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Hereditary liver disease: Gallstones

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Gallstones are common in Western countries and due to pain and complications pose a substantial burden on health care systems. In general, cholesterol gallstones are distinguished from bilirubin gallstones. Bilirubin gallstones form if the ion product of unconjugated bilirubin and calcium in gallbladder bile exceeds the solubilisation capacities of mixed micelles and vesicles. Cholesterol gallstones develop if the amount of cholesterol in gallbladder bile exceeds the maximum concentration that is soluble at the given concentration of bile salts and phospholipids. In addition, cholesterol gallstone formation requires hypomotility of the gallbladder and a mucin gel as nucleation matrix for monohydrate crystals. The individual risk of gallstone formation is determined by interactions of lithogenic alleles of gallstone susceptibility genes and multiple environmental factors. For asymptomatic gallstones, expectant management is recommended, whereas an episode of gallstone-associated pain substantially increases the risk of complications such as cholecystitis, cholangitis and pancreatitis and therefore necessitates cholecystectomy.

Epidemiology of cholelithiasis

Gallstones are calculi that most frequently form and reside in the gallbladder but in many cases become apparent because of their inherent risk to cause pain or complications. In principle, two major gallstone subtypes occur in the gallbladder, cholesterol gallstones and bilirubin or 'pigment' gallstones.[1] In Western populations, the majority of gallbladder stones are cholesterol gallstones.[1,2] In Europe, North- and South America, gallstones are extraordinarily common with prevalence rates in

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cross-sectional ultrasound-surveys surpassing 20% in selected studies.[3,4] However, the ultrasound-surveys revealed a wide range of cholelithiasis prevalence rates across different populations and continents. In contrast to the abundance of cholesterol gallstones in Europe, North- and South America, gallstones are rare in Asia and in Africa, where bilirubin gallstones are more common than cholesterol stones.[1] Highest frequencies of gallstones were found in American Indians with prevalence rates exceeding 30% in men and 60% in women.[5] In U.S., a large ultrasound based survey confirmed ethnic differences in cholelithiasis with gallstone prevalence rates being higher in Mexican Americans compared with non-hispanic whites whereas male and female non-hispanic blacks displayed the lowest gallstone frequencies.[6] Likewise, a study from Chile reported more frequent cholelithiasis in native Mapuche Indians and Hispanics compared to Maoris of the Easter Islands, who are of Polynesian descent.[7]

Pain caused by gallstones and complications of cholelithiasis frequently necessitate cholecystectomies rendering gallstone disease a substantial burden for health care systems in Western countries. In U.S., gallstones are one of the most costly of all digestive diseases with direct costs of gallstone disease only being surpassed by costs for gastro-oesophageal reflux disease.[8]

Risk factors for gallstone formation

Cholelithiasis is very rare in infants and infrequent in adolescents with the exception of the development of pigment gallstones owing to haemolysis. After childhood, gallstone prevalence increases linearly with age.[9,10] Furthermore, in most studies, women have higher gallstone prevalence rates than men and more frequently require gallbladder surgery.[4] In addition, the gallstone risk is increased further by the number of pregnancies.[10,11] In most surveys, a higher body mass index was associated with an increased gallstone risk.[10,11] However, some studies found obesity to be a stronger risk factor in women than in men.[12,13] Another association was noted between cholelithiasis and undiagnosed diabetes in women and men and surrogates of insulin resistance in women.[14] The correlation of insulin resistance and gallstones was consistently confirmed in several populations [15–17] suggesting that cholelithiasis might be another feature of the metabolic syndrome.[16,18] In line with these findings, a diet high in carbohydrates and with a high glycaemic load was found to be associated with cholecystectomies in women.[19] Paradoxically, gallstone formation is not only associated with obesity and insulin resistance but also with rapid weight loss. Independent whether achieved by consumption of a reduction diet or by surgical intervention, weight loss of more than 1.5 kg/week leads to a substantial gallstone risk with gallstone incidence exceeding 30% in both men and women. [20,21] In addition, in middle age women even moderate intentional weight loss associated with regain (“weight cycling”) was associated with a more than 30% increased risk of a cholecystectomy.[22]

Pathophysiology of gallstone formation

Bilirubin gallstones

Bilirubin gallstones principally consist of polymers of bilirubinate, the salt of unconjugated bilirubin, and calcium.[23] In most cases of bilirubin gallstones the causes for the changes leading to stone formation remain unknown. However, some conditions typically lead to bilirubin gallstone formation with a defined pathogenesis. Haemolysis causes unconjugated hyperbilirubinaemia with increased amounts of bilirubin transported to the liver for conjugation and increased biliary secretion of bilirubin conjugates.[24] This explains bilirubin gallstone formation in patients with chronic haemolytic anaemia.[25] Similarly, an induced enterohepatic circulation of bilirubin was proposed to increase bilirubin transport to the liver and biliary bilirubin secretion.[26] In health, bilirubin conjugates undergo fecal excretion. However, bile salt malabsorption leads to excess amounts of bile salts in the colon which can bind calcium to prevent formation of calcium-bilirubinate complexes, solubilise bilirubin conjugates and consequently promote passive colonic bilirubin absorption.[26] These proposed mechanisms may explain bilirubin gallstone formation in Crohn’s disease or following resection of the terminal ileum.[27,28] However, additional factors are likely to contribute to the

increased gallstone risk in patients with Crohn's disease.[29] Interestingly, alcoholism without cirrhosis was reported to be associated with pigment gallstone formation.[30] It was suggested that bile salt malabsorption due to structural damages of the intestinal mucosa may also contribute to the pathogenesis of alcohol-induced bilirubin gallstone formation.[26]

In liver cirrhosis, several factors combine to increase formation of bilirubin gallstones. Biliary bile salt and cholesterol levels in cirrhosis are low leading to a reduced capacity to bind and solubilise calcium and unconjugated bilirubin. Furthermore, the ratio of bilirubin diglucuronides to monoglucuronides is likely to be reduced owing to the reduced glucuronidation capacity of the cirrhotic liver leaving bilirubin conjugates in bile more prone to formation of unconjugated bilirubin from hydrolysis by β-glucuronidases.[31] Fig. 1 summarises key aspects of bilirubin gallstone formation and the pathogenetic concepts of underlying disorders.

Cholesterol gallstones

Cholesterol gallstones develop if the amount of cholesterol in gallbladder bile exceeds the maximum concentration that is soluble at the given concentration of bile salts and phospholipids. In addition, cholesterol gallstone formation requires a mucin gel as nucleation matrix for monohydrate crystals and in most instances occurs in a gallbladder with impaired motility.[32] Knowledge of the pathogenesis may explain cholesterol gallstone formation in some patients and allow the identification of an increased gallstone risk in others. Incidence of gallbladder sludge and gallstones in the last two trimester of pregnancy and in the first 4–6 weeks postpartum is substantial.[33] Among other factors, cholesterol relative to phospholipid and bile salt secretion rates are increased leading to bile supersaturated with cholesterol.[34] This finding may be due both to changes in female sex hormones during pregnancy and to insulin resistance in the last trimester of pregnancy.[35] Oestrogens seem to contribute to cholesterol supersaturation of bile, whereas progesterone impedes gallbladder contraction.[1,36] Compatible with these findings, the cholecystectomy risk is increased in women

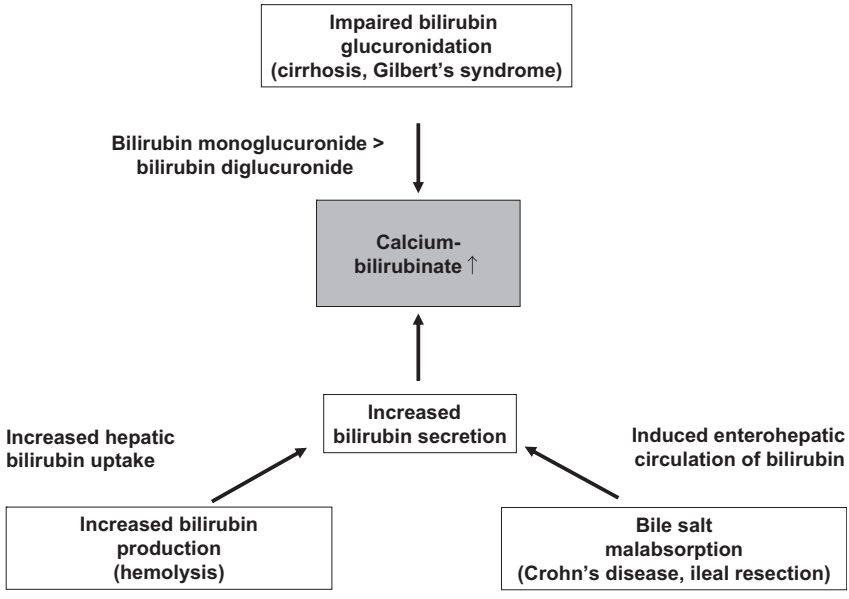

Fig. 1. Key factors of bilirubin gallstone formation and the pathogenesis of underlying disorders. Increased hepatic bilirubin uptake due to haemolysis and an induced enterohepatic bilirubin circulation due to bile salt malabsorption in Crohn's disease or following ileal resection result in increased biliary bilirubin secretion. Impaired bilirubin glucuronidation due to cirrhosis of the liver or Gilbert's syndrome results in higher proportions of monoglucuronidated bilirubin, which is prone to formation of unconjugated bilirubin from hydrolysis by β-glucuronidases.

taking postmenopausal hormone replacement therapy.[37] In contrast, the gallstone risk with modern low-dose anovulatory steroids, if any, is small.[1,38] Recently, the molecular mechanisms that increase cholesterol secretion following insulin resistance were elucidated in a mouse model of hepatic insulin resistance.[39] Insulin resistance was found to result in a more hydrophobic lithogenic bile salt profile and in increased biliary cholesterol secretion due to higher expression levels of the *Abcg5* and *Abcg8* genes that encode the heterodimeric apical hepatocellular cholesterol transporter.[39]

Dieting increases cholesterol gallstone formation in at least two ways: weight loss promotes higher biliary cholesterol secretion leading to bile supersaturated with cholesterol.[40] In addition, a diet low in lipids leads to a diminished release of cholecytokinin from enteroendocrine cells in the small intestine resulting in an impaired gallbladder contraction and an increased small bowel transit time allowing for higher cholesterol absorption rates.[41] The same mechanism explains gallstone formation following a therapy with octreotide, a somatostatin analogue that decreases cholecystokinin release from enteroendocrine cells.[42] Other medications increase the gallstone risk by changing biliary lipid secretion: fibrates increase cholesterol secretion, whereas cyclosporine and the endothelin receptor antagonist bosentan decrease secretion rates of bile salts.[1] Key factors of cholesterol gallstone formation and pathogenetic concepts of underlying causes that increase cholesterol gallstone risk are summarised in Fig. 2.

Genetic background of gallstone susceptibility

For decades, a genetic predisposition to gallstone formation was noted based on the results of epidemiological and family studies (summarised in Ref. [1]). More recently, a large twin study from Sweden confirmed the heritability of symptomatic cholelithiasis.[43] From higher concordance rates for symptomatic gallstones in monozygotic compared to dizygotic twins, genetic factors were calculated to account for 25% of the gallstone risk among the twins, the remainder is accounted for by environmental factors.[43] In the vast majority of cases, gallstone susceptibility is conferred from litogenic alleles of several lithogenic (*LITH*) genes and their interactions with environmental factors

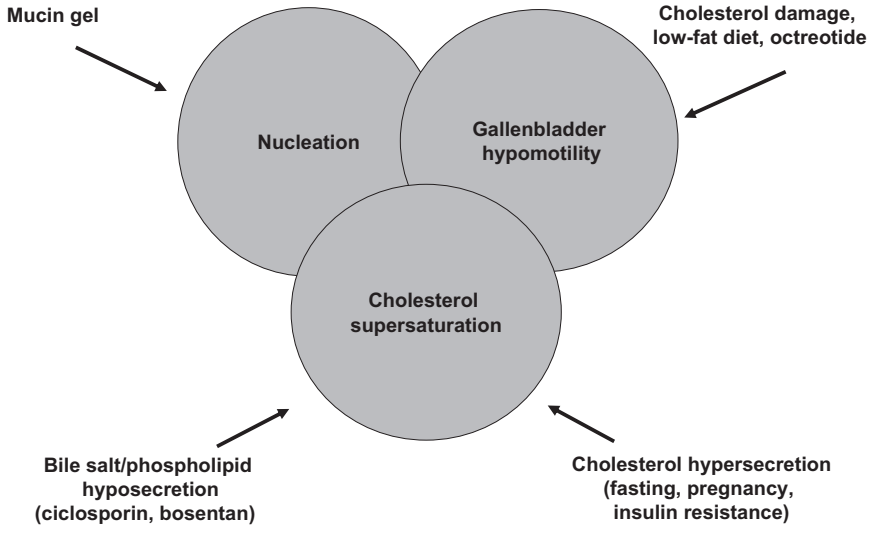

Fig. 2. Key factors of cholesterol gallstone formation and the pathogenesis of underlying risk factors. Fasting, insulin resistance and fibrates induce cholesterol hypersecretion, whereas drugs such as ciclosporin impair bile salt secretion resulting in cholesterol supersaturation of bile. Total parental nutrition, a diet low in fat and octreotide treatment lead to gallbladder hypomotility and stasis of bile in the gallbladder and prolonged time for nucleation. In addition, a mucin gel in the gallbladder is required as a nucleation matrix for cholesterol monohydrate crystals.

rendering cholelithiasis an example of a complex genetic trait.[44] However, in selected cases of 'oligogenic' cholelithiasis, mutations in single genes result in a marked increase of gallstone risk.[44]

A reduced capacity to conjugate bilirubin due to a polymorphism in the promoter of the UDP-glycuronosyl-transferase (*UGT1A1*) gene leads to Gilbert's syndrome, a common condition characterised by intermittent jaundice which also features higher proportions of biliary unconjugated and monoglucuronidated bilirubin and may predispose to pigment gallstone formation.[45,46] In patients with chronic haemolytic anaemia and cystic fibrosis, promoter polymorphisms of *UGT1A1* were associated with an increased gallstone risk underscoring the concept of a genetically determined reduced UDP-glycuronosyl-transferase activity as an important modifier in pigment gallstone formation.[47] Another persuasive example of an oligogenic predisposition to gallstone formation is the low phospholipid-associated cholelithiasis (LPAC) syndrome. In this syndrome, mutations in the *ABCB4* gene that encodes the apical phospholipids transporter of hepatocytes are associated with a low phospholipid concentration in bile, intrahepatic gallstones in younger patients, stone recurrence after cholecystectomy and a positive family history for gallstones and intrahepatic cholestasis of pregnancy.[48,49]

To date the most compelling *LITH* gene for common complex cholelithiasis is *ABCG8*. An individual single nucleotide polymorphism leading to the amino acid exchange p.D19H was unambiguously associated with an increased gallstone risk.[50,51] The ABCG5/ABCG8 heterodimer transports cholesterol as well as plant sterols and promotes biliary cholesterol secretion and limits intestinal cholesterol and plant sterol uptake.[52] The lithogenic 19H allele is associated with lower plasma plant sterol levels [53] supporting the concept of an increased gallstone risk from genetically determined higher biliary cholesterol secretion, as suggested from studies in the inbred mouse model of cholesterol cholelithiasis.[54] However, the lithogenic *ABCG8* variant explains only a fraction of the genetically determined gallstone risk only. Another genetic study identified a putative association of variants of the *NR1H4* gene that encodes the nuclear bile salt receptor FXR and cholelithiasis in selected populations.[55] Furthermore, a recent genetic study confirmed variants of the *SLC10A2* gene encoding the apical sodium-dependant bile acid transporter in the intestine as a risk factor for gallstone formation especially in male non-obese gallstone carriers.[56] These findings suggest that some genetic variants may account for an increased gallstone risk in certain ethnical populations or subgroups of patients only. Numerous additional genetic studies have investigated associations of several candidate genes with cholelithiasis but results were heterogeneous and positive associations await confirmation from independent analyses (summarised in Ref. [57]).

Natural history, symptoms and complications of gallstones

Most gallbladder stones remain asymptomatic. However, per year up to 2–3% of gallstone carriers experience intense pain attacks caused by an impacted gallstone in the gallbladder neck or cystic duct.[58] The gallstone-associated pain in most cases is located in the right upper quadrant of the abdomen or epigastrium, may radiate to the back or right shoulder, may be associated with nausea and vomiting and typically lasts longer than 15–30 min.[58] After a first episode of gallstone-associated pain most of the patients experience further pain attacks.[59] In addition, after a first episode of gallstone-associated pain the risk of complications such as cholecystitis, obstructive cholangitis and pancreatitis increases from 0.1 to 0.3% per year in asymptomatic gallstone carriers to 1–2% per year.[60] Conversely, half of the patients with biliary pancreatitis experienced a prior episode of gallstone-associated pain.[61] Other complications of cholelithiasis include gallbladder cancer with the highest risk in carriers of gallstones that are more than three centimetre in diameter, gallbladder polyps more than one centimetre in size and calcification of the gallbladder wall, known as porcelain gallbladder.[58] Whereas large gallstones are associated with an increased risk of gallbladder carcinoma, multiple small gallstones and preserved gallbladder contractility may increase the risk of stone migration to the common bile duct, stone impactation at the papilla and biliary pancreatitis.[62]

Diagnosis and treatment of symptomatic or complicated cholelithiasis

Symptoms of gallbladder-associated pain are in general non-specific rendering the discrimination of symptomatic and asymptomatic cholelithiasis difficult.[58] Suspicion of symptomatic cholelithaisis should prompt an ultrasound examination. Ultrasound has a high sensitivity for stone detection and

may indicate complications of cholelithiasis such as cholecystitis or gallbladder carcinoma and allows simultaneous examination of other organs that may be causing the symptoms.[58] An acute episode of biliary pain should be treated with analgetics and administration of non-steroidal anti-inflammatory drugs may reduce the risk of progression from biliary colic to cholecystitis.[63] Due to recurrent episodes of pain and the marked increase of complication rates, patients with symptomatic gallstones should undergo cholecystectomy. In contrast, since most asymptomatic gallstone carriers remain free of symptoms, in general cholcystectomy is not warranted for asymptomatic cholelithiasis.[64,65] However, there are some exceptions: patients with large gallstones, gallstones and gallbladder polyps and a porcelain gallbladder due to an increased risk of gallbladder cancer and patients with gallstones and weight loss surgery, resections in Crohn's disease and gastrectomies who are all at an increased risk of symptomatic cholelithiasis.[58]

Today, standard of care is laparoscopic cholecystectomy, which has complication rates similar to open cholecystectomy but is associated with a shorter hospital stay, faster convalescence and lower costs.[66] Laparoscopic cholecystectomy is also the treatment of choice for patients with acute cholecystitis and should be performed within 72 h of the onset of symptoms. Meanwhile, patient should be treated with antibiotics. Early laparoscopic cholecystectomy is associated with a shorter hospitalisation and 18% of patients had to undergo emergency cholecystectomy while awaiting elective surgery.[67] Since stones in the gallbladder and in the common bile duct often coexist, prior to gallbladder surgery the likelihood of choledocholithiasis should be assessed. In cases of a high likelihood common bile duct stones or confirmation of choleledocholithiasis by ultrasound, endoscopic ultrasound or magnetic resonance cholangiography, the standard approach by most hospitals is "therapeutic splitting". This concept includes the removal of stones from the common bile duct by retrograde endoscopic cholangiography followed by cholecystectomy (details discussed in Ref.[57]). In cases of obstructive cholangitis, antibiotic treatment and early, in case of cholangiosepis urgent, endoscopic stone removal or biliary drainage is necessary.[68] Patients with biliary pancreatitis benefit from early endoscopic stone removal if choledocholithiasis is accompanied by cholangitis, marked cholastasis or if the course of the pancreatitis is predicted to be severe.[57,69] After stone removal from the common bile duct, early elective cholecystectomy should be performed to prevent further complications.[57]

Primary and secondary prevention of gallstones

Dissolution therapy of gallbladder stones by ursodeoxycholic acid was largely abandoned and extracorporal shock wave lithotripsy combined with ursodeoxycholic acid is obsolete due to high rates of stone recurrence and the advent of laparoscopic cholecystectomy. However, the high gallstone incidence during weight loss can be markedly reduced by prophylactic ursodeoxycholic acid and independent of means of weight loss, every patient loosing more than 1.5 kg/week should take $\geq$500 mg of ursodeoxycholic acid for prevention of gallstone formation.[21,40] In contrast, ursodeoxycholic acid was of no benefit in patients with symptomatic gallstones awaiting surgery.[59]

In addition, epidemiological studies revealed lower rates of symptomatic cholelithiasis with coffee, nut and moderate alcohol consumption.[70–72] Furthermore, recreational physical activity was associated with a decreased risk of cholecystectomy.[73] Interestingly, an association was identified of vitamin C supplementation and a lower prevalence of gallstones, a finding that is supported by a case control study and an epidemiological study.[74–76] Recently, epidemiological studies reported an association of the use of statins, a group of drugs that inhibit HMG-CoA-reductase, the rate-limiting enzyme in cholesterol synthesis, and a reduction in symptomatic cholelithiasis followed by cholecystectomy.[77,78] However, de novo synthesised cholesterol does not appear to be preferentially secreted into bile and most biliary cholesterol is derived from HDL or chylomicron remnants.[44] Accordingly, earlier studies did not ascertain desaturation of bile from cholesterol or dissolution of gallstones by statins. It was suggested, that statins may have a role for gallstone prevention in persons with a defined genetic risk, e.g. the risk allele of *ABCG8*.[57] However, currently use of statins for the primary or secondary prevention of cholelithiasis cannot be recommended based on the data that are available to date.

Practice points

- Gallbladder stones are common and frequently cause pain and complications
- The individual risk to develop gallstones is genetically determined and results from interactions of susceptibility alleles of lithogenic genes and environmental factors
- Symptomatic gallstones are associated with a marked increase in complications and should prompt cholecystectomy; for asymptomatic gallstones, in general, expectant management is recommended

Research agenda

- Identification of the full set of lithogenic genes is needed for the understanding of the true pathophysiology of gallstone formation
- Individual prediction of gallstone risk based on genetic and environmental risk factors should prompt studies of specific drugs such as ursodeoxycholic acid for the prevention of stone formation in high risk individuals
- The natural history of gallstones needs to be further defined to identify those patients with gallbladder stones that benefit from prophylactic cholecystectomy

Summary

Due to pain and complications, gallstones pose a substantial burden on health care systems rendering identification of patients at risk and effective strategies for gallstone prevention highly desirable. Knowledge of the pathophysiology of cholelithiasis explains stone formation in some patients and allows identification of an increased gallstone risk in others. The risk of bilirubin gallstone formation is increased in patients with haemolysis, induced enterohepatic bilirubin circulation due to Crohn's disease or ileal resection or impaired bilirubin glucuronidation due to cirrhosis. The risk of cholesterol gallstone formation is increased by fasting, insulin resistance or fibrates that induce cholesterol hypersecretion or by drugs such as ciclosporin that impair bile salt secretion. In addition, total parental nutrition, a diet low in fat and octreotide lead to gallbladder hypomotility and stasis and a prolonged time for nucleation of cholesterol monohydrate crystals. In most cases, the individual risk of gallstone formation is determined by interactions of lithogenic alleles of gallstone susceptibility genes and multiple environmental factors. However, to date only the p.D19H variant of the *ABCG8* gene was unambiguously identified as a lithogenic genetic variant and identification of additional *LITH* genes is necessary for the assessment of individual genetic risk profiles for gallstone formation. For asymptomatic gallstones, expectant management is recommended whereas an episode of gallstone-associated pain substantially increases the risk of complications such as cholecystitis, cholangitis and pancreatitis and therefore necessitates cholecystectomy. Patients at a high risk for cholesterol gallstone formation e.g. due to rapid weight loss should take ursodeoxycholic acid for gallstone prevention. In contrast, for patients at risk for bilirubin gallstone formation to date no effective prevention is available.

Conflict of interest

None.

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