

Gallstone disease

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Gallstone disease is one of the most prevalent gastrointestinal diseases with a substantial burden to health care systems that is supposed to increase in ageing populations at risk. Aetiology and pathogenesis of cholesterol gallstones still are not well defined, and

strategies for prevention and efficient nonsurgical therapies are missing. This review summarizes current concepts on the pathogenesis of cholesterol gallstones with focus on the uptake and secretion of biliary lipids and special emphasis on recent studies into the genetic background.

Keywords: gastroenterology, hepatology.

Gallstones are a common clinical finding in the Western populations. Ultrasound studies indicate mean prevalence rates of 10–15% in adult European, and of 3–5% in African and Asian populations [1]. In the US, the prevalence rates range from 5% for nonHispanic black men to 27% for Mexican-American women [2]. In American Indians, gallstone disease is epidemic and found in 73% of adult female Pima Indians [3], and in 30% of male and 64% of female in other American Indians [4].

More than 80% of gallstone carriers are unaware of their gallbladder disease [5, 6], but about 1–2% per year of patients develop complications and need surgery [7]. In the US, gallstone disease has the most common inpatient diagnosis among gastrointestinal and liver diseases [8] and stands for \$5.8 billion direct costs, exceeded only by gastroesophageal reflux disease [9].

Risk factors

Female gender, fecundity, and a family history for gallstone disease are strongly associated with the formation of cholesterol gallstones [10] (Table 1). Obesity [11, 12] as well also other factors contributing to the metabolic syndrome [13] such as dyslip-

idemia (in particular hyperlipoproteinemia type IV [14–16] with hypertriglyceridemia and low HDL cholesterol), hyperinsulinemia-insulinresistance [17, 18] or overt type 2 diabetes are risk factors for the development of gallstones, itself supposed to be a complication of the metabolic syndrome [19]. Oestrogen-treatment enhances the risk, both in women when used for anticonception or hormone-replacement [20] and in men with prostatic cancer [21, 22]. Among specific dietary factors, short-time high cholesterol [23] as well as high-carbohydrate diets were associated with increased risk for gallstones [24, 25], and in highly prevalent areas, the intake of legume [26], while unsaturated fats [27], coffee [28], and moderate consumption of alcohol [24, 29] seem to reduce the risk. Also physical activity was found to decrease the risk for symptomatic gallstone disease, both for men and women [30, 31], and independent of weight reduction. On the other hand, rapid active weight loss [32, 33] and weight cycling [34, 35] strongly increase the risk for the development of gallstones. Thus, weight reductions should not exceed 1.5 kg per week [36]. Fibrates, used for the treatment of dyslipidemia, interfere with cholesterol and bile acid synthesis and increase cholesterol secretion into bile [37, 38]. However, in contrast to the prototype Clofibrate, during treatment with newer

Table 1 Characteristics of different types of gallstones and major risk factors for cholesterol gallstones

	Cholesterol stones	Brown pigment stone	Black pigment stone
Prevalence	80–90%	5–10%	<5%
Main composition	50–90% cholesterol	~50% bilirubin	>50% bilirubin
Colour	Yellow-grey	Brown	Dark brown-black
Aetiology	Cholesterol supersaturation	Increased deconjugation of bilirubin glucuronides	Increased biliary bilirubin load
Risk factors	Increasing age Female gender Family history Obesity Dietary factors: high caloric diets, high refined carbohydrate diets, low-fibre diets Dyslipidemia: hypertriglyceridemia Hormonal factors: pregnancy, contraceptive pills, oestrogen replacement therapy Life style factors: sedentary life style, rapid weight loss Medications: octreotide, (fibrates)	Biliary infections Abnormal biliary anatomy (Caroli's syndrome)	Haemolytic anaemia Liver cirrhosis Inefficient erythropoiesis Crohn's disease Cystic fibrosis

fibrates only a relatively small percentage do actually develop gallstones [39].

Bile formation

The formation of bile is essential for lipid digestion and the removal of excess cholesterol from the body either by direct excretion or after conversion to bile acids (Fig. 1). Bile mainly consists of water (90%) and three lipid species: cholesterol (4% of solutes by weight), phospholipids (24%) and bile salts (72%) [40]. For each of these compounds, specific ATP-binding-cassette transport proteins (ATP transporters) are expressed at the canalicular membrane domain of hepatocytes. ABCB4, in humans also known as multi-drug resistant p-glycoprotein MDR3, acts as a 'flip-pase' that translocates phospholipids from the inner to the outer leaflet of the membrane [41]. Mutations in the MDR3 gene were first described in progressive familial intrahepatic cholestasis (PFIC) type 3 [42] and later found in a number of hepatobiliary diseases [43], including cholesterol gallstone disease [44] and intrahepatic cholestasis of pregnancy (ICP) [45, 46]. ABCB11, also known as the bile salt export pump BSEP, is the main bile salt transporter [47]. This transporter is mutated in PFIC type 2 [48] and in benign recurrent intrahepatic cholestasis (BRIC) type

2 [49]. Cholesterol is transported by ABCG5 and ABCG8 that form obligate heterodimers [50]. Mutations of these transport proteins cause sitosterolemia [51].

Gene expression levels of ABCB4, ABCB11 and ABCG5/8 are regulated by at least two nuclear receptors (NR) initially found to regulate cholesterol and bile acid metabolism [52, 53]. The farnesoid or bile acid receptor FXR/BAR [54–57] regulates transcription of *ABCB4* [58] and *ABCB11* [59] while *ABCG5* and *ABCG8* are under control of the oxysterol or liver X receptors *LXR α/β* [60], perhaps mediated by FXR [61].

Once secreted, hepatic bile is modified by bicarbonate- and chloride-rich secretions of cholangiocytes, accompanied with water influx through aquaporin channels (recently reviewed in [62]). The chloride channel in cholangiocytes is the cystic fibrosis transmembrane conductance regulator (*ABCC7*, *CFTR*) that is mutated in cystic fibrosis [63, 64].

Cholesterol uptake into the liver is mainly mediated by the scavenger receptor B1 (SR-B1) for HDL [65] and to lesser extent by the apolipoprotein (Apo) B/E receptor for LDL [66] that is controlled by sterol-regulatory-element-binding protein (SREBP-1) [67].

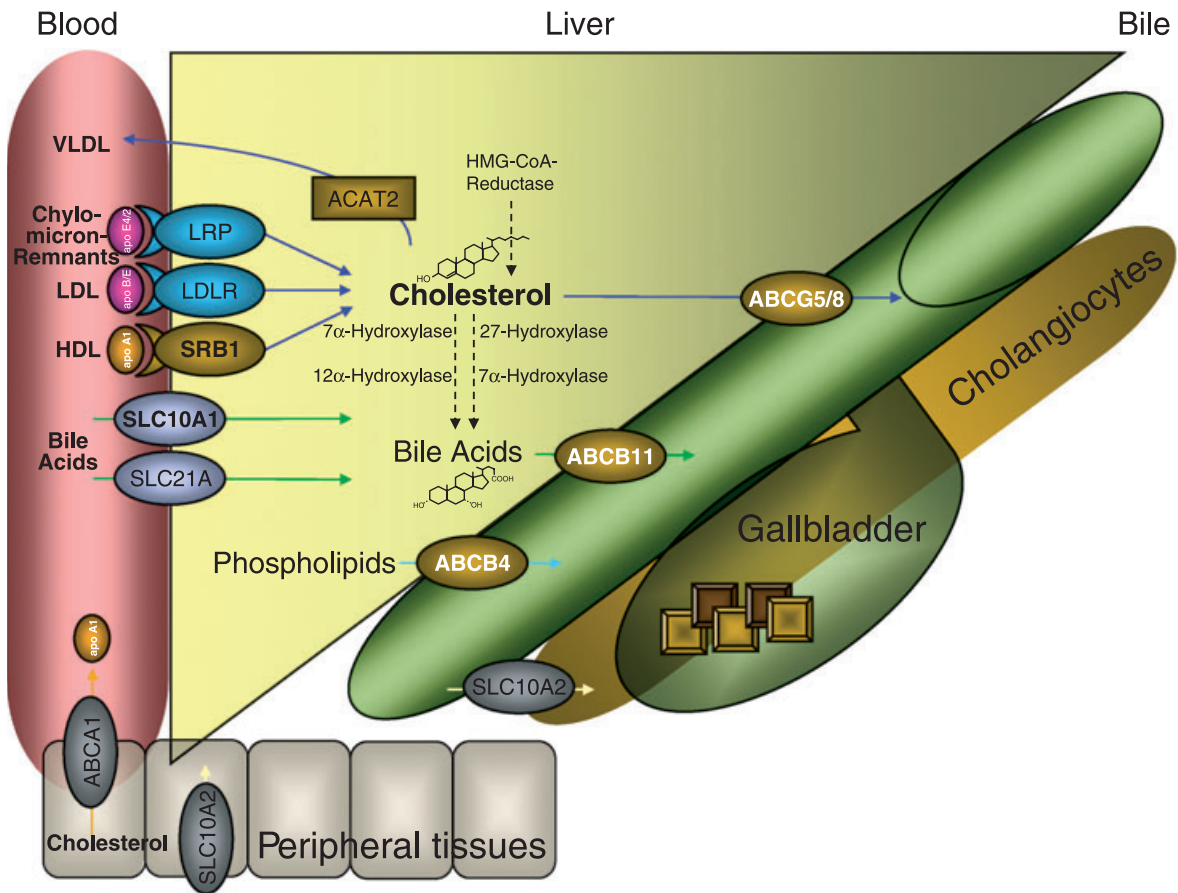


Fig. 1 Major components of cholesterol metabolism and bile formation. Intestinal cholesterol is transferred by the ABC-transporter ABCA1 to apoA1 particles that are taken up by the liver by high-density lipoprotein (HDL) receptor SRB1. Minor amounts of cholesterol derive from low-density lipoprotein (LDL) and chylomicron remnants and are taken up by LDL receptor (LDLR) and LDL-receptor-related protein (LRP). Hepatic de novo synthesis of cholesterol is under the control of hydroxy-methyl-glutaryl-(HMG-) CoA-reductase. Part of cholesterol is esterified by acyl CoA: cholesterol acyltransferase (ACAT) and secreted as very low-density lipoprotein (VLDL) cholesterol or stored in the liver as cholesterol esters. Cholesterol may be metabolized into bile acids in the classical, neutral pathway via 7α - and 12α -hydroxylase (CYP7A1 and CYP8B1) reactions or in smaller amounts via the alternative, acidic pathway via an initial 27-hydroxylase (CYP27A1) reaction. The key regulatory enzyme in bile acid synthesis is CYP7A1. Cholesterol and bile salts are excreted from the liver via ABCG5/8 and ABCB11 (bile salt export pump BSEP), respectively. The phospholipid flippase ABCB4 (MDR3) excretes phospholipids. Bile salts are mainly taken up by the liver via the sodium-dependent taurocholate transporter (NTCP) SLC10A1, and by organic anion transport proteins (OATPs) SLC21. The apical/ileal sodium-dependent bile salt transporter (ASBT/ISBT) SLC10A2 is expressed both in cholangiocytes and the intestine. Uptake, metabolism and excretion of cholesterol and bile acids are closely regulated to each other via stimulation or suppression of nuclear receptors.

LDL-receptor-related protein (LRP) [68] transfers cholesterol from chylomicron remnants that carry exogenous cholesterol from the intestine. Recently, also in the intestine transporters have been identified that are able to transfer cholesterol, the ABC transporter ABCA1 that is defective in Tangier disease [69] and the Niemann-Pick C1-like protein 1 (NPC1L1) [70]. The role of the

intestine in cholesterol absorption and its regulation has recently been reviewed by Lammert and Wang [71].

Transport systems for bile acids at the basolateral site of hepatocytes, i.e. the sodium-dependent taurocholate transport protein NTCP (SLC10A1), organic anion transport proteins OATPs (SLC21A), and in

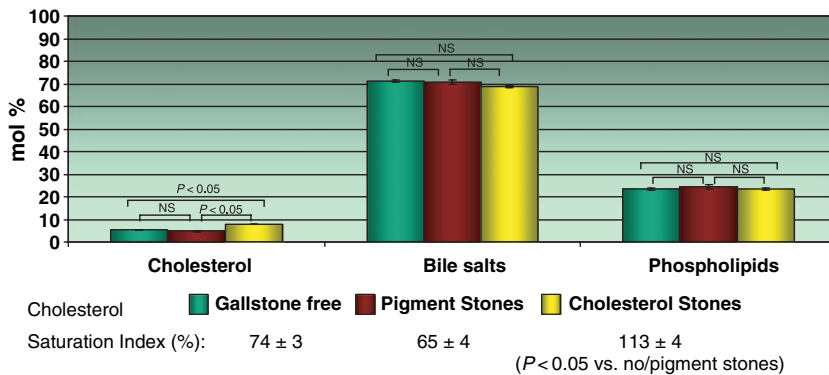


Fig. 2 Gallbladder bile composition of patients with cholesterol stones ($n = 145$) compared to pigment-stone ($n = 23$) and gallstone-free ($n = 87$) patients. Cholesterol stone patients have significantly higher cholesterol saturation index (CSI). Data from [99].

cholangiocytes, intestine and kidney, i.e., ASBT/ISBT, the ileal/apical sodium-dependent bile salt transporter (SLC10A2), MDR1 (ABCB1) and MRP2, 3 (ABCC2, 3), have recently been reviewed by Trauner and Boyer [72].

Pathophysiology

The majority (80–90%) of gallstones formed within the gallbladder consist mainly of cholesterol (70%) in a matrix of bile pigments, calcium salts and glycoproteins [73] (Fig. 2). Besides pure and mixed cholesterol stones, also pure pigment stones are found. Brown pigment stones are associated with infections of the biliary tract (bacterial and helminthic deconjugation of bilirubin glucuronides) and are more frequent in Asia. Black pigment stones mainly consist of calcium bilirubinate and are found in haemolytic anaemia or ineffective haematopoiesis and in patients with cystic fibrosis [74]. Increased enterohepatic cycling of bilirubin is the suggested cause of black pigment stones [75], also in patients with ileal dysfunction consistent with the finding of high levels of bilirubin in bile in patients with active ileal Crohn's disease or after ileal resection [76–79]. However, in these patients with bile salt malabsorption, other factors leading to biliary cholesterol supersaturation [78, 80] may preferably promote cholesterol gallstones.

For the formation of cholesterol gall bladder stones, three mechanisms are of major importance: (i) cholesterol supersaturation of bile; (ii) gallbladder hypomotility; and (iii) kinetic, pro-nucleating protein factors (Fig. 3).

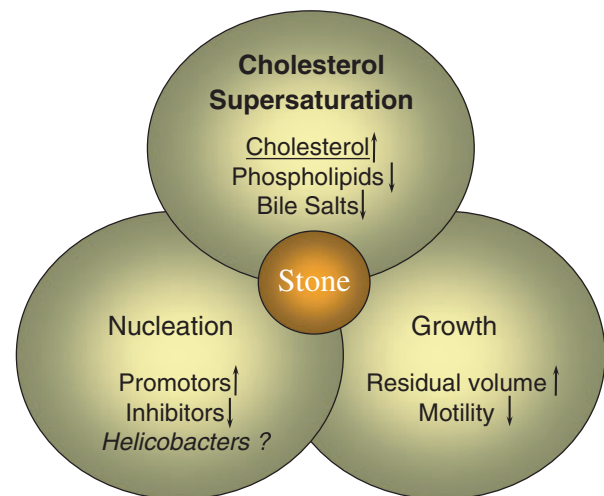


Fig. 3 Pathophysiology of cholesterol gallstone formation. Cholesterol crystals aggregate in bile supersaturated with cholesterol, nucleated in the presence of pro-nucleating factors such as mucin, and grow to stones in an enlarged gallbladder with hypomotility.

Cholesterol supersaturation

Cholesterol is only slightly soluble in aqueous media but is made soluble in bile through mixed micelles with bile salts and phospholipids, mainly phosphatidylcholine (lecithin) [81] (Fig. 4). Precipitation of cholesterol occurs when cholesterol solubility is exceeded (cholesterol saturation index >1). Ternary phase diagrams showing molar bile salt-cholesterol-phospholipid percentages [82–85] demonstrate that cholesterol crystals occur at low phospholipid : cholesterol ratios and at relative low phospholipid and high bile salt concentrations. Multilamellar vesicles

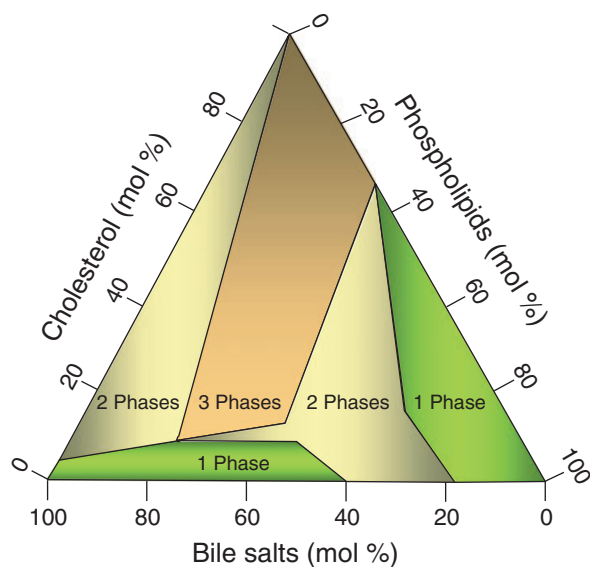


Fig. 4 Ternary phase diagram of major gallbladder lipids. Mixed micelles are found in Phase 1 mixtures of cholesterol, phospholipids and bile salts. Higher amounts of cholesterol or lower amounts of phospholipids and/or bile salts yield metastable (Phase 2 and 3) compositions, characterized by unilamellar and multilamellar vesicles that may give rise to cholesterol crystal.

then fuse and may aggregate as solid crystals. Thus, supersaturation of cholesterol in bile may be caused by hypersecretion of cholesterol, or from hyposecretion of bile salts or phospholipids.

The main cause of cholesterol supersaturation is hypersecretion of cholesterol [86] that in accordance with the epidemiology of gallstone disease increases with age [87]. Hypersecretion may be due to abnormalities in hepatic cholesterol metabolism, i.e. increased hepatic uptake, increased *de novo* synthesis and/or decreased conversion towards bile acids or cholesterol esters.

De novo synthesized cholesterol contributes only about 10% of biliary cholesterol [88]. The major part, more than 80% is of dietary origin [89, 90]. However, the effect on biliary cholesterol secretion was found to be different depending on the prevalence of gallstones. Only in gallstone subjects, cholesterol secretion increased [89].

In a number of studies in healthy and obese patients, with and without gallstones, measurements of the three key enzymes in hepatic cholesterol metabolism, i.e. *de novo* synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase; bile acid synthesis, cholesterol 7 α -hydroxylase (CYP7A1); and for formation of cholesteryl esters, acyl-coenzyme A: cholesterol acyltransferase (ACAT); did not identify a single metabolic defect for biliary cholesterol hypersecretion [91–94]. A recent study compared plasma levels for 7 α -hydroxy-4-cholesten-3-one and lathosterol, two strong indicators for hepatic bile acid and body cholesterol synthesis, in Chilean Hispanics and in Mapuche Indians [95]. These markers were significantly elevated in the Indian high-risk gallstone population. Whether this constellation is due to a primary defect or increased intestinal loss of bile salts is unknown [95].

Another factor often associated with cholesterol supersaturation in bile is excessive deoxycholic acid (DCA) in the bile acid pool that may be the result of prolonged intestinal transit [96, 97] and/or increased bacterial 7 α -dehydroxylation activity [98]. However, the concept that DCA contributes to cholesterol gallstone formation has been questioned [99, 100]. Nevertheless, it points to motility factors in the pathogenesis of gallstone disease.

Gallbladder hypomotility

As supersaturated bile often is found in healthy individuals [101], it is assumed that microcrystals formed are effectively flushed from the gallbladder during postprandial contractions. In cholesterol gallstone patients, altered interdigestive gallbladder emptying was observed [102], and patients with incomplete gallbladder emptying were found to have increased total lipid concentrations [103]. Impaired gallbladder motility is commonly seen in several risk groups for cholesterol gallstones, e.g. patients with diabetes mellitus, total parenteral nutrition (TPN), rapid weight loss (reviewed in [104]). On the other hand, once gallstones have formed, the risk for developing symptomatic gallstones disease seems to be higher for those patients who have efficient gallbladder emptying (>70% emptying after a test

meal) compared to those with sluggish motility (<55% emptying after a test meal, as estimated by ultrasonography) [105].

Treatment of acromegalic patients with octreotide, a long-acting somatostatin-analogue, impairs the postprandial release of duodenal cholecystokinin (CCK) that is the principal stimulus for gallbladder contraction. Thus, the risk for gallstones is substantially increased in these patients [106] that might be further increased by higher levels of DCA by impaired intestinal motility [107, 108]. For the prevention of gallstone development by gallbladder dysmotility, CCK injections have been recommended in patients receiving long-term TPN [109], and small fat containing meals during weight reducing diets [110]. Recent study in mice showed that fibroblast growth factor 15 (FGF-15; human homologue, FGF-19), a hormone made by the distal small intestine in response to bile acids suppressing *Cyp7a1* in the liver [111] as a counter player of CCK also controls gallbladder filling [112]. However, the importance of FGF-19 for gallbladder emptying and gallstone development in humans remains to be shown.

Kinetic factors

The formation of microcrystals in supersaturated bile is modulated by kinetic protein factors. From *in vitro* studies that used model bile systems, a number of inhibitory or promoting proteins have been described. However, only mucin, a glycoprotein mixture that is secreted by biliary epithelial cells, has consistently been defined as crystallization promoting protein in gallbladder sludge [113–115]. Although postulated from experiments with human bile [116], the role of inhibitory proteins [117] such as biliary secretory immunoglobulin A [118] remains elusive. Of note, cholesterol saturation and the amount of mucin [119] or total proteins [120] are not correlated to each other. Rather, a decreased degradation of mucin by lysosomal enzymes might promote cholesterol crystal and gallstone formation [121, 122].

Intestinal helicobacter

Recently, intestinal bacteria were found to promote cholesterol crystallization in a murine model of

gallstone formation. A variety of cholelithogenic enterohepatic *Helicobacter* species were identified [123], *H. pylori* infections, however, was not related to gallstone formation in susceptible animals [124]. In 22 out of 46 Chilean patients with chronic cholecystitis, hepatic *Helicobacter* species' mRNA [125], and in 25 out of 33 gallstones of Swedish patients *Helicobacter* DNA [126] could be identified. However, in humans, *Helicobacter* infection alone may not play a significant role in the formation of gallstones as the prevalence of *Helicobacter* species' DNA was the same in the gallbladder of patients with gallstone diseases and in controls [127] and data on the prevalence in patients with other hepatobiliary diseases are conflicting [128, 129].

Genetic factors

Gallstone disease (GD) is likely to result from a complex interaction of environmental factors and the effects of multiple undetermined genes [130]. Genetic factors that affect susceptibility to gallstone formation and gallbladder disease in humans are suggested by family studies [131–137] that identified gallstones mainly by using ultrasonography in first-degree relatives of patients with cholelithiasis two to four times more than in stone-free controls. In particular the high prevalence of cholesterol gallstone disease among American Indians and Hispanics indicates the prevalence of lithogenic genes [138, 139].

In 1999, Duggirala *et al.* [134] used pedigree data to explore the genetic susceptibility to symptomatic gallstone in a Mexican-American population of 32 families and estimated a heritability, i.e. the proportion of the phenotypic variance of the trait that is due to genetic effects, of $44 \pm 18\%$. However, this association study did not control for shared environmental effects [134]. A recent family study from the US performed a variance component analysis in 1038 individuals taken from 358 families and calculated the heritability of symptomatic GD to be $29 \pm 14\%$ [135].

Twin studies have been a valuable source of information on the genetic epidemiology of complex traits. They can be used to dissect complex genetics by

analysing the interaction of genotypes and phenotypes with gender, age, and lifestyle factors. In contrast to family studies, comparisons of the concordance rates of symptomatic GD between monozygotic (MZ) and dizygotic (DZ) pairs of twins provide information on whether the familial pattern is due to hereditary or environmental factors. A study in 35 Finnish twins pairs found pairwise correlations within the monozygotic but not the dizygotic pairs for biliary cholic and deoxycholic acids and serum precursor sterols, indicating the genetic determination of biliary lipid composition [140]. A recent large study in Swedish twins estimated the contribution of genetic factors to the development of symptomatic gallstone disease [141]. The Swedish Twin Registry was linked with the Swedish Inpatient and Causes of Death Registries for symptomatic gallstone disease and gallstone surgery-related diagnoses in 43 141 twin pairs born between 1900 and 1958. Concordance rates were significantly higher in monozygotic (12%) compared with dizygotic (6%) twins, both for males and females. The low concordance rates in monozygotic twins indicated the importance of environmental factors. Structural equation modelling was used to estimate the contributions of genetic as well as shared (e.g. diet in childhood, biliary infections) and nonshared environmental factor effects. Genetic effects accounted for 25% (95% CI 9–40%), shared environmental effects for 13% (95% CI 1–25%), and unique environmental effects for 62% (95% CI 56–68%) of the phenotypic variance among twins [141].

Due to its multifactorial pathogenesis, in humans gene abnormalities that are responsible for the development of cholesterol gallstone disease are difficult to identify. As yet, only in specific subgroups of patients monogenic predisposition for cholelithiasis has been ascribed to mutations in the genes that encode for CYP7A1 [142] or the phospholipid-flippase ABCB4 [44, 143]. Rosmorduc *et al.* found point mutations in *ABCB4* in 18 out of 32 patients in 'low phospholipid-associated cholelithiasis' or LPAC [143], a syndrome characterised by cholesterol gallstone disease before the age of 40 with both gallbladder stones, intrahepatic sludge and microlithiasis, and recurrent biliary symptoms after cholecystectomy. The responsible

defect, low biliary phospholipid secretion, was confirmed in *Mdr2*^{-/-} mice [144] that otherwise serve as a model system for human primary sclerosing cholangitis [145, 146].

During the last years, other candidate genes for cholesterol gallstone disease have been identified in studies in inbred mouse strains that differ in the susceptibility for cholesterol gallstone formation when fed a lithogenic diet containing 15% fat, 1% cholesterol and 0.5% cholic acid [147]. Using the quantitative trait loci analysis [148, 149], a murine gallstone map was developed describing the chromosomal organization of candidate gene loci [150]. Twenty-three candidate *lith* genes have been identified that are closely related to the regulation of synthesis, uptake and excretion of hepatobiliary lipids and proteins, e.g. genes that encode for sterol carrier protein, ABC transporters, nuclear receptors, mucin [151–158]. Likely candidate genes are *lith 1* (*Abcb11*), *lith 2* (*Abcc2*), *lith 7* (*Nr1h4*), *lith 9* (*Abcg5* and *Abcg8*), and *lith 13* (*Cckar*) [158]. In addition, genes that encode for immune-related factors, e.g. *Il4* have been postulated as *lith* genes [158, 159].

In humans, gene variants that are associated with cholesterol gallstone disease still have to be defined. Polymorphisms of genes encoding cholesterol transporting and metabolizing proteins Apolipoprotein B and E (*APOB*, *APOE*), cholesteryl ester transport protein (*CETP*), and *CYP7A1* were found to be associated with cholesterol gallstone disease, with striking ethnic differences (compiled in [160]), e.g. between Asian (China, Japan) and European populations (Finland, the Netherlands). A recent genomewide search found major susceptibility loci for gallbladder disease on chromosome 1 in Mexican Americans [161].

Diagnosis

Gallstone-associated symptoms are nonspecific. The symptom most closely associated with gallstone disease is 1–24 h lasting abdominal pain with radiation to the upper back. Onset more than an hour after meals support this diagnosis [162]. Gallstone disease is confirmed by ultrasonography in the fasting state

giving the correct diagnosis in >90% of cases [163]; however, bile duct stones may be missed by ultrasonography in up to 50% of cases. These are with similar sensitivity and specificity diagnosed by magnetic resonance cholangio-pancreaticography (MRCP, non-invasive) and by endoscopic ultrasound (EUS) or endoscopic retrograde cholangio-pancreaticography (ERCP). The latter procedure bears a substantial procedural risk but offers therapeutic options.

Therapy

Today, the treatment of choice of symptomatic gallstone disease is laparoscopic cholecystectomy [164, 165]. Mortality, complications and operative time do not differ between laparoscopic and open cholecystectomy; however, laparoscopic cholecystectomy is associated with a significantly shorter hospital stay and a quicker convalescence compared with the classical open cholecystectomy [166]. Small incision cholecystectomy may serve as a surgical alternative for laparoscopic cholecystectomy [167, 168]. Oral bile acid litholysis with chenodeoxycholic acid [169] and/or ursodeoxycholic acid (UDCA) [170, 171] have a very high recurrence rate [172] and therefore are an option for a very small group of cholesterol gallstones patients only. However, intrahepatic cholelithiasis in patients with MDR3 aberrations may profit from treatment with UDCA [143]. Extracorporeal shock-wave lithotripsy, combined with oral bile acid treatment [173], was launched some 20 years ago but was mostly abandoned because of the high risk of stone recurrence [174]. Contact stone dissolution with methyl tert-butyl ether [175] had never got entrance into clinical practice [176].

Complications

Complications of gallstone disease are inflammations of the gallbladder (cholecystitis), the biliary tract (cholangitis), and the pancreas (biliary pancreatitis). Persistent pain, fever, and jaundice indicating acute cholangitis are known as Charcot's Triad. Cholecystitis/cholangitis are treated with biliary secreted antibiotics; however, early laparoscopic cholecystectomy in acute cholecystitis shortens hospital stay without

increasing complication rates [177]. Bile duct stones are removed endoscopically (ERCP with endoscopic papillotomy, EPT) [178]. Gallbladder cancer is rare but closely related to gallbladder stones, nevertheless for cancer prevention, prophylactic cholecystectomy is not recommended [179]. However, empiric laparoscopic cholecystectomy seems to be a reasonable option for idiopathic pancreatitis [180] for which occult gallstone disease or biliary sludge [181] seem to be a frequent cause.

Prevention

Cholesterol gallstone disease may be prevented by life-style changes, in particular by reducing total caloric intake [182], but controlled studies are missing. Oral UDCA during weight-loss prevented cholesterol gallstone formation in man [183, 184], in contrast to Aspirin [183] that was previously found to be effective in the prairie dog [185]. An exciting new concept in the prevention of gallstone formation is the stimulation of nuclear receptors regulation cholesterol metabolism and secretion [186], as shown by the efficient prevention with synthetic FXR agonists in mice [187].

Conflict of interest statement

No conflict of interest was declared.

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