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Review Article

Genetics of Gallstone Disease and Their Clinical Significance: A Narrative Review

Christopher J. Costa[1*], Minh Thu T. Nguyen[2], Haleh Vaziri[2] and George Y. Wu[2]

[1]Department of Medicine, University of Connecticut Health Center, Farmington, CT, USA; [2]Division of Gastroenterology and Hepatology, University of Connecticut Health Center, Farmington, CT, USA

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Abstract

Gallstone (GS) disease is common and arises from a combination of genetic and environmental factors. Although genetic abnormalities specifically leading to cholesterol GSs are rare, there are clinically significant gene variants associated with cholesterol GSs. In contrast, most bilirubin GSs can be attributed to genetic defects. The pathogenesis of cholesterol and bilirubin GSs differs greatly. Cholesterol GSs are notably influenced by genetic variants within the ABC protein superfamily, including ABCG8, ABCG5, ABCB4, and ABCB11, as well as genes from the apolipoprotein family such as ApoB100 and ApoE (especially the E3/E3 and E3/E4 variants), and members of the MUC family. Conversely, bilirubin GSs are associated with genetic variants in highly expressed hepatic genes, notably UGT1A1, ABCC2 (MRP2), ABCC3 (MRP3), CFTR, and MUC, alongside genetic defects linked to hemolytic anemias and conditions impacting erythropoiesis. While genetic cases constitute a small portion of GS disease, recognizing genetic predisposition is essential for proper diagnosis, treatment, and genetic counseling.

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Introduction

Around 10–20% of the global adult population develop gallstones (GSs) in their lifetime, and 20% of these individuals eventually experience symptoms related to GSs.[1] Symptoms and complications from GS disease represent a leading cause for healthcare utilization and hospitalization. Hence, recognizing the underlying risk factors is imperative. The formation of GSs is a multifactorial process influenced by both environmental and genetic factors. For many decades, a genetic inclination towards GS formation was observed through epidemiological and family studies. Recent advances in genetic analysis and human genomic data have allowed for the identification of specific genetic variants; however, exact prevalence data is still unavailable given the rarity of these conditions. This review aims to discuss the known genetic causes of GS disease, including hepatic, biliary, and hematopoietic abnormalities.

Pathophysiology of GS Diseases

Approximately 90% of GSs are primarily cholesterol-based while the remaining stones are composed primarily of bilirubin or a mixture of cholesterol and bilirubin.[2] Cholesterol stones typically form due to a combination of genetic and environmental factors rather than specific genetic mutations.[1–5] In contrast, most bilirubin stones result from specific genetic defects.[1–8] The pathogenesis of cholesterol and bilirubin GSs differs substantially but shares a common principle: a hypersaturated solution leading to the precipitation of stones. Bile is composed of bile salts, phospholipids, cholesterol, conjugated bilirubin, electrolytes, and water. GS formation occurs when there is a reduction in the solubility of one or more components of bile leading to the precipitation and aggregation of insoluble components until they grow large enough to occlude biliary ducts.

The genetic predisposition to GS disease was first recognized in a series of families with heightened susceptibility. While this case series underscored the heritability of GS, potential genetic targets were not identified until later. Researchers successfully induced GSs in mice by exposing them to a diet rich in cholesterol and cholic acid, leading to stone formation in 75% of the animals after 8 months.[9] Khanuja *et al*. pinpointed the *Lith1* gene in an inbred C57B6 mouse strain which was mapped to chromosome 2. This discovery was distinct from the previously proposed target of β-Hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase and its regulatory elements.[10] Various genetic loci were identified, and eventually, mapping of quantitative trait loci, tangible evidence for the quantifiable associations between GS disease and ethnicity at the population level.[5]

Keywords: Gallstones; Cholelithiasis; ATP-binding cassette transporters; ABCG8 protein; Human; UDP-glucuronosyltransferase A1.

Abbreviations: ABC, ATP-binding cassette; APO, apolipoprotein; ATP, adenosine triphosphate; BMI, body mass index; CCK, cholecystokinin; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane receptor; CI, confidence interval; DNA, deoxyribonucleic acid; ERM, ezrin-radixin-moesin; GS, gallstone; GWAS, genome-wide association study; HBV, hepatitis B virus; HDL, high-density lipoprotein; HMG-CoA, β-hydroxy β-methylglutaryl-CoA; KO, knockout; LDL, low-density lipoprotein; LPAC, low phospholipid-associated cholelithiasis; MDR, multidrug resistance; miRNA, micro ribonucleic acid; mRNA, messenger ribonucleic acid; NHERF1, $Na^+/H+$ exchanger regulatory factor 1; OR, odds ratio; RBCs, red blood cells; SNPs, single nucleotide polymorphisms; T2DM, type 2 diabetes mellitus; UDCA, ursodeoxycholic acid; UGT, uridine 5′-diphospho-glucuronosyltransferase.

[*]**Correspondence to:** Christopher J. Costa, Department of Medicine, University of Connecticut Health Center, 263 Farmington Ave, Farmington, CT 06030, USA. ORCID: https://orcid.org/0000-0002-2648-0757. Tel: +1-941-250-4330, Fax: +1-860-679-6582, E-mail: chrcosta@uchc.edu

Cholesterol GSs

Cholesterol GSs arise from bile supersaturated with cholesterol. Daily required cholesterol is derived from dietary sources and absorbed by intestinal epithelial cells while the liver also synthesizes cholesterol *de novo*. Dietary cholesterol enters the intestinal tract with triglycerides undergoing hydrolysis by pancreatic lipase to form fatty acids and monoacylglycerols. Cholesterol exhibits limited solubility in bile. The distinctive amphipathic characteristics of bile acids enable them to effectively solubilize both cholesterol and phospholipids into small micelles. Enzymatic processes within intestinal mucosal cells then recombine fatty acids and monoacylglycerols into triglycerides, alongside cholesterol into large vesicles called chylomicrons. Cholesterol is subsequently transported to other tissues through the circulatory and lymphatic systems. Liver-produced apolipoproteins enclose hydrophobic cholesterol into chylomicrons, reducing its interaction with water molecules and facilitating transportation. Furthermore, apolipoproteins actively participate in the uptake and clearance of cholesterol by interacting with membrane receptors and other lipid transport proteins.[1]

Two clinically significant apolipoproteins are ApoB100, a primary constituent of chylomicrons, and ApoE, found in lipid vesicles, high-density lipoproteins (HDL), and low-density lipoproteins (LDL). Variants in protein-coding genes for ApoB100 and ApoE are linked to an elevated predisposition for GSs, as mutations result in less effective binding to lipoprotein receptors and an increase in serum cholesterol. Elevated levels of circulating cholesterol are a well-established risk factor for cholelithiasis.[4,6] Following hepatic uptake, cholesterol from chylomicron remnants serves as a substrate for bile acid synthesis and is subsequently secreted into bile in the forms of bile salt and unesterified cholesterol. Cholesterol GSs form when the cholesterol concentration in bile exceeds the maximum solubility threshold at the given levels of bile salts and phospholipids, referred to as the cholesterol saturation index.

Another important component in cholesterol homeostasis is the family of adenosine triphosphate (ATP)-binding cassette (ABC) transporters. ABC transporters are multi-faceted transmembrane proteins involved in transcellular transportation, intracellular signaling, transcriptional regulation, and a myriad of other processes.[11] These transporters regulate the excretion of cholesterol and are expressed in hepatocytes, enterocytes, and gallbladder epithelial cells. The proper functioning of ABC transporters, such as ABCG8/ABCG5, ABCB4, and ABCB11, enables the passage of cholesterol, bile acids, and other lipids into bile. Therefore, any changes in their protein expression or activity may lead to a modification in bile composition, potentially causing an elevation in cholesterol levels and a reduction in bile acids, consequently triggering stone formation due to decreased cholesterol solubilization. These small crystals often act as a nidus, facilitating the accumulation of additional cholesterol, ultimately giving rise to GSs.[2,3]

Cholesterol GSs can also form through the precipitation and nucleation of excess cholesterol from biliary micelles, a process influenced by mucins—large glycoproteins produced by goblet cells in mucous glands. These glycoproteins, originating from MUC genes, contribute to the viscoelastic protective property of mucus through polymer formation.[12] Factors like water content and salt concentrations affect mucin solubility. Adequate hydration is needed, as higher water content ensures mucins are hydrated enough to form gels. Imbalances in salt concentration, involving sodium and potassium ions, can disrupt the consistency of mucus.[13] Gallbladder secretory mucins protect against bile's detergent-like effects and may contribute to cholesterol GSs formation.[14] Mucin serves as a lithogenic agent by fostering a favorable environment for the nucleation of cholesterol monohydrate from supersaturated bile through the hydrophobic binding sites within its polypeptide core.[14] This hypothesis is supported by observations that nucleation occurs after the fusion of cholesterol and lipid-enriched vesicles from bile. Physiological concentrations of mucin can induce nearly complete fusion within hours. Prominent mucin hypersecretion is associated with cholesterol GSs. Lysolecithin and polysaturated free fatty acids stimulate gallbladder mucin synthesis and secretion through the prostanoid pathway.[15]

Genetic causes of cholesterol GSs

ABCG8/ABCG5

Three key ABC proteins, ABCG5/ABCG8, ABCB4, and ABCB11, have been shown to be involved in GS disease due to mutations (Fig. 1A). ABCG5/ABCG8 is a heterodimer protein that serves as a cholesterol transporter within the bile canalicular network directly influencing the cholesterol content of bile.[16,17] It is also expressed to a large degree on the apical membranes of small intestine enterocytes, an additional mechanism by which it impacts the overall cholesterol status. Of significance is a specific mutation: a nonsynonymous alteration within the ABCG8 gene where histidine replaces aspartic acid at position 19 (ABCG8 D19H). This gain-of-function mutation leads to an elevated concentration of cholesterol in bile.[18,19] It is estimated that approximately 12% of Europeans carry this risk variant. While substantial data centers around this variant, another alteration involving the same protein exists: ABCG5 R50C. This mutation where cysteine substitutes for arginine at position 50, results in increased transport activity and decreased cholesterol absorption.[2–4,8,20] The cumulative outcome of these mutations is amplified lithogenicity of bile, increasing the risk of GS formation.

Several investigations conducted at the population level have suggested the role of mutated ABCG5/ABCG8 in the premature onset of GSs while also highlighting the prevalence of these mutations within different ethnic groups. A recent meta-analysis of European studies suggested that the presence of this variant confers an increased risk for GS with an odds ratio of 1.78 (95% confidence interval [CI]: 1.70–1.86).[8] These mutations have been found closely linked to metabolic risk factors such as aberrations in lipid profiles, elevated body mass index (BMI), and the presence of coronary artery disease.[3,4,18,21]

Among Latin Americans, particularly those of Mapuche descent in Chile, the higher prevalence of GS disease and subsequent complications like gallbladder cancer has been attributed to the presence of the ABCG8 D19H (rs1887534) risk variant. Incidence rates are estimated to be 50% in women and 12% in men. To evaluate regional and population-specific variations, Bustos *et al*. conducted a genome-wide association study (GWAS) in over a thousand Chilean individuals. They identified 10 clinically significant variants of which only ABCG8 D19H had been reported previously, revealing an extensive array of genetic risk among individuals of Chilean Mapuche heritage. This study has limitations including the arbitrary selection of a German population as the control group and a larger proportion of patients with type 2 diabetes mellitus (T2DM) among Chilean GS cases compared to controls.[19]

In Asian populations, Liang *et al*. undertook a GWAS aimed at exploring the link between GS and the rs11887534 (D19H) variant using participants from the Taiwanese biobank. The analysis was stratified by gender, hormone exposure (including the use of combined hormonal birth control), and other demographic factors. Of the nearly 22,000 patients, more

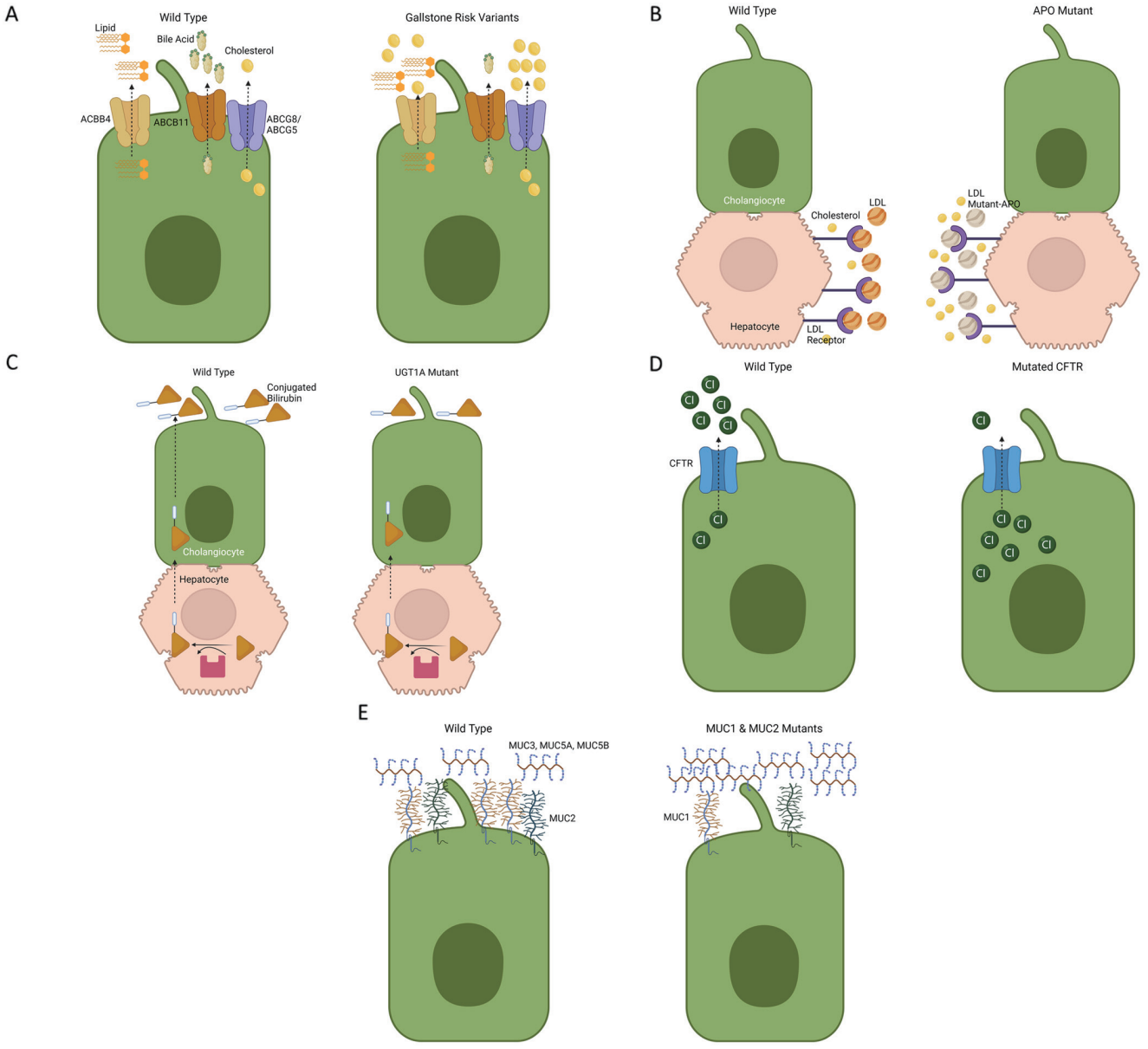

Fig. 1. Protein products of genes linked to GS disease and their functions in physiology and pathophysiology. (A) ABC transporters: The normal functioning of ABCG8/ABCG5, ABCB4, and ABCB11 enables the transport of cholesterol, bile acids, and lipids into bile (left). Mutations in any of these result in increased cholesterol and lipids, while decreasing bile acids in bile, thus precipitating stone formation (right). (B) APO: Apolipoproteins function to stabilize cholesterol-containing vesicles (left). Mutations in apolipoproteins result in less effective binding to their receptors and increased serum cholesterol (right). (C) UGT1A1: uridine 5′-diphospho-glucuronosyltransferase 1A1 conjugates bilirubin through glucuronidation, thereby increasing its solubility in aqueous bile (left). Pathologic mutations result in reduced conjugated bilirubin in bile, leading to an increase in stone formation (right). (D) CFTR: Cystic fibrosis transmembrane conductance regulator facilitates the transport of chloride ions across various cell membranes along with water (left). Mutated CFTR reduces the transport of chloride ions, leading to decreased water content and predisposing to stone formation (right). (E) Mucin mutations: MUC1 and MUC2, transmembrane mucins, regulate the secretion of mucins such as MUC3 and MUC5B (left). Mutations in MUC1 and MUC2, resulting in decreased expression, lead to increased intraluminal mucins (MUC3, MUC5A, MUC5B), precipitating GS formation (right). ABC, ATP-binding cassette; APO, apolipoproteins; UGT1A1, uridine 5′-diphospho-glucuronosyltransferase 1A1; CFTR, cystic fibrosis transmembrane conductance regulator. The figure was created using BioRender.com.

than half (approximately 14,000) were female, with only 3% being heterozygous or homozygous for the risk variant. The study showed that individuals carrying the risk variant, whether homozygous or heterozygous, had an increased risk for GSs. This risk was more pronounced among female participants. This study had a large sample size with well-documented baseline patient characteristics, a controlled geographic location to minimize environmental variables, and stratification by hormone usage—a known independent risk factor.[1,3,22] However, the study relied on self-reported GS carrier status which introduces potential inaccuracies, in addition to having baseline differences in lipid panel readings between male and female participants.[23]

A similar analysis conducted by Teng *et al*. in the Taiwanese population confirmed rs11887534 (D19H) as the most prevalent and strongly linked variant to GS disease. They

also discovered two other risk variants: rs6756629 and rs56132765.[20] When exploring potential connections between these variants and other conditions that could predispose to GSs, such as dyslipidemia and T2DM, no significant associations were found. This suggests that the identified risk variants within ABCG8/ABCG5 increase the risk for GSs independently.[20] However, further analysis will need to be conducted to determine whether these associations hold true on a larger global scale given that both above studies were conducted in small and homogenous populations.

ABCB4

The ABCG8/ABCG5 protein and its mutations exert the greatest impact on the genetic predisposition to GS disease in this group, but other members of this protein family also pose a risk. The ABCB4 gene and its protein product known as multidrug resistance (MDR) protein 3 translocate lipids across the cell membrane and into bile by floppase activity. This protein has been implicated in the genetic risk of GS disease through mechanisms previously discussed for ABCG8/ABCG5, namely the generation of cholesterol supersaturated bile. It is associated with low phospholipid-associated cholelithiasis (LPAC), progressive familial intrahepatic cholelithiasis type 3 (initially recognized in Amish patients in 1960 and referred to as Byler's syndrome at that time), intrahepatic cholestasis of pregnancy, and primary biliary cholangitis.[24–26]

Metabolic shifts may amplify the impact of the mutant gene, as demonstrated by a recent animal study revealing that thyroid hormone signaling can potentiate the effects of mutant variants.[27] Studies have shown that mice with a double knockout (KO) of this gene experienced fatal hepatic lipid accumulation. Gene therapy employing human ABCB4 messenger ribonucleic acid (mRNA) has the potential to prevent this outcome, indicating a causal role and a potential therapeutic avenue for human patients.[5,28,29] The identification of LPAC in humans has become a pivotal focus in comprehending the pathogenesis of GS disease.[28]

Diagnostic criteria for LPAC include the presence of cholesterol GSs, familial clustering, an early age of onset often before the age of 40, intrahepatic cholelithiasis, and disease recurrence despite cholecystectomy.[28] In a recent study conducted in France, 60 patients with established LPAC and ABCB4 variants were compared to 65 matched controls who required magnetic resonance imaging of the liver at a tertiary center. The study found that patients with mutant ABCB4 were at an increased risk for intrahepatic cholelithiasis, biliary duct dilatation, and signs of complications (*e.g.,* liver parenchyma heterogeneity, biliary ductal stenosis, abscess, and contrast enhancement of bile duct walls). Interestingly, these differences did not have a significant impact on the natural progression of the disease or the course of radiological features during the follow-up period, an average of 54 months.[30]

In a prospective study conducted in France, the significance of LPAC detection among patients with recurrent biliary symptoms and pancreatitis was evaluated. Data, including blood markers, use of ursodeoxycholic acid (UDCA), and recurrence rates, were obtained in patients who presented with acute pancreatitis with GSs on ultrasound imaging. The data highlighted a tendency for the cardinal symptoms to manifest in young male patients with a normal BMI range. This study demonstrated that LPAC is easily diagnosable and treatable. Early detection via ultrasonography and treatment with UDCA reduced symptom severity. An important limitation, however, lies in the non-uniform genetic analysis, which was conducted only on 7 out of the 24 patients included. This analysis identified mutations in previously implicated loci but did not uncover any within ABCB4.[31]

ABCB11

ABCB11 (bile salt exporter pump) is another ABC protein belonging to the MDR family sharing a large degree of homology with MDR1. It was identified after examining the mouse *Lith1* gene and its human correlates.[32] This protein is implicated in familial intrahepatic cholestasis. Its expression is predominantly localized to the bile canalicular membrane and demonstrates specificity for bile acids. ABCB11 plays an important role in governing the transfer of bile components across the membrane, with mechanisms not yet fully delineated. However, a higher quantity of cholesterol is secreted per bile acid molecule when bile acid concentrations are low.[32]

In a German study that specifically investigated *Lith1* and the distribution of its single nucleotide polymorphisms (SNPs) among over 800 patients who underwent cholecystectomy for biliary colic, none of the ABCB11 SNPs examined reached a significant level of risk in this population when comparing GS cases and controls.[33] However, a different study identified a notable association between the ABCB11 rs2287622 risk variant and intrahepatic cholelithiasis.[34]

Conversely, in patients of Asian ethnicity, two risk variants are implicated in worsening the clinical course: rs118109635 and rs497692, representing a missense mutation and a synonymous mutation, respectively. An analysis of Chinese patients with primary intrahepatic stones revealed a significant discrepancy in the occurrence of these variants between cases and controls. Subsequent evaluations of transcript and protein expression showed that both variants have a negative impact on the translation of the ABCB11 protein. Notably, only the rs497692 variant reduces the production of ABCB11 mRNA. This observation was further validated *in vitro* using cells transfected with both wild type and mutant vectors. Despite these correlations, the precise mechanism through which these mutations contribute to human disease remains uncertain. As a result, no definitive therapeutic target has been identified. Furthermore, the research is conducted within a moderately homogenous population, raising questions about the generalizability of the findings.[35]

Apolipoprotein family genes

There are two defined mutation sites within the ApoB100 protein (Fig. 1B). The first site is recognized by the EcoRI restriction enzyme, and the second site is recognized by XbaI. The mutations typically involve single base changes in their exons. In a 2013 meta-analysis involving 10 studies from various countries, researchers established a significant correlation between ApoB100 mutations and GS production. Mutations within XbaI carried the greatest risk, particularly in patients of Chinese ethnicity.[36] In a 2021 analysis focused on the XbaI rs693 variant, the presence of this mutation increased the risk of GS disease among the Chinese population, with an OR of nearly 3.[37] This elevated risk remained consistent across both sexes.

ApoE is present in various circulating lipoproteins. Large-scale association studies have connected ApoE alleles and SNPs within those alleles to the risk of GS disease. In a study using the United Kingdom's biobank, the E3/E3 variant of ApoE was found to be the most common. Only slightly higher risks were associated with cholelithiasis and cholecystitis in individuals with E3/E4 (prevalence reported in the classic meta-analysis: 21.3% in Caucasians, 31.8% in African Americans, 17.6% in Hispanic, and 15.5% in Japanese) and E4/E4 (prevalence: 1.8% in Caucasians, 2.1% in African Americans, 1.9% in Hispanic, and 0.8% in Japanese) genotypes, although none of the allelic combinations demonstrated significant increased risks.[38,39] In a meta-analysis of studies that reported

genotypes and ultrasound examinations, researchers investigated the potential associations between E4 variants and GS disease. Nearly 7,000 cases and controls were evaluated using a random-effects model, and the results indicated no statistically significant association between the two.[40]

In one of the studies from the aforementioned meta-analysis, Abu *et al*. focused on patients who underwent bariatric surgery. Among patients with no pre-operative GSs, the highest incidence was observed in patients with the E3/E4 genotype with a follow-up observation period of 12 months. However, this limited study included only 134 patients and was conducted in a population already at an elevated risk of developing GSs due to obesity.[41] In contrast, Martinez-Lopez *et al*. investigated the correlation between ApoE genotypes and GS disease within a cohort of nearly 500 Mexican patients. E3/E3 and E3/E4 mutations constituted 43% and 32% of the cases, respectively. Both were statistically different than the distribution among controls. However, the statistical significance of this finding is tempered by the study's reliance on a relatively small population, which limits generalizability.[42]

Bilirubin GSs

Bilirubin GSs, also known as black or brown pigment stones, constitute less than 10% of all GSs.[1] They are predominantly composed of bilirubinate polymers which are salts formed by unconjugated bilirubin and calcium. Bilirubin is derived from the catabolism of heme molecules. Initially, unconjugated (indirect) bilirubin circulates in the bloodstream until it reaches the liver, where it undergoes a transformation facilitated by UDP-glucuronosyltransferase (UGT). This enzymatic process involves the conjugation of glucuronic acid molecules, rendering bilirubin water-soluble.[43] Conjugated bilirubin is subsequently released into bile canaliculi and then carried to the gallbladder for storage as a component of bile. Several non-hepatic and hepatic genetic conditions can lead to increased production of bilirubin, resulting in increased hepatic bilirubin uptake.

The most common non-hepatic causes include sickle cell disease, thalassemia, and hereditary spherocytosis, among many others.[44] Crigler-Najjar syndrome (both type I and type II) are rare genetic disorders characterized by a severe deficiency in UGT, resulting in dangerously high levels of unconjugated bilirubin.[45,46] Studies have also shown that excessive mucin could be a factor in the formation of brown pigment GSs, as lipopolysaccharides from certain bacteria are potent stimulators of mucin secretion.[14] There are mouse models characterized by hemolysis due to dyserythropoiesis, and excessive mucin production leading to an increased risk of pigment or mixed GSs.[47–50]

Genetic causes of bilirubin GS

UGT1A1

One of the more predictable genetic risk factors for cholelithiasis involves the UGT family member A1 (UGT1A1) (Fig. 1C). This enzyme plays a crucial role in the glucuronidation of bilirubin, a process that allows bilirubin to dissolve in aqueous solutions and be transported in bile, ultimately enabling bilirubin excretion in feces. The same enzyme is implicated in the most common form of uncomplicated hyperbilirubinemia—Gilbert syndrome. While there are fewer ethnic associations with variants in this gene and its protein product, it exerts a substantial global impact on the genetic predisposition to GSs. When combined with the risk from ABCB8 D19H, it accounts for up to 15% of the overall risk for GS formation.[1] Moreover, mutations in the UGT1A1 gene seem to pose a greater risk factor in males than females. The mechanism by which unconjugated bilirubin precipitates GSs is not fully described. This is likely due to the poor water solubility of unconjugated bilirubin, which creates nucleation sites for cholesterol crystal formation, ultimately contributing to stone formation.[1,51]

In comparison to other ABC family genes, UGT1A1 has not been as extensively researched. However, it has been studied in a well-defined cohort of elderly patients from Sicily, Italy. Using exome-wide association techniques, three intronic variants of UGT1A1 were identified that were significantly associated with an increased risk of cholecystectomy for symptomatic cholelithiasis. As expected, these variants were also linked to increased serum bilirubin concentrations. This analysis relied on *in silico* analysis and self-reported cholecystectomy, which could potentially affect the results.[51]

In a more recent study, Zhuo *et al*. assessed the association of GSs and the polymorphism of certain risk variants of UGT1A1 in Chinese patients who had experienced acute liver failure due to hepatitis B virus (HBV) infection.[52] Their investigation compared the distribution of frequently implicated risk variants of UGT1A1 (UGT1A16, UGT1A127, UGT1A128, and UGT1A160) among these patients, individuals with HBV without GSs, those with acute liver failure without HBV, and healthy controls. Through their analysis, they discovered that not only did these groups differ in their serum bilirubin levels, but also that the variants UGT1A127 and UGT1A128 were most strongly associated with acute liver failure in those with GSs. This finding is surprising since UGT1A1 is typically more closely associated with severe unconjugated hyperbilirubinemia, suggesting a potential synergy between the pathologies of HBV-induced liver failure and the damage caused by UGT1A1 mutations. While this study raises interesting questions, it also has limitations in terms of generalizability and the presence of confounding factors related to liver injury in the context of HBV infection.[52]

ABCC2

In the class of ABC proteins, one noteworthy member is ABCC2, also referred to as MRP2. It serves as a transporter for bilirubin and bile salts and is prominently expressed on the apical membranes of both hepatocytes and gallbladder cholangiocytes.[53] The absence of ABCC2 from membranes has been identified as the underlying mutation responsible for Dubin-Johnson syndrome.[54] While this syndrome is present in various ethnic groups, epidemiological data collected from Iranian and Moroccan Jewish populations showed a prevalence of 1 case per 1,300 individuals.[55] Genetic mutations leading to Dubin-Johnson syndrome, conjugated hyperbilirubinemia, and resultant bilirubin gallstone disease are broad, with 68 variants listed in the Human Gene Mutation Database.

While mutations in this gene have not been extensively studied compared to the previously discussed genes, a recent cohort study provided further insight. This analysis involved 32 patients of European and North African descent referred for Dubin-Johnson syndrome genetic analysis, providing the most comprehensive mutation data for this condition. These unrelated individuals exhibited similar clinical presentations. Within this group of 30 patients, researchers identified 29 distinct mutations with the recurrent finding of a single SNP: c.2302C>T leading to tryptophan substitution for arginine. This same variant has appeared in other Dubin-Johnson syndrome cases, especially in Japan.[55] Given the heterogeneous population of the study, it suggests broad applicability of its findings. However, a notable limitation is the origin of the data from a tertiary center, raising the possibility of undiscovered mutations in cases that were either misidentified or

not referred.

In a Chinese case series, the p.G693R mutation in ABCC2 appeared in 2 unrelated patients. This mutation was then introduced into a plasmid and transfected into three different cell lines, two of which were derived from hepatic cancers. The analysis of protein expression levels revealed lower ABCC2 levels in all cell lines containing the mutated plasmid. This implies either increased degradation of the mutant protein product or poor translation of the mutant mRNA. Furthermore, these transfected cells exhibited a reduced ability to transport organic anions, a key function of ABCC2. After imaging for protein localization, it was found that mutant ABCC2 was primarily located in the cytoplasm rather than its usual membrane location.[56] These findings align with earlier research by Chai *et al*., which emphasized the essential role of ezrin-radixin-moesin (ERM) complexes in localizing MRP2 to the cell membrane of hepatocytes, bolstering the strength of these observed phenomena.[57] However, it's important to acknowledge that this *in vitro* assessment of p.G693R has significant weaknesses. The lack of mRNA transcript quantification opens the possibility of reduced transcription or increased degradation of the transcript as potential explanations for the reduced protein expression. Additionally, the use of a population-specific variant, which could arguably be individual-specific, may limit the generalizability of these findings.

ABCC2 works in concert with ABCC3 (MRP3), which also transports many of the same bile salts but is situated on the basolateral membrane of hepatocytes. While ABCC3 expression is minimal in healthy hepatocytes, it undergoes significant upregulation in cases of cholestasis, likely attributed to reduced ABCC2 functionality.[53,57,58]

Bilirubin GSs due to hemolytic and disorders of erythropoiesis

Bilirubin or pigment GSs stem from irregular bilirubin metabolism, primarily associated with chronic hemolytic anemias and ineffective erythropoiesis. Focus will be centered on the shared mechanisms through which these distinct pathological conditions trigger an accumulation of bilirubin, ultimately leading to the formation of these rare GSs.[1,59] Unlike the high occurrence of cholesterol GSs in Europe, North and South America, GSs are infrequent in Asia and Africa, where bilirubin GSs surpass cholesterol GSs in prevalence.[60]

In the context of inherited hemolytic anemias, including conditions such as hereditary spherocytosis, sickle cell anemia, and the thalassemias, mutations cause reduced stability of RBCs, leading to their lysis within the bloodstream and the subsequent release of excessive quantities of hemoglobin. Hereditary spherocytosis is attributed to defects in scaffolding proteins, with ankyrin-1 (*ANK1,* autosomal dominant), α-spectrin (*SPTA1*, autosomal recessive), and β-spectrin (*SPTB*, autosomal dominant) being the most affected proteins.[61] Ankyrin-1 mutations are responsible for more than half of the cases in both the USA and Europe, with an estimated prevalence of 1 in every 200 individuals.[61] Sickle cell anemia, as recently reviewed by Kavanaugh *et al*., is distinguished by mutations in the hemoglobin β-globulin protein. These mutations result in decreased aqueous solubility and abnormal polymerization of hemoglobin under hypoxic conditions, eventually causing lysis of RBCs.[62] Likewise, thalassemias (both α and β subtypes) involve defects in their respective hemoglobin globulin chains, resulting in instability and lysis of RBCs.[63,64] In each of these syndromes, the surplus hemoglobin transported to the liver triggers an increase in bilirubin levels. Initially, efforts are made to conjugate bilirubin, but once the enzymes become overwhelmed, a portion remains unconjugated.

There is ongoing research to investigate whether GSs from hemolytic anemia exhibit different clinical features. In a ten-year study in China focused on patients admitted with symptomatic GSs and hemolytic anemias, it was found that individuals with hemolytic anemia harbored characteristic changes in their circulating lipid profiles when compared to controls presenting with cholesterol GSs. Notably, patients with hemolytic anemias had reduced levels of HDL, LDL, and total cholesterol compared with controls. Most of the anemia cases were due to hereditary spherocytosis. Moreover, the patients with hemolytic anemias tended to be younger at the time of presentation, underscoring the early onset of these anemias and their impacts. Additionally, the severity of anemia was found to be a predictor of the age of the first symptomatic GS presentation. This study included nearly nine hundred patients, all of whom underwent ultrasound examination to confirm GSs.[65]

CFTR

Mutations in CFTR, which functions as a membrane chloride channel, constitute the underlying etiology in cystic fibrosis (CF) (Fig. 1D). Mutations in this protein result in abnormal anion transport across the membrane. CF follows an autosomal recessive inheritance pattern, requiring two mutated alleles for the full development of the condition. The carrier rates for a single mutated allele vary by ethnicity, with the highest prevalence in individuals of Northern European Caucasian descent.

Pathophysiological mechanisms contributing to the increased risk of cholelithiasis in patients with CFTR mutations are actively being explored. Evidence from animal models suggests a consistent disruption in the regulation of bile components, leading to a heightened concentration and crystallization of substances that form GSs. Research conducted on mice with the ΔF508 mutation (the most common genetic mutation in humans) shed light on these mechanisms. While there are no observable differences in gallbladder structure or inflammation between the mutant mice and the control group, an analysis of bile revealed several findings. In the KO mice, bile exhibited a more acidic pH, elevation of both conjugated and unconjugated bilirubin, and reduced bile salts coupled with increased cholesterol levels. These alterations increase the tendency of bile to form GSs. Intriguingly, the mouse model also displayed a greater susceptibility to pigment stones, despite an increase in cholesterol secretion. Furthermore, these characteristics were examined in an alternate CF KO model, where there was no increased cholesterol secretion. This suggests that the most common human gene mutation uniquely facilitates the transport of cholesterol.[47]

Miller and his team conducted a genetic analysis within the United States population using the Truven Marketscan claims cohort. They identified more than 19,000 individuals with carrier status and about 20,000 patients with CF. For each of these, they selected 5 matched controls, resulting in a study population of nearly 120,000 individuals. In their investigation, they assessed both the classic manifestations of CF (*e.g.,* male infertility, meconium ileus, and bronchiectasis) in addition to related conditions, including T2DM, cholelithiasis, and obstructive jaundice. Results revealed that carriers of the CF gene were more prone to the classic and associated signs of CF compared to the control group, but their risk was lower than that of individuals with full-blown CF. Regarding cholelithiasis, the study demonstrated that CF carriers faced an increased risk compared to the control group, showing an OR of 1.14 (95% confidence interval: 1.04–1.25, p=0.002). In contrast, CF patients exhibited a significantly higher OR of approximately 2.5 for cholelithiasis.[66] The strengths of

the study include its large sample size, thorough matching, and strong use of controls. However, it relies on self-reported cholelithiasis data, introducing uncertainty.

Further support for the role of CFTR in cholelithiasis comes from SNPs analysis conducted on a small, homogenous group of high-altitude Tibetans found that SNPs located within the coding regions of the CFTR sequence may confer a reduced risk of GSs. Cases were defined as participants with confirmed GSs through ultrasound while controls were individuals without GSs. Whole exon sequencing was employed for the analysis. The study identified two SNPs, namely rs79074685 and rs201880593 that were significantly associated with a lowered likelihood of cholelithiasis. While intriguing, the research focused on a small and genetically distinct population, raising questions about the extent to which these findings can be generalized to the larger population. Moreover, the inferences of these specific SNPs remain unclear, which restricts the applicability of the authors' discoveries.[67] CFTR is unique among other previously discussed GS genes in that specific gene therapy is available and approved for use in the USA.

MUC

Various mucin genes have been implicated in GS formation. Among human gallbladder epithelial cells, MUC3, MUC5AC, MUC5B, and MUC6 are expressed, with MUC3 and MUC5B exhibiting the highest expression in cholesterol-associated gallbladder disease.[68] Notably, MUC1, MUC3, and MUC4 are transmembrane, while MUC5AC, MUC5B, and MUC6 are secreted (Fig. 1E).[14] These genes encode apoproteins with amino acid repeats that act as attachment sites for oligosaccharides, contributing to the matrix and membrane-forming abilities of mucins. Bile mucins possess glycosylated and non-glycosylated regions responsible for binding oligosaccharides and bilirubin, respectively. Disruptions in these genes can significantly affect the solubility and stability of bile. Limited human studies have revealed differences in mucin expression in bile.

Yoo *et al*. investigated MUC protein expression rates using immunohistochemistry in patients with cholesterol stones, cholesterol polyps, and gallbladder carcinoma. Histological sampling of post-operative specimens showed no significant differences in the expression levels of MUC5B and MUC3 concerning the presence or absence of cholesterol GSs. This suggests a lack of correlation between these genes and a predisposition for cholesterol GSs. However, the cholesterol polyp group exhibited notably increased expression of MUC3 and MUC5B compared to controls. The clinical significance of these observations is limited due to the small sample size in the study, which comprised only twenty Korean patients. Both the cholesterol stone group (n=5) and cholesterol polyp group (n=6) were relatively small.[68] These findings align with earlier data by Vilkin *et al*., derived from ERC aspirates, which indicated no variance in bile mucin expression levels between patients with and without GSs. Likewise, since this study was conducted with a sample size of 29 Israeli individuals, it also suffered from limited statistical power.[12] However, taken together, these concordant findings suggest no significant correlations exist between mucin expressions and GS disease in these populations.

Interestingly, in Chinese men, a population-level SNP analysis yielded positive associations between individual variants in MUC1 and MUC2. When present together, the combined OR for GSs was 4.68 (p=0.0008), suggesting that specific variants within the population might influence the risk of GS disease.[69] These findings reinforce earlier work done in MUC1 KO mouse models by Wang *et al*. In a series of experiments, they demonstrated that these KO mice had increased expressions of other mucins (namely MUC3, MUC4, MUC5AC, and MUC5B) in the presence of a lithogenic diet, when compared to wildtype mice.[70,71] Their findings suggest that aberrations in MUC1 may drive an increased risk for GSs, particularly among at-risk populations and in the context of lithogenic diets.

MicroRNA in gallstone disease

MicroRNAs (miRNAs) have been shown to play a role in the pathogenesis of GSs. These small transcripts, comprising 20–30 nucleotides, function as regulators of mRNA transport and translation, exerting control over both spatial and temporal aspects of translation.[72] Recent observations have illuminated variations in the expression of both mRNAs and miRNAs between individuals with and without GSs. Notably, Yang *et al*. identified more than 500 mRNA transcripts and 17 miRNAs with differential expressions. This differentiation was based on target sequences that had been previously identified, as well as suspected sequences derived from closely related non-human primates.[73]

One particular miRNA, miR-210, exhibited increased expression in gallbladders containing GSs. This miRNA targets the ATP11A gene, which encodes one of the ABC transporters sharing the same name. As highlighted earlier, ABC transporters are pivotal in determining the cholesterol content of bile, thereby impacting the lithogenic potential of bile. Interestingly, miR-210 is regulated by HIF-1α in response to hypoxia, a critical cellular reaction to stress and a pathway which has also been linked to cholelithiasis.[1,2,8,74] The research group observed a reverse correlation between the levels of mir-210 and ATP11A mRNA. This association suggests an important role for mir-210 in the pathogenesis of the disease. However, it remains uncertain whether the alterations in expression precede the onset of cholelithiasis or occur subsequently. Given this miRNA's involvement in various stress responses, it is possible that the observed changes are a direct response to the presence of cholelithiasis.[73]

Genetically-tailored therapy for GSs

The management of GSs is well-described in the literature. Regardless of stone composition, the primary treatment for individuals with symptomatic disease involves laparoscopic cholecystectomy if they are suitable candidates. Our emphasis will shift toward exploring the treatment considerations tailored to individuals whose GSs are predominantly influenced by genetic factors. For example, for young asymptomatic carriers of ABCB4 mutations (predisposing to LPAC syndrome), they may benefit from preventive UDCA therapy.[75] However, within the general population, preventative medications like UDCA, statins, or ezetimibe are generally not recommended due to non-favorable cost-benefit ratios.[1]

Preclinical data suggests that viral-mediated delivery of genes for ABC4 may prevent the progression of progressive familial intrahepatic cholestasis. Researchers employed a modified adeno-associated virus to deliver the rescue gene, coupled with a small molecule inhibitor of the immune response to limit antibody formation to the viral vector. This approach resulted in a significant improvement in biliary transit in treated mice. Although studied in double KO mice, mirroring human pathology, the technique holds promise for future applications. If gene therapy advances in the human population or employs a different vehicle, this presents an exciting potential therapy.[76]

Perhaps the most promising treatments for genetically-linked GS disease stem from hematopoietic disorder treatments. Recently, the Food and Drug Administration in the

Table 1. Summary table of the genetic variants, their functions, pathologic variants, and corresponding references

Gene	Relevant Studies
ABCG8/ABCG5	19,20,23
Function: Transportation of cholesterol into bile	
Pathology: Mutation leads to gain-of-function, causing increased cholesterol in bile	
ABCB4	28,30,31
Function: Transportation of bile acids into bile	
Pathology: Decreased bile acids per cholesterol molecule; LPAC	
ABCB11	33–35
Function: Transportation of lipids into bile	
Pathology: Familial intrahepatic cholestasis	
UGT1A1	51,52
Function: Bilirubin glucuronidation	
Pathology: Decreased conjugated bilirubin in bile	
APO	37,38,40
Function: Maintain cholesterol vesicles	
Pathology: Increased serum cholesterol	
CFTR	47,66,67
Function: Transport chloride ions	
Pathology: Decreased chloride and water content in bile, increasing concentration of stone-forming substances	
MUC 1	69–71
Function: Transmembrane mucin serving barrier function	
Pathology: Mutation associated with increased GS risk in Chinese patients	
MUC 2	69–71
Function: Transmembrane mucin serving barrier function	
Pathology: Mutation associated with increased GS risk in Chinese patients	
MUC 3	12,14,68
Function: Secreted mucin in bile	
Pathology: Mutation associated with increased risk for cholesterol polyp	
MUC 5B	12,14,68
Function: Secreted mucin in bile	
Pathology: Mutation associated with increased risk for cholesterol polyps	

United States approved two gene therapies for sickle cell disease. These therapies utilize CRIPSR/Cas9 technology, a type of virally mediated transfection, to insert functional copies of the hemoglobin gene into hematopoietic cells. The CRISPR technology demonstrated the ability to increase wildtype hemoglobin in patients with sickle cell disease, sufficiently reducing vaso-occlusive crises (98% CI: 0.87–1), albeit with a notable adverse event profile (up to 45%), including blood cancers and, relevant to this review, cholelithiasis in 11% of participants.[77,78]

In the future, the paradigm will likely shift towards a more preventative approach to GS disease, perhaps one utilizing gene therapy. This shift could involve the creation of a personalized risk score that accounts for both genetic and environmental risk factors, enabling the accurate stratification of patients into moderate and high-risk categories. For example, individuals whose risk profile is primarily influenced by environmental factors can prioritize the control of exogenous triggers such as obesity and diet since GSs recurrence is less likely when these factors are managed. Conversely, individuals with a predominant genetic influence in their risk profile may consider surgical management once symptoms develop, given the inherently high risk of GSs recurrence. However, these emerging strategies are still speculative and require further research to validate.

Conclusion

Cholelithiasis and its consequent symptomatic GS disease arise from a complex interplay of factors. Certain populations face an elevated risk for GS development due to specific genetic mutations. A significant proportion of these mutations influence cholesterol synthesis or transport in various ways (Table 1).[12,14,19,20,23,28,30,31,33–35,37,38,40,47,51,52,66–71] Notably,

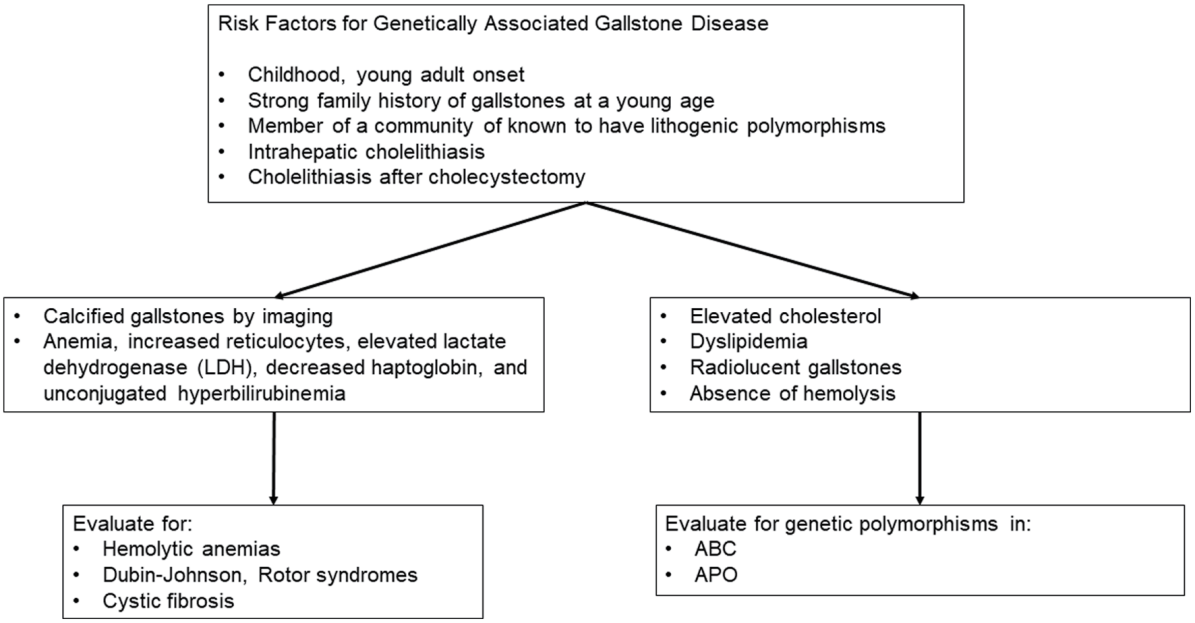

Fig. 2. Recommended approach for diagnosing genetically linked GSs, including a suggested algorithmic approach to identify patients at an increased risk for genetically associated GSs and diagnostic workup. ABC, ATP-binding cassette; APO, apolipoprotein. The figure was created using BioRender.com.

the most impactful genes are those belonging to the ABC transporter family, particularly the ABCG8 cholesterol transporter. Different genes play important roles in the pathogenesis of GS disease across various ethnicities. For instance, within the South American population, specifically individuals with Mapuche heritage in Chile, the presence of variants within the ABCG8 gene is linked to the highest risk of gallstone formation. Similarly, for individuals of Asian descent, the UGT1A1 mutation serves as their distinctive risk variant. While cholelithiasis is commonly observed, particularly among older adults, certain features should prompt consideration of a genetic basis for this condition (Fig. 2). Genetic testing could be beneficial for patients who are young, have a strong family history of GS disease, belong to high-risk communities, and experience recurrent cholelithiasis even after cholecystectomy. With the increasing accessibility and diminishing costs of sequencing and genetic investigation, we anticipate a greater integration of these technologies into routine diagnostic procedures in the foreseeable future. While the identification of these genetic variants may not yet directly influence treatment strategies, there could be potential ramifications for both family members and future generations.

Several emerging genes are being studied through genome-wide association studies and similar investigations. A considerable portion of these genes operate outside the conventional pathways related to cholesterol synthesis, degradation, and transport. This suggests the possibility of discovering additional therapeutic targets beyond the established avenues. The foundational research initiated in murine models is evolving alongside the application of increasingly sophisticated molecular biology techniques. As our understanding of these intricate pathways deepens and our capacity to manipulate genetic expression *in vivo* advances, novel therapeutic options will hopefully emerge for individuals affected by genetic risk factors. In the future, this extensive study population could serve as a valuable resource for identifying both single-gene and polygenetic risk variants associated with cholelithiasis.

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Conflict of interest

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Author contributions

CJC conducted extensive literature searches, drafted the manuscript, and created all figures and the table. GYW conceptualized, and edited the review. MTN and HV provided edits. All authors provided revisions and approved the final version.

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