EDITORIAL

Intestinal Absorption, Hepatic Synthesis, and Biliary Secretion of Cholesterol: Where Are We for Cholesterol Gallstone Formation?

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With a 10%-15% prevalence, gallstone disease is one of the most prevalent and costly digestive diseases in Western countries.[1,2] About two-thirds of gallstones are cholesterol gallstones,[3] while the remaining are pigment stones that contain less than 30% cholesterol. The prevalence of gallstones increases with age and is associated with a number of major risk factors.[1,4] Overall, cholesterol gallstone disease is deemed as the gallbladder/bile expression of the metabolic syndrome, as it is often associated with obesity, type 2 diabetes, dyslipidemia, and hyperinsulinemia. The combination of multiple disturbances affecting cholesterol homeostasis in bile is essential for cholesterol gallstone formation. The interactions of five primary defects (Fig. 1) result in rapid cholesterol nucleation and crystallization in bile, the key step for gallstone formation[1,5]: (1) *LITH* genes and genetic defects; (2) unphysiological sustained supersaturation of bile with cholesterol due to hepatic hypersecretion; (3) enhanced intestinal cholesterol absorption; (4) accelerated phase transitions of cholesterol; and (5) prolonged gallbladder stasis due to disrupted gallbladder motility accompanied with immunomediated gallbladder inflammation, as well as hypersecretion of mucins and accumulation of mucin gel in the gallbladder lumen.[1,6,7] Growth of solid, platelike cholesterol monohydrate crystals to form gallstones is a consequence of persistent hepatic hypersecretion of biliary cholesterol together with enhanced gallbladder mucin secretion and incomplete evacuation by the gallbladder due to its impaired motility function.

In this issue of HEPATOLOGY, Krawczyk et al.[8] report that patients with cholesterol-enriched stones displayed a high biliary cholesterol output and relatively low absorption efficiency of intestinal cholesterol. Apparently, this metabolic trait could precede gallstone formation, which may be a feature of those ethnic groups at (possibly genetic) high risk of gallstones. In their case-control studies, the authors examined four cohorts, each including gallstone patients and matched controls: one cohort of German subjects (112 patients and 152 controls), two cohorts of Chilean ethnic groups (100 Hispanic patients and 100 controls), and one cohort of Amerindian Mapuches (20 patients and 20 controls). Using chromatography/mass spectrometry, serum levels of surrogates were measure as markers of both intestinal cholesterol absorption (phytosterols: sitosterol and campesterol) and hepatic *de novo* synthesis (cholesterol precursors: lathosterol and desmosterol). In addition, serum sterol levels were employed as markers for evaluating increased risk of gallstones in an 8-year ultrasonographic follow-up study (Hispanics, 35 gallstone patients and 35 controls). Sterol levels were also measured in gallbladder bile from a subgroup of patients and controls (n = 17 each). Common variants of *ABCG5/G8* were genotyped. Cholesterol gallstone patients had distinctive serum sterol profiles (i.e., low levels of phytosterols, high levels of cholesterol precursors, and low ratios of phytosterols:cholesterol precursors). Differences were more pronounced in women than in men and in Chilean Hispanics than in Germans. In bile, both phytosterols and cholesterol were increased and relative lipid compositions of gallbladder bile plotted above the micellar phase boundary. In the follow-up study, individuals with incident stones had significantly lower serum phytosterol levels even before the appearance of gallstones. Based on the ratios of phytosterols:cholesterol precursors, the following sequences were established: Amerindians<Hispanics<Germans. The overall genotype distributions of the variants of *ABCG5/G8* did not differ between cases with gallstones and stone-free controls, and therefore, these results could not fully explain the sterol metabolic trait of gallstone disease across the cohorts.

Abbreviations: ABC, ATP-binding cassette; HDL, high-density lipoprotein; NPC1L1, Niemann-Pick C1-like 1 protein.

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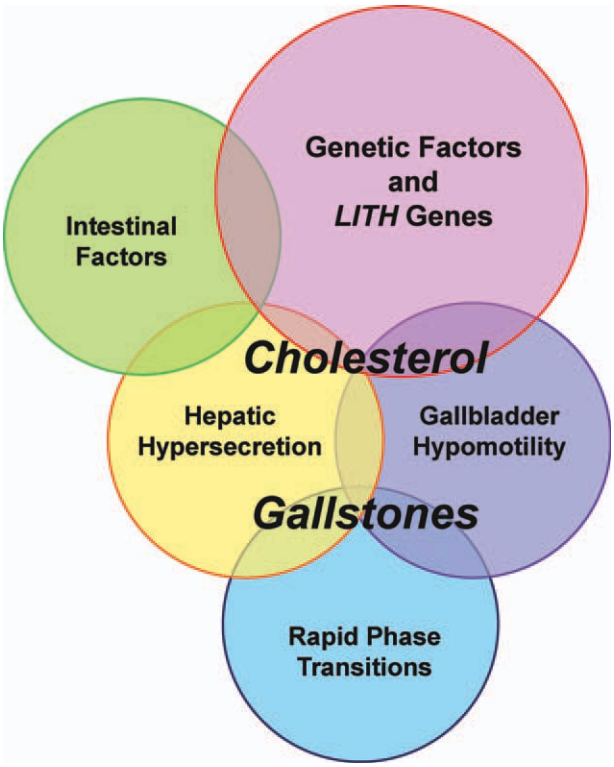

Fig. 1. Venn diagram of five primary defects: genetic factors and *LITH* genes, hepatic hypersecretion, gallbladder hypomotility, rapid phase transitions, and intestinal factors. Hepatic cholesterol hypersecretion into bile is the principal defect and is the outcome in part of a complex genetic predisposition. The downstream effects include gallbladder hypomotility and rapid phase transitions. A major result of gallbladder hypomotility is alteration in the kinetics of the enterohepatic circulation of bile salts, resulting in increased cholesterol absorption and reduced bile salt absorption that lead to abnormal enterohepatic circulation of bile salts and diminished biliary bile salt pool size. Gallbladder hypomotility facilitates cholesterol nucleation/crystallization and allows the gallbladder to retain cholesterol monohydrate crystals. Although a large number of *Lith* genes have been identified in mouse models of cholesterol gallstones, the identification of human *LITH* genes and their contributions to the formation of cholesterol gallstones require further investigation. Reproduced with modifications and with permission.[9]

Cholesterol homeostasis in humans is the consequence of the fine regulatory mechanisms involving intestinal absorption and hepatic *de novo* synthesis, as well as biliary secretion and fecal excretion of cholesterol. In enterocytes, sterol uptake and secretion is mediated by two brush border transport proteins. The Niemann-Pick C1-like 1 protein (NPC1L1) acts as an intestinal sterol influx transporter that actively facilitates the uptake of cholesterol.[9-11] Two ATP-binding cassette (ABC) transporters, ABCG5 and ABCG8, work as a heterodimeric efflux pump, for mediating cholesterol transport back to the intestinal lumen. Because in humans both NPC1L1 and ABCG5/G8 are also expressed in the hepatocyte at the canalicular membrane, both transporters contribute to the overall balance of cholesterol absorption/secretion at two different sites: intestine and liver.[12,13]

Where exactly excess biliary cholesterol comes from (i.e., intestinal absorption, hepatic *de novo* synthesis, reverse cholesterol transport by high-density lipoprotein [HDL]) is still an open issue and should be interpreted together with the function of these cholesterol transporters at different levels. Krawczyk et al. suggested that, at least in their particular setting, reduced cholesterol absorption, which is associated with increased cholesterol synthesis, may drive impaired cholesterol homeostasis in gallstone disease, with higher cholesterol clearance. The authors also speculated that the early events might be the biliary/intestinal cholesterol efflux (sterol clearance) followed by increased synthesis of cholesterol, since they did not occur simultaneously. Overall, however, such events should result in "sustained" rather than "fluctuating" supersaturation of bile with cholesterol. Genetic factors and *LITH* genes play a role in the pathogenesis of cholesterol gallstones.[10] In principle, a gain-of-function of ABCG5/G8 in humans might recapitulate both steps, leading to decreased intestinal absorption versus increased biliary output of cholesterol. The ABCG8 p.D19H and p.T400K coding variants might play a role as putative susceptibility variants for gallstone formation in humans.[14-16] Despite much evidence in this respect, the authors could not confirm such an interesting hypothesis, possibly due to the small cohort size.

The issue becomes even more intriguing when considering that high cholesterol content is typical of Westernized diets and that the small intestine is a unique organ providing dietary and reabsorbed biliary cholesterol to the body.[9] Furthermore, high efficiency of intestinal cholesterol absorption may occur, as shown in several inbred strains of mice.[7,17] Slow/sluggish intestinal motility is a feature in gallstone disease and is an additional factor predisposing to increased cholesterol absorption, more hydrophobe absorption, and secondary bile salt synthesis by the intestinal anaerobic microbiota. Thus, high dietary cholesterol matched with increased intestinal cholesterol absorption both appear to be key and independent risk factors for the formation of cholesterol gallstones.[9] This mechanism might be actively operating in subgroups of subjects who are at lower genetic risk of developing gallstones but are victims of environmental dietary factors. Indeed, the potent and selective inhibitor of NPC1L1 ezetimibe reduced biliary cholesterol secretion by suppressing intestinal cholesterol absorption and protected gallbladder motor function by desaturating bile, thus preventing the formation of cholesterol gallstones in mice.[11,18] The results are straightforward, since in mice NPC1L1 is expressed only in the intestine. In

hamsters and humans, however, NPC1L1 is also detected at a significantly lower expression level in the liver compared with the intestine. Because NPC1L1 is a cholesterol transporter that is expressed on the canalicular membrane of hepatocytes, it could function to limit cholesterol excretion, presumably by reabsorbing cholesterol from bile.[19] However, it was found that ezetimibe can significantly reduce hepatic secretion of biliary cholesterol in cholesterol-fed hamsters.[20] Furthermore, in gallbladder biles of Mexican patients with gallstones, ezetimibe reduced biliary cholesterol saturation and retarded cholesterol crystallization.[11] These results strongly suggest that the secretion efficiency of biliary cholesterol is most likely determined by the net effect between the efflux and influx of cholesterol molecules across the canalicular membrane of hepatocyte, which could be regulated by ABCG5/G8 and the NPC1L1 pathways.[11] It is highly likely that because biliary cholesterol secretion is a unique path for excretion of cholesterol from the body in humans and hamsters, hepatic ABCG5/G8 may play a stronger role in the regulation of biliary cholesterol secretion than NPC1L1. In addition, in the gut-liver axis, the intestinal NPC1L1 plays a significant role in providing dietary and reabsorbed biliary cholesterol to the body, and the inhibition of its functions by ezetimibe significantly reduces cholesterol absorption. Consequently, the bio-availability of cholesterol from intestinal sources for biliary secretion is decreased significantly.[9] Moreover, intestinal absorption of dietary cholesterol and reabsorption of biliary cholesterol could play a major role in a subgroup of patients with cholesterol gallstones. Such aspects need to be prospectively investigated.

Because of some gallstone patients with increased hepatic *de novo* cholesterol synthesis, the results of Krawczyk et al. suggested a potential therapeutic role for statins. Statins decrease hepatic cholesterol biosynthesis by inhibiting the rate-limiting 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase pathway and may therefore lower the risk of cholesterol gallstones by reducing the cholesterol concentration in bile. Although this was the case with pravastatin in a previous report[21] and in a recent large cohort study investigating the statin use and risk of gallstone disease followed by cholecystectomy,[22] evidence remains scarce and speculative. In general, the contribution of *de novo* synthesis on the formation of lithogenic bile and cholesterol gallstones appears to be modest.[23] In a small group of cholesterol gallstone patients, it was found that statins neither influenced biliary cholesterol secretion nor reduced cholesterol saturation index in gallbladder bile.[24] They did not influence cholesterol crystal detection time in these patients, either.[25] Lastly, simvastatin reduced plasma cholesterol concentrations, but could not prevent gallstone formation and biliary cholesterol crystallization in the prairie dog model of cholesterol gallstones.[26] The combination therapy of statins with the hydrophilic ursodeoxycholic acid yielded either limited[27] or similar[28] dissolution rates versus ursodeoxycholic acid alone in patients with radiolucent cholesterol gallstones. These issues also remain unsettled in the study of Krawczyk et al., since none of the patients were treated with statins.

Krawczyk et al. showed that the ratio of phytosterol:-cholesterol precursors in serum was even more predictive than "orthodox" variables determining the typical metabolic syndrome. Also, the ratio was consistent with the gallstone prevalence in different geographical areas and populations: $<20\%$ in Germans (and similar in Italians[29]), $\sim 27\%$ in Hispanics, and 35% in Mapuches. Whether serum phytosterol levels may become additional predictive biomarkers for increased gallstone risk even at a younger age (as is the case for other aspects of cholesterol metabolism) is still a matter of debate. Of note, nonalcoholic fatty liver disease (another "fellow traveler" with the metabolic syndrome) also showed similar cholesterol metabolic profiles compared with gallstone disease.[30]

The process referred to as reverse cholesterol transport (i.e., cholesterol from peripheral [extrahepatic] tissues returning to the liver) needs to be considered as well. HDL delivers cholesterol to the hepatocyte for selective uptake by scavenger receptor class B type I, an HDL receptor[31] that contributes to the hepatic cholesterol pool used for bile acid synthesis and excretion of cholesterol in the bile and feces. Thus, knowing the ultimate interaction between complex pathways involving cholesterol absorption, transport, synthesis, and secretion in subgroups of patients precipitating solid cholesterol crystals in bile and forming gallstones obviously requires further attention.

In conclusion, Krawczyk et al. investigated subtle mechanisms governing cholesterol homeostasis in the body (intestine, liver, and bile) with respect to cholesterol gallstone disease. Whether intestinal cholesterol absorption is decreased in certain gallstone patients and associated with a gain-of-function of the cholesterol intestinal and biliary transporter ABCG5/G8 (as well as of a number of other *LITH* genes) is a matter of research. Serum phytosterol levels might become additional predictive biomarkers for evaluating increased risk of gallstones. A new strategy aiming at inhibiting both hepatic synthesis and intestinal absorption of cholesterol for reducing its biliary output might be envisioned for a genetically defined subgroup of individuals at a high risk for gallstones. Overall, data need to be integrated with those suggesting that the absorption of intestinal cholesterol

indeed plays a role in the pathogenesis of gallstone disease, and that other groups of patients might benefit from drugs (such as ezetimibe) inhibiting this process.[11]

Although the ultimate and major sources of biliary cholesterol remain to be established in different populations, a more and more intriguing story about cholesterol cholelithiasis is developing and linking with complex metabolic disturbances and genetics. This will require appropriate preventive and medicinal approaches in the future.

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References

1. Portincasa P, Moschetta A, Palasciano G. Cholesterol gallstone disease. Lancet 2006;368:230-239.
2. Sandler RS, Everhart JE, Donowitz M, Adams E, Cronin K, Goodman C, et al. The burden of selected digestive diseases in the United States. Gastroenterology 2002;122:1500-1511.
3. Wang DQH, Cohen DE, Carey MC. Biliary lipids and cholesterol gallstone disease. J Lipid Res 2009;50(Suppl):S406-S411.
4. Katsika D, Grjibovski A, Einarsson C, Lammert F, Lichtenstein P, Marschall HU. Genetic and environmental influences on symptomatic gallstone disease: a Swedish study of 43,141 twin pairs. HEPATOLOGY 2005; 41:1138-1143.
5. Wang DQH, Afdhal NH. Gallstone disease. In: Feldman M, Friedman LS, Brand LJ, eds. Sleisenger and Fordtran's Gastrointestinal and Liver Disease. 9th ed. Philadelphia, PA: Elsevier; 2010:1089-1120.
6. Portincasa P, Di Ciaula A., Wang HH, Palasciano G, van Erpecum KJ, Moschetta A, et al. Coordinate regulation of gallbladder motor function in the gut-liver axis. HEPATOLOGY 2008;47:2112-2126.
7. Wang DQH, 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.
8. Krawczyk M, Lutjohann D, Schirin-Sokhan R, Villaroel L, Nervi F, Pimentel F, et al. Phytosterol and cholesterol precursor levels indicate increased cholesterol excretion and biosynthesis in gallstone patients. HEPATOLOGY 2012;55:1507-1517.
9. de Bari O, Neuschwander-Tetri BA, Liu M, Portincasa P, Wang DQH. Ezetimibe: its novel effects on the prevention and the treatment of cholesterol gallstones and nonalcoholic fatty liver disease. J Lipids 2012;2012:302847.
10. Wang HH, Portincasa P, Afdhal NH, Wang DQH. *Lith* genes and genetic analysis of cholesterol gallstone formation. Gastroenterol Clin North Am 2010;39:185-207.
11. Wang HH, Portincasa P, Mendez-Sanchez N, Uribe M, Wang DQH. Effect of ezetimibe on the prevention and dissolution of cholesterol gallstones. Gastroenterology 2008;134:2101-2110.
12. Yu L, Hammer RE, Li-Hawkins J, von Bergmann K, Lutjohann D, Cohen JC, et al. Disruption of *Abcg5* and *Abcg8* in mice reveals their crucial role in biliary cholesterol secretion. Proc Natl Acad Sci USA 2002;99:16237-16242.
13. Davis HR Jr, Zhu LJ, Hoos LM, Tetzloff G, Maguire M, Liu J, et al. Niemann-Pick C1 like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. J Biol Chem 2004;279:33586-33592.
14. Katsika D, Magnusson P, Krawczyk M, Grunhage F, Lichtenstein P, Einarsson C, et al. Gallstone disease in Swedish twins: risk is associated with ABCG8 D19H genotype. J Intern Med 2010;268:279-285.
15. Buch S, Schafmayer C, Volzke H, Becker C, Franke A, von Eller-Eberstein H, et al. A genome-wide association scan identifies the hepatic cholesterol transporter ABCG8 as a susceptibility factor for human gallstone disease. Nat Genet 2007;39:995-999.
16. Grunhage F, Acalovschi M, Tirziu S, Walier M, Wienker TF, Ciocan A, et al. Increased gallstone risk in humans conferred by common variant of hepatic ATP-binding cassette transporter for cholesterol. HEPATOLOGY 2007;46:793-801.
17. Wang DQH, 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.
18. Zuniga S, Molina H, Azocar L, Amigo L, Nervi F, Pimentel F, et al. Ezetimibe prevents cholesterol gallstone formation in mice. Liver Int 2008;28:935-947.
19. Tang W, Jia L, Ma Y, Xie P, Haywood J, Dawson PA, et al. Ezetimibe restores biliary cholesterol excretion in mice expressing Niemann-Pick C1-like 1 only in liver. Biochim Biophys Acta 2011;1811:549-555.
20. Valasek MA, Repa JJ, Quan G, Dietschy JM, Turley SD. Inhibiting intestinal NPC1L1 activity prevents diet-induced increase in biliary cholesterol in Golden Syrian hamsters. Am J Physiol Gastrointest Liver Physiol 2008;295:G813-G822.
21. Smit JWA, van Erpecum KJ, Stolk MFJ, Geerdink RA, Cluysenaer OJ, Erkelens DW, et al. Successful dissolution of cholesterol gallstone during treatment with pravastatin. Gastroenterology 1992;103:1068-1070.
22. Bodmer M, Brauchli YB, Krahenbuhl S, Jick SS, Meier CR. Statin use and risk of gallstone disease followed by cholecystectomy. JAMA 2009; 302:2001-2007.
23. Turley SD, Dietschy JM. The contribution of newly synthesized cholesterol to biliary cholesterol in the rat. J Biol Chem 1981;256: 2438-2446.
24. Smit JWA, van Erpecum KJ, Renooij W, Stolk MFJ, Edgar P, Doornewaard H, et al. The effects of the 3-hydroxy, 3-methylglutaryl coenzyme A reductase inhibitor pravastatin on bile composition and nucleation of cholesterol crystals in cholesterol gallstone disease. HEPATOLOGY 1995;21:1523-1529.
25. Smit JWA, van Erpecum KJ, Portincasa P, Renooij W, Erkelens DW, vanBerge-Henegouwen GP. Effects of simvastatin and cholestyramine on bile lipid composition and gall bladder motility in patients with hypercholesterolaemia. Gut 1995;37:654-659.
26. Davis KG, Wertin TM, Schriver JP. The use of simvastatin for the prevention of gallstones in the lithogenic prairie dog model. Obes Surg 2003;13:865-868.
27. Tazuma S, Kajiyama G, Mizuno T, Yamashita G, Miura H, Kajihara T, et al. A combination therapy with simvastatin and ursodeoxycholic acid is more effective for cholesterol gallstone dissolution than is ursodeoxycholic acid monotherapy. J Clin Gastroenterol 1998;26:287-291.
28. Sackmann M, Koelbl R, Pauletzki J, Cremer P, Klueppelberg UG, von RC, et al. Simvastatin added to ursodeoxycholic acid does not enhance disappearance of gallstone fragments after shock wave therapy. Z Gastroenterol 1995;33:585-589.
29. Attili AF, Carulli N, Roda E, Barbara B, Capocaccia L, Menotti A, et al. Epidemiology of gallstone disease in Italy: prevalence data of the multicenter italian study on cholelithiasis (M.I.C.O.L.). Am J Epidemiology 1995;141:158-165.
30. Simonen P, Kotronen A, Hallikainen M, Sevastianova K, Makkonen J, Hakkarainen A, et al. Cholesterol synthesis is increased and absorption decreased in non-alcoholic fatty liver disease independent of obesity. J Hepatol 2011;54:153-159.
31. Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. Science 1996;271:518-520.