

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: The authors have conducted a review to understand the recent advances in the understanding of gallstones. However, some such reviews have already been published in the literature. Secondly, most of the cited articles are more than 3-5 years old. Please revisit the literature and revise the manuscript accordingly. I have attached the following recent articles with my reviewer comments. Wang HH, Portincasa P, Liu M, Wang DQ. Genetic Analysis of ABCB4 Mutations and Variants Related to the Pathogenesis and Pathophysiology of Low Phospholipid-Associated Cholelithiasis. *Genes (Basel)*. 2022 Jun 11;13(6):1047. doi: 10.3390/genes13061047. PMID: 35741809; PMCID: PMC9222727. Sun H, Warren J, Yip J, Ji Y, Hao S, Han W, Ding Y. Factors Influencing Gallstone Formation: A Review of the Literature. *Biomolecules*. 2022 Apr 6;12(4):550. doi: 10.3390/biom12040550. PMID: 35454138; PMCID: PMC9026518. Feng R, Zhang T, Kayani MUR, Wang Z, Shen Y, Su KL, Bielike K, Chen L. Patients with Primary and Secondary Bile Duct Stones Harbor Distinct Biliary Microbial Composition and Metabolic Potential. *Front Cell Infect Microbiol*. 2022 Apr 25; 12:881489. doi: 10.3389/fcimb.2022.881489. PMID: 35548466; PMCID: PMC9082501.

**Response:** Thank you for your patient and careful comments, which have well demonstrated your unique academic insights and profound academic attainments, bringing great enlightenment and help to our scientific research ideas. We have thoroughly revised the manuscript according to your requirements, hoping to meet your requirements. Here's my point-to-point response to your questions:

1. Although there have been several reviews on cholesterol gallstones, previous reviews have focused on genetic factors, cholesterol hypersecretion, and gallbladder dyskinesia during gallstone formation. Our review focuses on the important roles of immune and microbial factors in the formation of cholesterol gallstones.
2. We have supplemented and improved the highlights of the latest cutting-edge research results based on the three papers you provided and the other recent related literatures as follows:

#### **Immune disorders lead to cholesterol gallstones**

At the same time, mucin may be positively correlated with calcification of cholesterol gallstones[22].

#### **The role of intestinal flora dysregulation in cholesterol gallstones**

A recent study comparing the biliary microbiota of lithiasis and non-lithiasis groups found that the *Alcaligenaceae* reached higher relative abundance in lithiasis samples[41]. In this family, *Alcaligenes recti* has been reported to be involved in the metabolism of various bile acids.

#### **Genetic susceptibility to cholesterol gallstones**

In addition to these two mechanisms, there are other factors that contribute to the formation of cholesterol gallstones, such as genetic factors and gallbladder dyskinesia[48].

Mutations and variants of ABCB4 inhibit the secretion of phospholipids from the liver to the

bile ducts, resulting in a decrease or deficiency of phospholipids in bile and the formation of cholesterol gallstones, known as low phospholipid-associated cholelithiasis (LPAC). A recent study compared the chemical composition of fresh gallbladder bile between ABCB4 KO and WT mice and found cholesterol supersaturation and the presence of cholesterol crystals in gallbladder bile in the former but not in the latter. The results of this study demonstrate the critical role of ABCB4 in phospholipid transport and the important role of ABCB4 mutations in the formation of cholesterol gallstones[53].

#### **Cholesterol gallstones formation by impaired gallbladder motility**

A recent study showed that 78 of 959 patients (8%) who underwent laparoscopic Roux-en-Y gastric bypass (RYGB) or sleeve gastrectomy developed symptomatic gallstone disease within 24 months[63]. In patients without gallstones before RYGB surgery, ursodeoxycholic acid treatment reduced the occurrence of symptomatic gallstone disease compared with placebo[64].

### **3. We have updated some outdated references according to your request as follows:**

Previous references	Updated references
Aerts R, Penninckx F. The burden of gallstone disease in Europe. <i>Aliment Pharmacol Ther</i> 2003; 18 Suppl 3: 49-53 [PMID: 14531741]	Wang Y, Qi M, Qin C, Hong J. Role of the biliary microbiome in gallstone disease. <i>Expert Rev Gastroenterol Hepatol</i> 2018; 12(12): 1193-1205 [PMID: 30791792 DOI: 10.1080/17474124.2018.1533812]
Paumgartner G, Sauerbruch T. Gallstones: pathogenesis. <i>Lancet</i> 1991; 338: 1117-1121 [PMID: 1682550]	Rudling M, Laskar A, Straniero S. Gallbladder bile supersaturated with cholesterol in gallstone patients preferentially develops from shortage of bile acids. <i>J Lipid Res</i> 2019; 60(3): 498-505 [PMID: 30610083 DOI: 10.1194/jlr.S091199]
Carey MC. Critical tables for calculating the cholesterol saturation of native bile. <i>J Lipid Res</i> 1978; 19: 945-955 [PMID: 731129]	Dosch AR, Imagawa DK, Jutric Z. Bile Metabolism and Lithogenesis: An Update. <i>Surg Clin North Am</i> 2019; 99(2): 215-229 [PMID: 30846031 DOI: 10.1016/j.suc.2018.12.003]
van Geenen EJM, van der Peet DL, Bhagirath P, Mulder CJJ, Bruno MJ. Etiology and diagnosis of acute biliary pancreatitis. <i>Nat Rev Gastroenterol Hepatol</i> 2010; 7: 495-502 [PMID: 20703238 DOI: 10.1038/nrgastro.2010.114]	Boxhoorn L, Voermans RP, Bouwense SA, Bruno MJ, Verdonk RC, Boermeester MA, van Santvoort HC, Besselink MG. Acute pancreatitis. <i>Lancet</i> 2020; 396(10252): 726-734 [PMID: 32891214 DOI: 10.1016/S0140-6736(20)31310-6]
Everhart JE, Yeh F, Lee ET, Hill MC, Fabsitz R, Howard BV, Welty TK. Prevalence of gallbladder disease in American Indian populations: findings from the Strong Heart Study. <i>Hepatology</i> (Baltimore, Md) 2002; 35: 1507-1512	Wang Y, Qi M, Qin C, Hong J. Role of the biliary microbiome in gallstone disease. <i>Expert Rev Gastroenterol Hepatol</i> 2018; 12(12): 1193-1205 [PMID: 30791792 DOI: 10.1080/17474124.2018.1533812]

[PMID: 12029637]	
Miquel JF, Covarrubias C, Villaroel L, Mingrone G, Greco AV, Puglielli L, Carvallo P, Marshall G, Del Pino G, Nervi F. Genetic epidemiology of cholesterol cholelithiasis among Chilean Hispanics, Amerindians, and Maoris. <i>Gastroenterology</i> 1998; 115: 937-946 [PMID: 9753497]	Wang Y, Qi M, Qin C, Hong J. Role of the biliary microbiome in gallstone disease. <i>Expert Rev Gastroenterol Hepatol</i> 2018; 12(12): 1193-1205 [PMID: 30791792 DOI: 10.1080/17474124.2018.1533812]
Shaffer EA. Epidemiology and risk factors for gallstone disease: has the paradigm changed in the 21st century? <i>Curr Gastroenterol Rep</i> 2005; 7: 132-140 [PMID: 15802102]	Wang Y, Qi M, Qin C, Hong J. Role of the biliary microbiome in gallstone disease. <i>Expert Rev Gastroenterol Hepatol</i> 2018; 12(12): 1193-1205 [PMID: 30791792 DOI: 10.1080/17474124.2018.1533812]
Lee DK, Tarr PI, Haigh WG, Lee SP. Bacterial DNA in mixed cholesterol gallstones. <i>Am J Gastroenterol</i> 1999; 94: 3502-3506 [PMID: 10606311]	Wang Y, Qi M, Qin C, Hong J. Role of the biliary microbiome in gallstone disease. <i>Expert Rev Gastroenterol Hepatol</i> 2018; 12(12): 1193-1205 [PMID: 30791792 DOI: 10.1080/17474124.2018.1533812]
Swidsinski A, Lee SP. The role of bacteria in gallstone pathogenesis. <i>Front Biosci</i> 2001; 6: E93-103 [PMID: 11578976]	Wang Y, Qi M, Qin C, Hong J. Role of the biliary microbiome in gallstone disease. <i>Expert Rev Gastroenterol Hepatol</i> 2018; 12(12): 1193-1205 [PMID: 30791792 DOI: 10.1080/17474124.2018.1533812]
Swidsinski A, Ludwig W, Pahlig H, Priem F. Molecular genetic evidence of bacterial colonization of cholesterol gallstones. <i>Gastroenterology</i> 1995; 108: 860-864 [PMID: 7875489]	Wang Y, Qi M, Qin C, Hong J. Role of the biliary microbiome in gallstone disease. <i>Expert Rev Gastroenterol Hepatol</i> 2018; 12(12): 1193-1205 [PMID: 30791792 DOI: 10.1080/17474124.2018.1533812]
Wu X-T, Xiao L-J, Li X-Q, Li J-S. Detection of bacterial DNA from cholesterol gallstones by nested primers polymerase chain reaction. <i>World J Gastroenterol</i> 1998; 4: 234-237 [PMID: 11819284]	Wang Y, Qi M, Qin C, Hong J. Role of the biliary microbiome in gallstone disease. <i>Expert Rev Gastroenterol Hepatol</i> 2018; 12(12): 1193-1205 [PMID: 30791792 DOI: 10.1080/17474124.2018.1533812]
Kawai M, Iwahashi M, Uchiyama K, Ochiai M, Tanimura H, Yamaue H. Gram-positive cocci are associated with the formation of completely pure cholesterol stones. <i>Am J Gastroenterol</i> 2002; 97: 83-88 [PMID: 11808974]	Wang Y, Qi M, Qin C, Hong J. Role of the biliary microbiome in gallstone disease. <i>Expert Rev Gastroenterol Hepatol</i> 2018; 12(12): 1193-1205 [PMID: 30791792 DOI: 10.1080/17474124.2018.1533812]

Reviewer #2:

**Scientific Quality:** Grade C (Good)

**Language Quality:** Grade C (A great deal of language polishing)

**Conclusion:** Accept (General priority)

**Specific Comments to Authors:** The schema is good. This review is well written, and is informative for journal readers.

**Response:** Thank you for your patient and careful comments, which have well demonstrated your unique academic insights and profound academic attainments. Thank you again for giving us the opportunity to present our research results.