vital role in the formation of cholesterol gallstones in patients with NAFLD.

Bile flow is determined by both bile acid-dependent and bile acid-independent pathways, in which transhepatocyte transport of water and ionic/non-ionic solutes through the transcellular and the para-cellular routes facilitates the formation of bile [64]. Claudin 2 (Cldn2) is a paracellular channel-forming protein that is highly expressed in the hepatocyte and cholangiocyte [65–67]. Matsumoto *et al.* observed [68] that compared to wild-type mice, Cldn2 knockout mice display reduced bile flow rate by ~50%, which is attributable to a decrease in transepithelial electrical conductance and water permeability in the hepatobiliary system. This significantly increases total lipid concentrations in hepatic and gallbladder bile. Furthermore, osmotic gradient-driven water flow is dramatically reduced in hepatocyte bile canaliculi and bile ducts isolated from Cldn2 knockout mice. After 4 weeks on lithogenic diets, gallstones are formed in Cldn2 knockout, but not wild-type, mice. These findings indicate that claudin 2 is critical for bile formation and biliary lipid homeostasis. Dysfunctional claudin 2 enhances cholelithogenesis by impairing paracellular water movement into bile, leading to concentrated bile that greatly facilitates cholesterol crystallization [69–71].

Similar to patients with sitosterolemia [72–75], hepatic cholesterol output is significantly reduced, but not completely eliminated in mice with the deletion of either *Abcg5* or *Abcg8* alone, or both genes [76–80]. These findings indicate that an ABCG5/G8-independent pathway could exist for regulating biliary cholesterol secretion in humans and mice. Wang *et al.* [81] reported that the ABCG5/G8-independent pathway accounts for 30% to 40% of hepatic cholesterol output in the lithogenic state and plays a pivotal role in regulating biliary secretion of cholesterol in response to high dietary cholesterol. In the absence of ABCG5/G8, this pathway is involved in biliary cholesterol secretion and gallstone formation. Different from ABCG5/G8, this pathway is not activated by the LXR agonist through the LXR signaling pathway. This finding indicates that the importance of both ABCG5/G8-dependent and ABCG5/G8-independent pathways in hepatic cholesterol secretion and gallstone formation.

Clinical studies have found that intestinal cholecystokinin (CCK) secretion and gallbladder emptying in response to a fatty meal are impaired before celiac patients start the gluten-free diet [82–87]. However, it has not been really appreciated whether celiac disease is associated with gallstones because few studies are performed to investigate the impact of celiac disease

on gallstone formation [88]. After a mouse model with the lack of endogenous CCK is created to study the biliary characteristics of celiac disease, Wang *et al.* found [88–90] that CCK knockout mice display rapid cholesterol crystallization and gallstone formation with impaired gallbladder emptying and with excess amounts of intestine-derived cholesterol for biliary hypersecretion through reducing small intestinal transit time and increasing intestinal cholesterol absorption. These mouse experiments provide important clues to study the prevalence and pathogenesis of gallstones in patients with celiac disease [88]. Moreover, the potent CCK-1 receptor antagonist devazepide increases susceptibility to gallstone formation by impairing gallbladder emptying function, disrupting biliary cholesterol metabolism and enhancing intestinal cholesterol absorption in mice [91].

Although bile may contain both pro-nucleating and antinucleating agents and an imbalance in their abundance may lead to rapid cholesterol crystallization in gallbladder bile of patients with cholesterol gallstones [92–97], this concept still needs extensive studies to prove it. Mucin is the first biliary protein found to play a lithogenic role in accelerating cholesterol crystallization in animal and human studies [98–101]. Although many other biliary proteins have been proposed to be either pro-nucleating or antinucleating agents for influencing cholesterol crystallization in bile, their *in vivo* roles, if any, in the pathogenesis of gallstones remain largely unclear [102]. Niemann-Pick C2 (NPC2), a soluble lysosomal protein, is expressed in human and mouse liver and regulates hepatic cholesterol secretion by stimulating Abcg5/g8-mediated cholesterol transport [103]. After secreted into bile [104–106], it promotes cholesterol crystallization and gallstone formation in mice transgenic human NPC2 [107]. In contrast, Npc2 knockout mice show a reduction in hepatic cholesterol secretion and gallstone formation at 2 weeks on a lithogenic diet [106]. These results imply that biliary proteins could influence gallstone formation by modulating cholesterol crystallization likely through a liquid crystalline pathways in bile [108–111].

High efficiency of intestinal cholesterol absorption and high dietary cholesterol such as the Western diet are two independent risk for gallstones in mice [112]. Further studies found that numerous factors that modulate intestinal cholesterol uptake and absorption, cholesterol esterification, lipid transport within the enterocytes, and chylomicron assembly and secretion could influence cholesterol crystallization and gallstone formation by regulating delivery of the intestinal origin of cholesterol to the liver for secretion into bile [113, 114–119]. Moreover, the importance of intestinal microbiota and the immune system in the pathogenesis of gallstones needs to be investigated extensively in the future.

Obviously, many factors that change hepatic synthesis, transhepatocyte transport, and biliary secretion of cholesterol, phospholipids, and bile acids could influence gallstone formation by causing cholesterol hypersecretion and cholesterol-supersaturated bile [120–127]. Nuclear receptors such as liver X receptor (LXR), farnesoid X receptor (FXR), pregnane X receptor (PXR), constitutive androstane receptor (CAR), and ER α have been found to influence gallstone formation through these regulatory pathways of lipid metabolism in the liver [128–131]. More importantly, disruption of the liver-specific insulin receptor in mice increases susceptibility to gallstones by increasing hepatic cholesterol secretion through the forkhead transcription factor FoxO1 and Abcg5/g8 and leading to a lithogenic bile acid profile through reduction of enzymatic activity of cholesterol 7 α -hydroxylase and bile acid

synthesis of classical pathway [127]. However, the pathogenic mechanisms underlying the roles of insulin resistance in gallstone formation remain elusive.

CONCLUSION

Based on findings from the genetic studies of gallstones in mice, a novel concept regarding the genetic mechanisms of gallstone disease has been established, which has opened the door for investigating the orthologous human *LITH* genes and for exploring their cholelithogenic mechanisms in humans [132]. For future research, mouse studies will have to focus on the roles of nuclear receptors, intestinal microbiota, dyslipidemia, hyperinsulinemia, NAFLD, obesity, diabetes, aging, and sedentary lifestyle in the mechanisms underlying the formation of cholesterol gallstones. These results from mouse experiments will provide novel strategies for the treatment of this very prevalent hepatobiliary disease worldwide.

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
  - ■ of outstanding interest
- 1 ■ ■. Lammert F, Gurusamy K, Ko CW, et al. Gallstones. *Nat Rev Dis Primers*. 2016; 2:16024. This review summarized recent progresses in the pathogenesis, prevention, and treatment of gallstone disease. [PubMed: 27121416]
  - 2 ■ ■. Wang, DQ., Portincasa, P. Gallstones: recent advance in epidemiology, pathogenesis, diagnosis and management. New York: Nova Biomedical; 2017. p. 1-676. A comprehensive textbook elucidated major aspects of the epidemiology, pathogenesis, diagnosis, and management of gallstone disease
  3. Wang DQ, Cohen DE, Carey MC. Biliary lipids and cholesterol gallstone disease. *J Lipid Res*. 2009; 50(Suppl):S406–S411. [PubMed: 19017613]
  4. Ruhl CE, Everhart JE. Gallstone disease is associated with increased mortality in the United States. *Gastroenterology*. 2011; 140:508–516. [PubMed: 21075109]
  5. Peery AF, Crockett SD, Barritt AS, et al. Burden of gastrointestinal, liver, and pancreatic diseases in the United States. *Gastroenterology*. 2015; 149:1731e3–1741.e3. [PubMed: 26327134]
  6. Tepperman J, Caldwell FT, Tepperman HM. Induction of gallstones in mice by feeding a cholesterol-choleic acid containing diet. *Am J Physiol*. 1964; 206:628–634. [PubMed: 14160996]
  7. Khanuja B, Cheah YC, Hunt M, et al. Lith1, a major gene affecting cholesterol gallstone formation among inbred strains of mice. *Proc Natl Acad Sci U S A*. 1995; 92:7729–7733. [PubMed: 7644485]

8. Wang DQ, Lammert F, Cohen DE, et al. Cholic acid aids absorption, biliary secretion, and phase transitions of cholesterol in murine cholelithogenesis. *Am J Physiol.* 1999; 276(3 Pt 1):G751–760. [PubMed: 10070053]
9. Wang DQ, Tazuma S, Cohen DE, Carey MC. Feeding natural hydrophilic bile acids inhibits intestinal cholesterol absorption: studies in the gallstone-susceptible mouse. *Am J Physiol Gastrointest Liver Physiol.* 2003; 285:G494–G502. [PubMed: 12748061]
10. Wang DQ, Tazuma S. Effect of beta-muricholic acid on the prevention and dissolution of cholesterol gallstones in C57L/J mice. *J Lipid Res.* 2002; 43:1960–1968. [PubMed: 12401895]
11. Fujihira E, Kaneta S, Ohshima T. Strain difference in mouse cholelithiasis and the effect of taurine on the gallstone formation in C57BL/C mice. *Biochem Med.* 1978; 19:211–217. [PubMed: 656082]
12. Alexander M, Portman OW. Different susceptibilities to the formation of cholesterol gallstones in mice. *Hepatology.* 1987; 7:257–265. [PubMed: 3557305]
13. Wang DQ, Paigen B, Carey MC. Phenotypic characterization of Lith genes that determine susceptibility to cholesterol cholelithiasis in inbred mice: physical-chemistry of gallbladder bile. *J Lipid Res.* 1997; 38:1395–1411. [PubMed: 9254065]
14. Wang DQ, Afdhal NH. Genetic analysis of cholesterol gallstone formation: searching for Lith (gallstone) genes. *Curr Gastroenterol Rep.* 2004; 6:140–150. [PubMed: 15191694]
15. Lammert F, Carey MC, Paigen B. Chromosomal organization of candidate genes involved in cholesterol gallstone formation: a murine gallstone map. *Gastroenterology.* 2001; 120:221–238. [PubMed: 11208732]
16. Lyons MA, Wittenburg H. Cholesterol gallstone susceptibility loci: a mouse map, candidate gene evaluation, and guide to human LITH genes. *Gastroenterology.* 2006; 131:1943–1970. [PubMed: 17087948]
17. Gregory SG, Sekhon M, Schein J, et al. A physical map of the mouse genome. *Nature.* 2002; 418:743–750. [PubMed: 12181558]
18. Waterston RH, Lindblad-Toh K, Birney E, et al. Mouse Genome Sequencing Consortium. Initial sequencing and comparative analysis of the mouse genome. *Nature.* 2002; 420:520–562. [PubMed: 12466850]
19. Korstanje R, Paigen B. From QTL to gene: the harvest begins. *Nat Genet.* 2002; 31:235–236. [PubMed: 12089518]
20. Lander ES, Schork NJ. Genetic dissection of complex traits. *Science.* 1994; 265:2037–2048. [PubMed: 8091226]
21. Darvasi A. Experimental strategies for the genetic dissection of complex traits in animal models. *Nat Genet.* 1998; 18:19–24. [PubMed: 9425894]
22. Cornall RJ, Aitman TJ, Hearne CM, Todd JA. The generation of a library of PCR-analyzed microsatellite variants for genetic mapping of the mouse genome. *Genomics.* 1991; 10:874–881. [PubMed: 1916820]
23. Hearne CM, McAleer MA, Love JM, et al. Additional microsatellite markers for mouse genome mapping. *Mamm Genome.* 1991; 1:273–282. [PubMed: 1794058]
24. Aitman TJ, Hearne CM, McAleer MA, Todd JA. Mononucleotide repeats are an abundant source of length variants in mouse genomic DNA. *Mamm Genome.* 1991; 1:206–210. [PubMed: 1794049]
25. Wang DQ, Lammert F, Paigen B, Carey MC. Phenotypic characterization of Lith genes that determine susceptibility to cholesterol cholelithiasis in inbred mice: pathophysiology of biliary lipid secretion. *J Lipid Res.* 1999; 40:2066–2079. [PubMed: 10553010]
26. Lammert F, Wang DQ, Paigen B, Carey MC. Phenotypic characterization of Lith genes that determine susceptibility to cholesterol cholelithiasis in inbred mice: integrated activities of hepatic lipid regulatory enzymes. *J Lipid Res.* 1999; 40:2080–2090. [PubMed: 10553011]
27. van Erpecum KJ, Wang DQ, Lammert F, et al. Phenotypic characterization of Lith genes that determine susceptibility to cholesterol cholelithiasis in inbred mice: soluble pronucleating proteins in gallbladder and hepatic biles. *J Hepatol.* 2001; 35:444–451. [PubMed: 11682027]
28. Paigen B, Schork NJ, Svenson KL, et al. Quantitative trait loci mapping for cholesterol gallstones in AKR/J and C57L/J strains of mice. *Physiol Genomics.* 2000; 4:59–65. [PubMed: 11074014]

29. Wittenburg H, Lyons MA, Li R, et al. QTL mapping for genetic determinants of lipoprotein cholesterol levels in combined crosses of inbred mouse strains. *J Lipid Res.* 2006; 47:1780–1790. [PubMed: 16685081]
30. Paigen, B., Carey, MC. Gallstones. New York: Oxford University Press; 2002. p. 298–335.
31. Wang HH, Portincasa P, Afdhal NH, Wang DQ. Lith genes and genetic analysis of cholesterol gallstone formation. *Gastroenterol Clin North Am.* 2010; 39:185–207. vii–viii. [PubMed: 20478482]
32. Wittenburg H, Lyons MA, Li R, et al. Association of a lithogenic Abcg5/Abcg8 allele on Chromosome 17 (Lith9) with cholesterol gallstone formation in PERA/EiJ mice. *Mamm Genome.* 2005; 16:495–504. [PubMed: 16151694]
33. Wittenburg H, Lyons MA, Li R, et al. FXR and ABCG5/ABCG8 as determinants of cholesterol gallstone formation from quantitative trait locus mapping in mice. *Gastroenterology.* 2003; 125:868–881. [PubMed: 12949731]
34. Grunhage F, Acalovschi M, Tirziu S, et al. Increased gallstone risk in humans conferred by common variant of hepatic ATP-binding cassette transporter for cholesterol. *Hepatology.* 2007; 46:793–801. [PubMed: 17626266]
35. Wang Y, Jiang ZY, Fei J, et al. ATP binding cassette G8 T400K polymorphism may affect the risk of gallstone disease among Chinese males. *Clin Chim Acta.* 2007; 384:80–85. [PubMed: 17612515]
36. Jiang ZY, Parini P, Eggertsen G, et al. Increased expression of LXR alpha, ABCG5, ABCG8, and SR-BI in the liver from normolipidemic, nonobese Chinese gallstone patients. *J Lipid Res.* 2008; 49:464–472. [PubMed: 18007013]
37. Kuo KK, Shin SJ, Chen ZC, et al. Significant association of ABCG5 604Q and ABCG8 D19H polymorphisms with gallstone disease. *Br J Surg.* 2008; 95:1005–1011. [PubMed: 18457353]
38. Rudkowska I, Jones PJ. Polymorphisms in ABCG5/G8 transporters linked to hypercholesterolemia and gallstone disease. *Nutr Rev.* 2008; 66:343–348. [PubMed: 18522623]
39. Katsika D, Magnusson P, Krawczyk M, et al. Gallstone disease in Swedish twins: risk is associated with ABCG8 D19H genotype. *J Intern Med.* 2010; 268:279–285. [PubMed: 20497293]
40. von Kampen O, Buch S, Nothnagel M, et al. Genetic and functional identification of the likely causative variant for cholesterol gallstone disease at the ABCG5/8 lithogenic locus. *Hepatology.* 2013; 57:2407–2417. [PubMed: 22898925]
41. von Schonfels W, Buch S, Wolk M, et al. Recurrence of gallstones after cholecystectomy is associated with ABCG5/8 genotype. *J Gastroenterol.* 2013; 48:391–396. [PubMed: 22869156]
42. Everhart JE, Khare M, Hill M, Maurer KR. Prevalence and ethnic differences in gallbladder disease in the United States. *Gastroenterology.* 1999; 117:632–639. [PubMed: 10464139]
43. Wang HH, Afdhal NH, Wang DQ. Estrogen receptor alpha, but not beta, plays a major role in 17beta-estradiol-induced murine cholesterol gallstones. *Gastroenterology.* 2004; 127:239–249. [PubMed: 15236189]
44. Wang HH, Afdhal NH, Wang DQ. Overexpression of estrogen receptor alpha increases hepatic cholesterologenesis, leading to biliary hypersecretion in mice. *J Lipid Res.* 2006; 47:778–786. [PubMed: 16380638]
45. Wang HH, Liu M, Clegg DJ, et al. New insights into the molecular mechanisms underlying effects of estrogen on cholesterol gallstone formation. *Biochim Biophys Acta.* 2009; 1791:1037–1047. [PubMed: 19589396]
46. de Bari O, Wang HH, Portincasa P, et al. The deletion of the estrogen receptor alpha gene reduces susceptibility to estrogen-induced cholesterol cholelithiasis in female mice. *Biochim Biophys Acta.* 2015; 1852:2161–2169. [PubMed: 26232687]
47. Lyons MA, Korstanje R, Li R, et al. Single and interacting QTLs for cholesterol gallstones revealed in an intercross between mouse strains NZB and SM. *Mamm Genome.* 2005; 16:152–163. [PubMed: 15834632]
48. Krawczyk M, Wang DQ, Portincasa P, Lammert F. Dissecting the genetic heterogeneity of gallbladder stone formation. *Semin Liver Dis.* 2011; 31:157–172. [PubMed: 21538282]

49. Carmeci C, Thompson DA, Ring HZ, et al. Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer. *Genomics*. 1997; 45:607–617. [PubMed: 9367686]
50. de Bari O, Wang TY, Liu M, et al. Estrogen induces two distinct cholesterol crystallization pathways by activating ERalpha and GPR30 in female mice. *J Lipid Res*. 2015; 56:1691–1700. This article reported that GPR30 plays a key role in the formation of estrogen-induced gallstones, which is independent of that of ERα. It may help explain why gallstone prevalence is higher in women than in men. [PubMed: 26152119]
51. Jaruvongvanich V, Sanguankee A, Upala S. Significant association between gallstone disease and nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Dig Dis Sci*. 2016; 61:2389–2396. [PubMed: 26993825]
52. Koller T, Kollerova J, Hlavaty T, et al. Cholelithiasis and markers of nonalcoholic fatty liver disease in patients with metabolic risk factors. *Scand J Gastroenterol*. 2012; 47:197–203. [PubMed: 22182015]
53. Loria P, Lonardo A, Lombardini S, et al. Gallstone disease in nonalcoholic fatty liver: prevalence and associated factors. *J Gastroenterol Hepatol*. 2005; 20:1176–1184. [PubMed: 16048564]
54. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci U S A*. 1995; 92:5510–5514. [PubMed: 7539918]
55. Semenza GL. Involvement of oxygen-sensing pathways in physiologic and pathologic erythropoiesis. *Blood*. 2009; 114:2015–2019. [PubMed: 19494350]
56. Semenza GL. Regulation of oxygen homeostasis by hypoxia-inducible factor 1. *Physiology (Bethesda)*. 2009; 24:97–106. [PubMed: 19364912]
57. Majmundar AJ, Wong WJ, Simon MC. Hypoxia-inducible factors and the response to hypoxic stress. *Mol Cell*. 2010; 40:294–309. [PubMed: 20965423]
58. Kietzmann T, Cornesse Y, Brechtel K, et al. Perivenous expression of the mRNA of the three hypoxia-inducible factor alpha-subunits, HIF1alpha, HIF2alpha and HIF3alpha, in rat liver. *Biochem J*. 2001; 354:531–537. [PubMed: 11237857]
59. Kondo K, Sugioka T, Tsukada K, et al. Fenofibrate, a peroxisome proliferator-activated receptor alpha agonist, improves hepatic microcirculatory patency and oxygen availability in a high-fat-diet-induced fatty liver in mice. *Adv Exp Med Biol*. 2010; 662:77–82. [PubMed: 20204774]
60. Asai Y, Yamada T, Tsukita S, et al. Activation of the hypoxia inducible factor 1alpha subunit pathway in steatotic liver contributes to formation of cholesterol gallstones. *Gastroenterology*. 2017; 152:1521e8–1535.e8. This paper investigated the mechanism underlying the role of HIF1A in mechanistic links between nonalcoholic fatty liver disease and gallstone disease. [PubMed: 28088462]
61. Calamita G, Ferri D, Gena P, et al. Water transport into bile and role in bile formation. *Curr Drug Targets Immune Endocr Metabol Disord*. 2005; 5:137–142. [PubMed: 16089345]
62. Marinelli RA, LaRusso NF. Aquaporin water channels in liver: their significance in bile formation. *Hepatology*. 1997; 26:1081–1084. [PubMed: 9362345]
63. Masyuk AI, LaRusso NF. Aquaporins in the hepatobiliary system. *Hepatology*. 2006; 43(2 Suppl 1):S75–S81. [PubMed: 16447276]
64. Boyer JL. Bile formation and secretion. *Compr Physiol*. 2013; 3:1035–1078. [PubMed: 23897680]
65. Wada M, Tamura A, Takahashi N, Tsukita S. Loss of claudins 2 and 15 from mice causes defects in paracellular Na⁺ flow and nutrient transport in gut and leads to death from malnutrition. *Gastroenterology*. 2013; 144:369–380. [PubMed: 23089202]
66. Rosenthal R, Milatz S, Krug SM, et al. Claudin-2, a component of the tight junction, forms a paracellular water channel. *J Cell Sci*. 2010; 123(Pt 11):1913–1921. [PubMed: 20460438]
67. Van Itallie CM, Holmes J, Bridges A, et al. The density of small tight junction pores varies among cell types and is increased by expression of claudin-2. *J Cell Sci*. 2008; 121:298–305. [PubMed: 18198187]
68. Matsumoto K, Imasato M, Yamazaki Y, et al. Claudin 2 deficiency reduces bile flow and increases susceptibility to cholesterol gallstone disease in mice. *Gastroenterology*. 2014; 147:1134–1145.e1110. [PubMed: 25068494]

69. van Erpecum KJ. Biliary lipids, water and cholesterol gallstones. *Biol Cell*. 2005; 97:815–822. [PubMed: 16232124]
70. Portincasa P, Palasciano G, Svelto M, Calamita G. Aquaporins in the hepatobiliary tract. Which, where and what they do in health and disease. *Eur J Clin Invest*. 2008; 38:1–10.
71. Calamita G, Ferri D, Bazzini C, et al. Expression and subcellular localization of the AQP8 and AQP1 water channels in the mouse gall-bladder epithelium. *Biol Cell*. 2005; 97:415–423. [PubMed: 15859952]
72. Miettinen TA. Phytosterolaemia, xanthomatosis and premature atherosclerotic arterial disease: a case with high plant sterol absorption, impaired sterol elimination and low cholesterol synthesis. *Eur J Clin Invest*. 1980; 10:27–35. [PubMed: 6768564]
73. Salen G, Shore V, Tint GS, et al. Increased sitosterol absorption, decreased removal, and expanded body pools compensate for reduced cholesterol synthesis in sitosterolemia with xanthomatosis. *J Lipid Res*. 1989; 30:1319–1330. [PubMed: 2600539]
74. Lutjohann D, Bjorkhem I, Beil UF, von Bergmann K. Sterol absorption and sterol balance in phytosterolemia evaluated by deuterium-labeled sterols: effect of sitostanol treatment. *J Lipid Res*. 1995; 36:1763–1773. [PubMed: 7595097]
75. Gould RG, Jones RJ, LeRoy GV, et al. Absorbability of beta-sitosterol in humans. *Metabolism*. 1969; 18:652–662. [PubMed: 5799288]
76. Wang HH, Patel SB, Carey MC, Wang DQ. Quantifying anomalous intestinal sterol uptake, lymphatic transport, and biliary secretion in *Abcg8*($-/-$) mice. *Hepatology*. 2007; 45:998–1006. [PubMed: 17393508]
77. Yu L, Hammer RE, Li-Hawkins J, et al. Disruption of *Abcg5* and *Abcg8* in mice reveals their crucial role in biliary cholesterol secretion. *Proc Natl Acad Sci U S A*. 2002; 99:16237–16242. [PubMed: 12444248]
78. Yu L, Li-Hawkins J, Hammer RE, et al. Overexpression of *ABCG5* and *ABCG8* promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. *J Clin Invest*. 2002; 110:671–680. [PubMed: 12208868]
79. Plosch T, Bloks VW, Terasawa Y, et al. Sitosterolemia in ABC-transporter *G5*-deficient mice is aggravated on activation of the liver-X receptor. *Gastroenterology*. 2004; 126:290–300. [PubMed: 14699507]
80. Wang J, Mitsche MA, Lutjohann D, et al. Relative roles of *ABCG5/ABCG8* in liver and intestine. *J Lipid Res*. 2015; 56:319–330. [PubMed: 25378657]
81. Wang HH, Li X, Patel SB, Wang DQ. Evidence that the adenosine triphosphate-binding cassette *G5/G8*-independent pathway plays a determinant role in cholesterol gallstone formation in mice. *Hepatology*. 2016; 64:853–864. [PubMed: 27014967]
82. Calam J, Ellis A, Dockray GJ. Identification and measurement of molecular variants of cholecystokinin in duodenal mucosa and plasma. Diminished concentrations in patients with celiac disease. *J Clin Invest*. 1982; 69:218–225. [PubMed: 7033291]
83. Maton PN, Selden AC, Fitzpatrick ML, Chadwick VS. Defective gallbladder emptying and cholecystokinin release in celiac disease. Reversal by gluten-free diet. *Gastroenterology*. 1985; 88:391–396. [PubMed: 3965328]
84. Low-Beer TS, Harvey RF, Davies ER, Read AF. Abnormalities of serum cholecystokinin and gallbladder emptying in celiac disease. *N Engl J Med*. 1975; 292:961–963. [PubMed: 1117928]
85. Hopman WP, Rosenbusch G, Hectors MP, Jansen JB. Effect of predigested fat on intestinal stimulation of plasma cholecystokinin and gall bladder motility in coeliac disease. *Gut*. 1995; 36:17–21. [PubMed: 7890230]
86. Fraquelli M, Bardella MT, Peracchi M, et al. Gallbladder emptying and somatostatin and cholecystokinin plasma levels in celiac disease. *Am J Gastroenterol*. 1999; 94:1866–1870. [PubMed: 10406250]
87. Masclee AA, Jansen JB, Driessen WM, et al. Gallbladder sensitivity to cholecystokinin in coeliac disease. Correlation of gallbladder contraction with plasma cholecystokinin-like immunoreactivity during infusion of cerulein. *Scand J Gastroenterol*. 1991; 26:1279–1284. [PubMed: 1763298]

88. Wang HH, Liu M, Li X, et al. Impaired intestinal cholecystokinin secretion, a fascinating but overlooked link between coeliac disease and cholesterol gallstone disease. *Eur J Clin Invest.* 2017; 47:328–333. [PubMed: 28186337]
89. Wang HH, Liu M, Portincasa P, et al. Lack of endogenous cholecystokinin promotes cholelithogenesis in mice. *Neurogastroenterol Motil.* 2016; 28:364–375. [PubMed: 26604077]
90. Wang HH, Portincasa P, Liu M, et al. Effect of gallbladder hypomotility on cholesterol crystallization and growth in CCK-deficient mice. *Biochim Biophys Acta.* 2010; 1801:138–146. [PubMed: 19836465]
91. Wang HH, Portincasa P, Wang DQ. The cholecystokinin-1 receptor antagonist devazepide increases cholesterol cholelithogenesis in mice. *Eur J Clin Invest.* 2016; 46:158–169. [PubMed: 26683129]
92. Lamont JT, Carey MC. Cholesterol gallstone formation. 2. Pathobiology and pathomechanics. *Prog Liver Dis.* 1992; 10:165–191. [PubMed: 1296229]
93. Afdhal NH, Smith BF. Cholesterol crystal nucleation: a decade-long search for the missing link in gallstone pathogenesis. *Hepatology.* 1990; 11:699–702. [PubMed: 2184117]
94. Abei M, Kawczak P, Nuutinen H, et al. Isolation and characterization of a cholesterol crystallization promoter from human bile. *Gastroenterology.* 1993; 104:539–548. [PubMed: 8425697]
95. Holzbach RT, Kibe A, Thiel E, et al. Biliary proteins. Unique inhibitors of cholesterol crystal nucleation in human gallbladder bile. *J Clin Invest.* 1984; 73:35–45. [PubMed: 6690482]
96. Holzbach RT. Nucleation of cholesterol crystals in native bile. *Hepatology.* 1990; 12:155S–159S. discussion 159S–161S. [PubMed: 2210644]
97. Kibe A, Holzbach RT, LaRusso NF, Mao SJ. Inhibition of cholesterol crystal formation by apolipoproteins in supersaturated model bile. *Science.* 1984; 225:514–516. [PubMed: 6429856]
98. Lee SP, LaMont JT, Carey MC. Role of gallbladder mucus hypersecretion in the evolution of cholesterol gallstones. *J Clin Invest.* 1981; 67:1712–1723. [PubMed: 7240416]
99. Afdhal NH, Gong D, Niu N, et al. Cholesterol cholelithiasis in the prairie dog: role of mucin and nonmucin glycoproteins. *Hepatology.* 1993; 17:693–700. [PubMed: 8477974]
100. LaMont JT. Gallbladder mucin glycoprotein hypersecretion in experimental cholelithiasis: role of mucin gel in nucleation of cholesterol gallstones. *Adv Exp Med Biol.* 1982; 144:231–234. [PubMed: 7080915]
101. LaMont JT, Smith BF, Moore JR. Role of gallbladder mucin in pathophysiology of gallstones. *Hepatology.* 1984; 4:51S–56S. [PubMed: 6546237]
102. Wang DQ, Cohen DE, Lammert F, Carey MC. No pathophysiologic relationship of soluble biliary proteins to cholesterol crystallization in human bile. *J Lipid Res.* 1999; 40:415–425. [PubMed: 10064729]
103. Yamanashi Y, Takada T, Yoshikado T, et al. NPC2 regulates biliary cholesterol secretion via stimulation of ABCG5/G8-mediated cholesterol transport. *Gastroenterology.* 2011; 140:1664–1674. [PubMed: 21315718]
104. Klein A, Amigo L, Retamal MJ, et al. NPC2 is expressed in human and murine liver and secreted into bile: potential implications for body cholesterol homeostasis. *Hepatology.* 2006; 43:126–133. [PubMed: 16374838]
105. Zanlungo S, Rigotti A, Nervi F. Hepatic cholesterol transport from plasma into bile: implications for gallstone disease. *Curr Opin Lipidol.* 2004; 15:279–286. [PubMed: 15166783]
106. Balboa E, Morales G, Aylwin P, et al. Niemann-pick c2 protein expression regulates lithogenic diet-induced gallstone formation and dietary cholesterol metabolism in mice. *Lipids.* 2012; 47:13–25. [PubMed: 22038687]
107. Acuna M, Gonzalez-Hodar L, Amigo L, et al. Transgenic overexpression of Niemann-Pick C2 protein promotes cholesterol gallstone formation in mice. *J Hepatol.* 2016; 64:361–369. This article examined the importance of a biliary NPC2 protein in cholesterol crystallization. [PubMed: 26453970]
108. Busch N, Matiuck N, Sahlin S, et al. Inhibition and promotion of cholesterol crystallization by protein fractions from normal human gallbladder bile. *J Lipid Res.* 1991; 32:695–702. [PubMed: 1856612]

109. Halpern Z, Dudley MA, Kibe A, et al. Rapid vesicle formation and aggregation in abnormal human biles. A time-lapse video-enhanced contrast microscopy study. *Gastroenterology*. 1986; 90:875–885. [PubMed: 3949117]
110. Halpern Z, Dudley MA, Lynn MP, et al. Vesicle aggregation in model systems of supersaturated bile: relation to crystal nucleation and lipid composition of the vesicular phase. *J Lipid Res*. 1986; 27:295–306. [PubMed: 3734627]
111. Holan KR, Holzbach RT, Hermann RE, et al. Nucleation time: a key factor in the pathogenesis of cholesterol gallstone disease. *Gastroenterology*. 1979; 77:611–617. [PubMed: 467918]
112. Wang DQ, Zhang L, Wang HH. High cholesterol absorption efficiency and rapid biliary secretion of chylomicron remnant cholesterol enhance cholelithogenesis in gallstone-susceptible mice. *Biochim Biophys Acta*. 2005; 1733:90–99. [PubMed: 15749059]
113. Xie Y, Cifarelli V, Pietka T, et al. Cd36 knockout mice are protected against lithogenic diet-induced gallstones. *J Lipid Res*. 2017; 58:1692–1701. This paper investigated the role of Cd36 in the regulation of canalicular and biliary cholesterol transport and secretion, as well as its effects on gallstone formation. [PubMed: 28634191]
114. Wang HH, Portincasa P, Mendez-Sanchez N, et al. Effect of ezetimibe on the prevention and dissolution of cholesterol gallstones. *Gastroenterology*. 2008; 134:2101–2110. [PubMed: 18442485]
115. de Bari O, Wang HH, Portincasa P, et al. Ezetimibe prevents the formation of oestrogen-induced cholesterol gallstones in mice. *Eur J Clin Invest*. 2014; 44:1159–1168. [PubMed: 25303682]
116. Zuniga S, Molina H, Azocar L, et al. Ezetimibe prevents cholesterol gallstone formation in mice. *Liver Int*. 2008; 28:935–947. [PubMed: 18783541]
117. Kikuchi T, Orihara K, Oikawa F, et al. Intestinal CREBH overexpression prevents high-cholesterol diet-induced hypercholesterolemia by reducing Npc1l1 expression. *Mol Metab*. 2016; 5:1092–1102. [PubMed: 27818935]
118. Buhman KK, Accad M, Novak S, et al. Resistance to diet-induced hypercholesterolemia and gallstone formation in ACAT2-deficient mice. *Nat Med*. 2000; 6:1341–1347. [PubMed: 11100118]
119. Wang HH, Wang DQ. Reduced susceptibility to cholesterol gallstone formation in mice that do not produce apolipoprotein B48 in the intestine. *Hepatology*. 2005; 42:894–904. [PubMed: 16175613]
120. Lammert F, Wang DQ, Hillebrandt S, et al. Spontaneous cholecysto- and hepatolithiasis in Mdr2^{-/-} mice: a model for low phospholipid-associated cholelithiasis. *Hepatology*. 2004; 39:117–128. [PubMed: 14752830]
121. Li J, Wang Y, Matye DJ, et al. Sortilin 1 modulates hepatic cholesterol lipotoxicity in mice via functional interaction with liver carboxylesterase 1. *J Biol Chem*. 2017; 292:146–160. [PubMed: 27881673]
122. Xie Y, Fung HY, Newberry EP, et al. Hepatic Mttp deletion reverses gallstone susceptibility in L-Fabp knockout mice. *J Lipid Res*. 2014; 55:540–548. [PubMed: 24474819]
123. Xie Y, Newberry EP, Kennedy SM, et al. Increased susceptibility to diet-induced gallstones in liver fatty acid binding protein knockout mice. *J Lipid Res*. 2009; 50:977–987. [PubMed: 19136665]
124. Huang W, Bansode RR, Xie Y, et al. Disruption of the murine protein kinase C β gene promotes gallstone formation and alters biliary lipid and hepatic cholesterol metabolism. *J Biol Chem*. 2011; 286:22795–22805. [PubMed: 21550971]
125. Xie Y, Blanc V, Kerr TA, et al. Decreased expression of cholesterol 7 α -hydroxylase and altered bile acid metabolism in Apobec-1^{-/-} mice lead to increased gallstone susceptibility. *J Biol Chem*. 2009; 284:16860–16871. [PubMed: 19386592]
126. Amigo L, Castro J, Miquel JF, et al. Inactivation of hepatic microsomal triglyceride transfer protein protects mice from diet-induced gallstones. *Gastroenterology*. 2006; 131:1870–1878. [PubMed: 17064699]
127. Biddinger SB, Haas JT, Yu BB, et al. Hepatic insulin resistance directly promotes formation of cholesterol gallstones. *Nat Med*. 2008; 14:778–782. [PubMed: 18587407]

128. Moschetta A, Bookout AL, Mangelsdorf DJ. Prevention of cholesterol gallstone disease by FXR agonists in a mouse model. *Nat Med.* 2004; 10:1352–1358. [PubMed: 15558057]
129. Uppal H, Zhai Y, Gangopadhyay A, et al. Activation of liver X receptor sensitizes mice to gallbladder cholesterol crystallization. *Hepatology.* 2008; 47:1331–1342. [PubMed: 18318438]
130. He J, Nishida S, Xu M, et al. PXR prevents cholesterol gallstone disease by regulating biosynthesis and transport of bile salts. *Gastroenterology.* 2011; 140:2095–2106. [PubMed: 21354151]
131. Cheng S, Zou M, Liu Q, et al. Activation of constitutive androstane receptor prevents cholesterol gallstone formation. *Am J Pathol.* 2017; 187:808–818. [PubMed: 28283178]
132. Wang, DQ., Afdhal, NH. Gallstone disease. In: Feldman, M.Friedman, LS., Brandt, L., editors. *Sleisenger and Fordtran's gastrointestinal and liver disease.* 10. Philadelphia, PA: Elsevier Saunders; 2014. p. 1100-1133.

KEY POINTS

- Cholesterol gallstone disease is caused by complex genetic and environmental factors. It is one of the most common and costly digestive diseases in Western countries. At least 20 million Americans (~12% of adults) have gallstones.
- Compelling evidence from pathophysiological, physical–chemical, and genetic studies shows that cholesterol gallstone disease is determined by multiple *Lith* genes, which is a dominant trait. However, no mode of inheritance fitting to the Mendelian pattern could be found in most cases. The overarching pathogenic factor is persistent hepatic hypersecretion of cholesterol into bile, leading to cholesterol-supersaturated gallbladder bile.
- Understanding the molecular genetics of gallstone disease in mice will push for the identification of human *LITH* genes and for the understanding of the pathogenic mechanisms of each of *LITH* genes in humans.
- Further mouse studies will investigate the roles of nuclear receptors, intestinal microbiota, dyslipidemia, hyperinsulinemia, NAFLD, obesity, diabetes, aging, and sedentary lifestyle in the formation of cholesterol gallstones.
- With such knowledge of the genetic and environmental factors involved in gallstone pathogenesis, mouse studies will provide important insight into the complex pathogenic mechanisms of gallstone disease. This will facilitate the development of a novel, effective, and noninvasive therapy for patients with gallstones.

FIGURE 1.

Representative photomicrographs of cholesterol crystallization and gallstone formation observed by phase contrast and polarizing light microscopy in gallbladder bile of gallstone-susceptible C57L/J mice during the 8-week period of feeding a lithogenic diet: (a) mucin gel; (b) small liquid crystals; (c) aggregated liquid crystals; (d) fused liquid crystals with Maltese-cross birefringence and focal conic textures; (e) arc-like (possible anhydrous cholesterol) crystal; (f) filamentous crystals; (g) tubular crystal fracturing at ends to produce cholesterol monohydrate crystals; (h) typical cholesterol monohydrate crystal, with 79.2° and 100.8° angles; (i) agglomerated cholesterol monohydrate crystals; (j and k) sandy stones; and (l) gallstones. Reproduced with permission from reference [13].

FIGURE 2.

The gallstone (*Lith*) gene map shows the quantitative trait loci (QTL) regions containing *Lith* genes, as well as candidate genes for cholesterol gallstone disease on mouse chromosomes. A vertical line represents one chromosome, with the centromere at the top. Genetic distances from the centromere (horizontal black lines) are indicated to the left of the chromosomes in centimorgans (cM). The locations of gallstone QTLs (*Lith* genes) and candidate genes are indicated by horizontal black lines with the gene symbols to the right (see Table 1 for the list of gene symbols and names). Reproduced with permission from reference [31].

FIGURE 3.

Interactions of five primary defects promote the formation of cholesterol gallstones: (i) genetic factors and *Lith* genes; (ii) hepatic hypersecretion; (iii) gallbladder hypomotility; (iv) rapid phase transitions; and (v) intestinal factors. Persistent hepatic cholesterol hypersecretion is the consequence of complex genetic predispositions, leading to the formation of cholesterol-supersaturated bile and accelerating cholesterol crystallization. Impaired gallbladder motility results in the production and accumulation of excess mucin gel, promoting the formation of biliary sludge and the growth of microlithiasis. These alterations also disrupt the kinetics of the enterohepatic circulation of bile acids (intestinal factors), leading to a diminished intestinal absorption and pool size of bile acids. Increased

cholesterol absorption delivers dietary and re-absorbed biliary cholesterol to the liver for secretion into bile. Abnormal intestinal microbiota may disrupt cholesterol and bile acid metabolism in the liver, intestine, and bile, as well as impair gallbladder emptying and refilling. Reproduced with permission from reference [31].

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Table 1

2017 Inventory of the candidate gallstone genes in mice and humans

Gene symbol	Gene name	Mouse chromosome	cM	Human orthologue
A. Liver				
(i) Lipid membrane transporters				
<i>ABCG5</i> and <i>ABCG8</i>	ATP-binding cassette, sub-family G (WHITE), member 5 and member 8	17	55.02	2p21
<i>ABCB4</i> (<i>MDR2</i>)	ATP-binding cassette, sub-family B (MDR/TAP), member 4 (phosphatidylcholine transporter; multiple drug resistance 2)	5	3.43	7q21.1
<i>ABCB11</i> (<i>BSEP</i> , <i>SPGP</i>)	ATP-binding cassette, sub-family B (MDR/TAP), member 11 (bile salt export pump; sister of P-glycoprotein)	2	39.69	2q24
<i>ABCC2</i> (<i>CMOAT</i> , <i>MRP2</i>)	ATP-binding cassette, sub-family C (CFTR/MRP), member 2 (canalicular multispecific organic anion transporter; multidrug resistance-related protein 2)	19	36.67	10q24
<i>SLC10A1</i> (<i>NTCP</i>)	solute carrier family 10 (sodium/bile acid cotransporter family), member 1 (sodium/taurocholate cotransporting polypeptide)	12	37.21	14q24.1
<i>SLC21A1</i> (<i>OATP1</i>)	solute carrier organic anion transporter family, member 1a1 (organic anion transporting polypeptide 1)	6	73.09	n/a
<i>SLC22A1</i> (<i>OCT1</i> , <i>ORCT</i>)	solute carrier family 22 (organic cation transporter), member 1 (organic cation transporter 1)	17	8.63	6q25.3
<i>ATP8B1</i> (<i>FIC1</i>)	ATPase, class I, type 8B, member 1 (familial intrahepatic cholestasis type 1)	18	37.49	18q21.31
<i>NPC1L1</i>	Niemann-Pick C1-like 1	11	3.93	7p13
(ii) Lipid regulatory enzymes				
<i>CYP7A1</i>	cytochrome P450, family 7, subfamily a, polypeptide 1 (cholesterol 7 α -hydroxylase)	4	2.91	8q11-q12
<i>CYP7B1</i>	cytochrome P450, family 7, subfamily b, polypeptide 1 (oxysterol 7 α -hydroxylase)	3	4.98	8q21.3
<i>CYP27A1</i>	cytochrome P450, family 27, subfamily a, polypeptide 1 (sterol 27-hydroxylase)	1	38.54	2q35
<i>HMGCR</i>	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	13	50.65	5q13.3-q14
<i>SOAT2</i> (<i>ACAT2</i>)	sterol O-acyltransferase 2 (acyl-coenzyme A:cholesterol acyltransferase 2)	15	57.33	12q13.13
<i>UGT1A1</i>	uridine diphosphate (UDP)-glucuronosyltransferase 1 family, polypeptide A1	1	44.55	2q37
(iii) Intracellular lipid transporters				
<i>CAV1</i>	caveolin 1	6	7.73	7q31.1
<i>CAV2</i>	caveolin 2	6	7.72	7q31.1
<i>FABP1</i>	fatty acid binding protein 1, liver	6	32.14	2p11
<i>NPC1</i>	Niemann-Pick type C1	18	6.15	18q11.2
<i>OSBP</i>	oxysterol binding protein	19	8.58	11q12-q13
<i>PCTP</i>	phosphatidylcholine transfer protein	11	54.7	17q21-q24

Gene symbol	Gene name	Mouse chromosome	cM	Human orthologue
<i>SCP2</i>	sterol carrier protein 2	4	50.2	1p32
<i>CETP</i>	cholesteryl ester transfer protein	n/a	n/a	16
(iv) Lipid regulatory transcription factors				
<i>NR2B1 (RXRA)</i>	nuclear receptor subfamily 2, group B, member 1 (retinoid X receptor alpha)	2	19.38	9q34.3
<i>NR1H3 (LXRA)</i>	nuclear receptor subfamily 1, group H, member 3 (liver X receptor alpha)	2	50.52	11p11.2
<i>NR1H2 (LXRB)</i>	nuclear receptor subfamily 1, group H, member 2 (liver X receptor beta)	7	28.83	19q13.3
<i>NR1H4 (FXR)</i>	nuclear receptor subfamily 1, group H, member 4 (farnesoid X receptor)	10	44.98	12q23.1
<i>NR1C1 (PPARA)</i>	peroxisome proliferator activated receptor alpha	15	40.42	22q13.31
<i>NR1C2 (PPARD)</i>	peroxisome proliferator activator receptor delta	17	14.64	6p21.2
<i>NR1C3 (PPARG)</i>	peroxisome proliferator activated receptor gamma	6	53.41	3p25
<i>NROB2 (SHP)</i>	nuclear receptor subfamily 0, group B, member 2 (small heterodimer partner)	4	66.25	1p36.1
<i>NR1I3 (CAR)</i>	nuclear receptor subfamily 1, group I, member 3 (constitutive androstane receptor)	1	79.21	1q23.3
<i>SREBF1</i>	sterol regulatory element binding transcription factor 1	11	37.81	17p11.2
<i>SREBF2</i>	sterol regulatory element binding transcription factor 2	15	38.49	22q13
<i>SCAP</i>	SREBF chaperone (SREBF cleavage activating protein)	9	59.91	3p21.31
<i>ESR1 (ERα)</i>	estrogen receptor 1 (alpha)	10	2.03	6q25.1
<i>ESR2 (ERβ)</i>	estrogen receptor 2 (beta)	12	33.52	14q23.2
<i>GPER1 (GPR30)</i>	G protein-coupled estrogen receptor 1	5	78.58	7p22.3
<i>AR</i>	androgen receptor	X	42.82	Xq12
<i>ADRB3</i>	adrenergic receptor, beta 3	8	15.94	8p12
(v) Lipoprotein receptors and related genes				
<i>APOB</i>	apolipoprotein B	12	2.53	2p24-p23
<i>APOE</i>	apolipoprotein E	7	9.94	19q13.2
<i>APOA5</i>	apolipoprotein A-V	9	25.37	11q23
<i>LDLR</i>	low density lipoprotein receptor	9	7.87	19p13.2
<i>LRP1</i>	low density lipoprotein receptor-related protein 1	10	74.52	12q13.3
<i>LRP2 (GPR330)</i>	low density lipoprotein receptor-related protein 2 (glycoprotein 330; megalin)	2	40.74	2q31.1
<i>SCARB1 (SRB1)</i>	scavenger receptor class B, member 1	5	64.11	12q24.31
<i>LPL</i>	lipoprotein lipase	8	33.88	8p22

Gene symbol	Gene name	Mouse chromosome	cM	Human orthologue
<i>LIPC (HPL)</i>	hepatic lipase	9	39.57	15q21-q23
<i>LCAT</i>	lecithin cholesterol acyltransferase	8	53.06	16q22.1
<i>LRPAP1</i>	low density lipoprotein receptor-related protein associated protein 1	5	18.01	4p16.3
<i>PLTP</i>	phospholipid transfer protein	2	85.27	20q13.12
B. Gallbladder				
(i) Hormone receptors				
<i>CCKIR (CCKAR)</i>	cholecystokinin-1 receptor (cholecystokinin-A receptor)	5	29.52	4p15.2
<i>ESR1 (ERα)</i>	estrogen receptor 1 (alpha)	10	2.03	6q25.1
<i>ESR2 (ERβ)</i>	estrogen receptor 2 (beta)	12	33.52	14q23.2
<i>GPER1 (GPR30)</i>	G protein-coupled estrogen receptor 1	5	78.58	7p22.3
<i>PGR</i>	progesterone receptor	9	2.46	11q22-q23
(ii) Mucin				
<i>MUC1</i>	mucin 1	3	39.02	1q21
<i>MUC2</i>	mucin 2	7	87.1	11p15.5
<i>MUC3</i>	mucin 3	5	76.22	n/a
<i>MUC4</i>	mucin 4	16	23.3	3q29
<i>MUC5AC</i>	mucin 5ac	7	87.23	11p15.5
<i>MUC5B</i>	mucin 5b	7	87.29	11p15.5
<i>MUC6</i>	mucin 6	7	87.03	11p15.5
(iii) Lipid membrane transporters				
<i>ABCG5 and ABCG8</i>	ATP-binding cassette, sub-family G (WHITE), member 5 and member 8	17	55.02	2p21
<i>SCARB1 (SRBI)</i>	scavenger receptor class B, member 1	5	64.11	12q24.31
(iv) Lipid regulatory enzymes				
<i>HMGCR</i>	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	13	50.65	5q13.3-q14
<i>SOAT2 (ACAT2)</i>	sterol O-acyltransferase 2 (acyl-coenzyme A:cholesterol acyltransferase 2)	15	57.33	12q13.13
C. Small intestine				
(i) Lipid membrane transporters				
<i>ABCG5 and ABCG8</i>	ATP-binding cassette, sub-family G (WHITE), member 5 and member 8	17	55.02	2p21
<i>SCARB1 (SRBI)</i>	scavenger receptor class B, member 1	5	64.11	12q24.31

Gene symbol	Gene name	Mouse chromosome	cM	Human orthologue
<i>SLC10A2 (IBAT)</i>	solute carrier family 10, member 2 (ileal bile salt transporter)	8	2.16	13q33
(ii) Lipid regulatory enzymes				
<i>HMGR</i>	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	13	50.65	5q13.3-q14
<i>SOAT2 (ACAT2)</i>	sterol O-acyltransferase 2 (acyl-coenzyme A:cholesterol acyltransferase 2)	15	57.33	12q13.13
(iii) Intracellular lipid transporters				
<i>CAV1</i>	caveolin 1	6	7.73	7q31.1
<i>CAV2</i>	caveolin 2	6	7.72	7q31.1
<i>FABP6 (ILLBP)</i>	fatty acid binding protein 6, ileal	11	25.81	5q33.3-q34
<i>OSBP</i>	oxysterol binding protein	19	8.58	11q12-q13
<i>SCP2</i>	sterol carrier protein 2	4	50.2	1p32
<i>NPC1L1</i>	Niemann-Pick C1-like 1	11	3.93	7p13
<i>APOB</i>	apolipoprotein B	12	2.53	2p24-p23
<i>APOA1</i>	apolipoprotein A-I	9	25.36	11q23-q24
<i>APOA4</i>	apolipoprotein A-IV	9	25.36	11q23
<i>APOC3</i>	apolipoprotein C-III	9	25.36	11q23
(iv) Lipid regulatory transcription factors				
<i>NR2B1 (RXRA)</i>	nuclear receptor subfamily 2, group B, member 1 (retinoid X receptor alpha)	2	19.38	9q34.3
<i>NR1H3 (LXRA)</i>	nuclear receptor subfamily 1, group H, member 3 (liver X receptor alpha)	2	50.52	11p11.2
<i>NR1H2 (LXRB)</i>	nuclear receptor subfamily 1, group H, member 2 (liver X receptor beta)	7	28.83	19q13.3
<i>NR1H4 (FXR)</i>	nuclear receptor subfamily 1, group H, member 4 (farnesoid X receptor)	10	44.98	12q23.1
<i>NR1C1 (PPARA)</i>	peroxisome proliferator activated receptor alpha	15	40.42	22q13.31
<i>NR1C2 (PPARD)</i>	peroxisome proliferator activator receptor delta	17	14.64	6p21.2
<i>NR1C3 (PPARG)</i>	peroxisome proliferator activated receptor gamma	6	53.41	3p25
(v) Hormone receptors				
<i>CCK</i>	cholecystokinin	9	72.43	3p22.1
<i>CCKIR (CCKAR)</i>	cholecystokinin-1 receptor (cholecystokinin-A receptor)	5	29.52	4p15.2
<i>ESR1 (ERα)</i>	estrogen receptor 1 (alpha)	10	2.03	6q25.1
<i>ESR2 (ERβ)</i>	estrogen receptor 2 (beta)	12	33.52	14q23.2
<i>GPER1 (GPR30)</i>	G protein-coupled estrogen receptor 1	5	78.58	7p22.3

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cM, centimorgan; n/a, not available; p, short arm of the chromosome; q, long arm of the chromosome.

Map position is based on conserved homology between mouse and human genomes and assigned indirectly from localization in other species. Information on homologous regions was retrieved from the mouse/human homology databases maintained at the Jackson Laboratory (http://www.informatics.jax.org/searches/marker_form.shtml) and the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/HomoloGene>). Reproduced with permission from reference [31].