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The Genetics of Complex Cholestatic Disorders

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Abstract

Cholestatic liver diseases are caused by a range of hepatobiliary insults and involve complex interactions among environmental and genetic factors. Little is known about the pathogenic mechanisms of specific cholestatic diseases, which has limited our ability to manage patients with these disorders. However, recent genome-wide studies have provided insight into the pathogenesis of gallstones, primary biliary cirrhosis, and primary sclerosing cholangitis. A lithogenic variant in the gene that encodes the hepatobiliary transporter ABCG8 has been identified as a risk factor for gallstone disease; this variant has been associated with altered cholesterol excretion and metabolism. Other variants of genes encoding transporters that affect the composition of bile have been associated with cholestasis, namely ABCB11, which encodes the bile salt export pump, and ABCB4, which encodes hepatocanalicular phosphatidylcholine floppase. Many genes associated with gallstones have also been linked with vanishing bile duct syndromes and other cholestatic disorders. In contrast, studies have associated primary biliary cirrhosis and primary sclerosing cholangitis with genes encoding major histocompatibility complex proteins and identified loci associated with microbial sensing and immune regulatory pathways outside this region, such as genes encoding IL12, STAT4, IRF5, IL2 and its receptor (IL2R), CD28, and CD80. These discoveries have raised interest in the development of reagents that target these gene products. We review recent findings from genetic studies of patients with cholestatic liver disease. Future characterization of genetic variants in animal models, stratification of risk alleles by clinical course, and identification of interacting environmental factors will increase our understanding of these complex cholestatic diseases.

Complex cholestatic diseases include a range of disorders affecting small and large bile ducts and the gallbladder.¹ To date, development of rational interventions for individuals with specific cholestatic disorders has been hampered by gaps in understanding disease pathogenesis. However, recent developments in identifying genetic influences (see

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Conflicts of interest

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Appendix for definitions) have begun to address an unmet need for rational treatment. The immune-mediated biliary disorders, primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), represent the most important small and large bile duct diseases. The incidence and prevalence rates for PSC vary from 0 to 1.3 per 100,000 inhabitants/year and 0 to 16.2 per 100,000 inhabitants, respectively, whereas the incidence and prevalence of PBC range from 0.3 to 5.8 per 100,000 inhabitants/year and 1.9 to 40.2 per 100,000 inhabitants, respectively.²⁻⁴ PBC and PSC have been observed in all heritages, and geographic variations are evident with an increased prevalence in northern latitudes. Clustering of PBC has also been reported geographically, for example, in coastal First Nations of British Columbia, where disease has been recorded to be as high as 1 in 4 within generations of well-characterized multiplex families.⁵ In contrast, cholesterol gallstone disease is far more common and we have a much clearer understanding of the pathophysiology. Several factors combine to promote gallstone formation, such as supersaturation of bile with cholesterol or bilirubin, gallbladder hypomotility, and an imbalance of crystallization promoters (eg, mucin) and inhibitor proteins.⁶ Nevertheless, the incidence of gallstones differs markedly worldwide, reaching 50% in the American Indian population, 15% to 20% in the European population, approximately 10% in the Asian population, and less so in African populations.⁷ These differences are not fully explained by environmental factors such as physical inactivity or high-calorie, high-carbohydrate, and low-fiber diets or medications.^{8,9}

The dynamic genetic interactions that contribute to disease manifest at various levels. Some genes determining disease risk may only do so by imparting variability in how individuals respond to a particular environmental challenge. Others may express the consequence of genetic variation in a graded manner in as much as a single gene can be responsible for a wide phenotype spectrum depending on background genetic variability. A good example is provided by recognizing how *ABCB4* variants can lead to disease ranging from mild elevations of γ -glutamyltransferase (GGT) levels to early development of biliary cirrhosis in childhood as well as cholestasis of pregnancy and intrahepatic gallstones with premature cholelithiasis (Figure 1A). The clinical spectrum of disease associated with this single gene aptly shows the mechanistic complexity of cholestatic disease. Genetic variation in structural proteins may also contribute to disease even if not directly related to the primary process mechanistically. For example, the association of keratin variants with PBC¹⁰ suggests how mutations in structural proteins can act as genetic modifiers and may be related to an accelerated liver disease phenotype alongside the overall genetic complexity of disease.

Heritability and the Role of the Major Histocompatibility Complex

Heritability is difficult to quantify and confounded by sharing of environmental triggers within close relatives. It is usually estimated by concordance rates in monozygotic versus dizygotic twins or by dividing the prevalence of disease among siblings with that of the general population (ie, sibling relative risk, λ_s). Collectively, such estimates suggest that the development of PBC and PSC is comparable to the 10-fold to 20-fold increased relative risk observed with other immune-mediated conditions such as inflammatory bowel disease (IBD). More precise estimates have been derived from countries with registries recording outcomes for disease for the population as a whole. For example, studies performed in ~30,000 Swedish twins with symptomatic gallstone disease have estimated the heritability to be approximately 25%.¹¹

Before the advent of genome-wide association studies (GWAS), variants within the major histocompatibility complex (MHC) on chromosome 6p21 were the only robust candidates that were clearly associated with PBC and PSC. More than 30 years ago, PSC was linked

with HLAB8, a known haplotypic marker for ancestral haplotype (AH) 8.1.¹² For PBC, *DRB1*0801* was shown to be over-represented in those of European descent and *DRB1*0803* in Japanese subjects.¹³ A predominant role of the MHC region has subsequently been confirmed in genome-wide studies of PBC¹⁴ and PSC.¹⁵ Specific associations continue to be refined by ongoing studies.^{16,17} This is comparable to other autoimmune diseases such as type 1 diabetes, in which the genetic influence of the MHC has been estimated to contribute to more than 40% of the heritability.¹⁸

Some insight into risk-related alleles in the class II region of patients with PSC has been provided by fine mapping of the *HLA-DRB1* genotypes with 3-dimensional modeling of the HLA-DR β 1 chain.¹⁵ These studies have identified key amino acids within the class II heterodimer that influence the range of peptides incorporated into the binding pocket of patients with PSC. Analogous studies in type 1 diabetes have provided a model whereby the disease-related alleles have an increased affinity for binding of insulin peptides into the HLA-DQ β 1 chain with the capacity of promoting an autoimmune response.¹⁹ Another model of HLA-mediated immune injury has been suggested by abacavir hypersensitivity.²⁰ Following the specific interaction of drug with HLA-B*57:01, the immune repertoire of endogenous peptides in the binding pocket is redirected, leading to activation of abacavir-specific T cells that drive polyclonal CD8 T-cell activation.²¹ In the context of cholestasis, the HLA-B*57:01 association identified in a genome-wide association study for flucloxacillin-induced liver injury provides another example.²² Intriguingly, while HLA is the strongest association for amoxicillin-clavulanate-induced liver injury,²³ variants in a classic autoimmune-related gene, *PTPN22*, were also implicated.

A major distinction between PBC and PSC is the presence of highly conserved humoral and cellular immune responses to pyruvate dehydrogenase complex E2 (PDC-E2) in patients with PBC. The loss of tolerance to the mitochondrial proteins has been attributed to the aberrant expression of proteins resembling PDC-E2 on the cell surface of biliary epithelium.²⁴ The immunodominant T-cell epitopes that bind with HLA-DRB4 have been mapped to the lipoyl domain of PDC-E2²⁵ and found to be similar to the immunodominant B-cell antigens, but the structural interaction of HLA-DRB8 and PDC-E2 has yet to be resolved. Also, our lack of knowledge of environmental factors that trigger the immune biliary diseases hinders our ability to study whether the disease-related HLA either lacks the ability to bind and help eradicate pathogens or specifically binds the environmental agent, triggering an autoimmune response.

Several GWAS studies have shown that the MHC is the major determinant in controlling viral infection with human immunodeficiency virus²⁶ and chronic hepatitis B virus infection.²⁷ This is not surprising given that more than 250 proteins encoded within the MHC region play a major role in response to pathogens. As a result, the complexity of the HLA and the complex pattern of linkage disequilibrium between disease-associated variants on the various extended HLA haplotypes make it difficult to discern the disease-related causal variants. For example, the PSC-associated AH8.1 has an extensive linkage throughout the MHC with *HLA-A1*, *Cw7*, *B8*, *TNFAB*a2b3*, *TNFS*5*, *C2*C*, *Bf*s*, *C4A*Q0*, *C4B*1*, *DRB1*0301*, *DRB3*0101*, *DQA1*0501*, and *DQB1*0201*.²⁸ The AH8.1 has thus been linked with diminished complement levels, inability to clear immune complexes, increased susceptibility to infectious disease, and more rapid progression of bacterial and viral infections.²⁸ However, it is not possible to determine the effects of single genes in the haplotype because of the strong linkage disequilibrium.

Of interest, AH8.1 is overrepresented in many other autoimmune diseases besides PSC, including autoimmune hepatitis. Patients with AH8.1 have differential cytokine responses to mitogens and lipopolysaccharide with increased tumor necrosis factor (TNF)- α levels,

diminished interferon gamma levels, and predominant T-helper (Th)2 cytokine production. Although it is tempting to speculate that patients with AH8.1 have inefficient responses to pathogens and are more likely to have auto-immune responses, there are also data to suggest a potential benefit to survival. For example, the frequency of AH8.1 is approximately 10% in Europe and the haplotype is more frequently found in male nonagenarians.²⁹ It could be argued that AH8.1 has been maintained within the population by the protective effects for men at the cost of increased risk of autoimmune disease in women. Thus, specific haplotypes may be beneficial by affecting the ability of the host to present antigens and clear infections but on the downside may contribute to autoimmune responses and “collateral” immune damage.

GWAS

The main goal of GWAS is to identify common genetic variation associated with well-phenotyped disease in a nonbiased fashion. Inherent to the properties of association-based statistics, the premise for conducting GWAS is based on the assumption that disease risk is associated with relatively common variants with a minor allele frequency of greater than 2% to 5%. The process is based on the principle of linkage disequilibrium whereby reasonable genomic coverage is accomplished by genotyping careful selections of 500,000 to 5,000,000 alleles at single nucleotide polymorphisms for any given individual. The readout is to identify frequency differences in polymorphisms between cases and controls. As a result, the studies mandate a large sample size to detect robust associations because false-positive findings will arise due to chance alone. The occurrence of linkage disequilibrium is used to advantage in that not all polymorphisms have to be genotyped to detect an association. On the downside, it is very difficult to ascertain which of a series of alleles in linkage disequilibrium is actually the causative variant on a haplotype. The larger the population studied for any given heterogeneous complex disorder, the more associated variants that can be confirmed. For example, GWAS in Crohn's disease and ulcerative colitis are now based on more than 75,000 cases and controls and have subsequently identified 163 inflammatory bowel disease loci.³⁰ However, each locus contributes only a small risk to disease and the variants identified tend to be in noncoding regions of genes, implying subtle effects on gene expression rather than overt structural/functional changes.³¹

Not surprisingly, many of the risk alleles identified in immune-related conditions by GWAS are found in conjunction with genes related to immune function, both within and outside the MHC. Of note, disease-specific risk alleles related to the affected organ have seldom been identified with rare exceptions, such as insulin gene polymorphisms in type 1 diabetes.¹⁸ One obvious trend is that many risk loci are shared between multiple autoimmune diseases. For example, more than 160 risk loci have been found as common to both Crohn's disease and ulcerative colitis, suggesting a hypothesis that these variants represent a continuum in the risk of inflammatory bowel disease.³⁰ Of interest, unrelated inflammatory diseases also share up to 50% of loci outside the MHC region. For instance, the detection of shared causal variants associated with the interleukin (IL)-12/IL-23 signaling pathway has been observed in patients with psoriasis, Crohn's disease, and multiple sclerosis (Figure 2).³² Although in most studies there is no obvious biological correlation with loci and disease, the studies provide a signpost of where to begin the search.

GWAS in PBC

Several GWAS have been performed using subjects with PBC and controls from North America, Italy, the United Kingdom, and Japan.^{14,33–36} In keeping with other autoimmune diseases, it seems likely that there will be few, if any, PBC-specific genetic associations (Table 1). Many of the PBC-related variants have been identified in other GWAS of

immune-related diseases, with a different mosaic of disease-specific risk contributing to the pathogenesis of PBC (Figure 2). Overall, the data suggest important contributions from a number of immune pathways to the development of PBC, particularly including a strong implied role for the interleukin (IL)-12 cytokine pathway and downstream JAK-STAT signaling (Figure 3).^{14,33–35,37} This needs to be seen in the context of a large preexisting body of evidence supporting immune models of disease. The present list of associated genes sustains a model heavily focused toward the effects of balancing immunoregulatory pathways, in particular Th cell lineages including Th1 and Th17 cells. Such unbiased, hypothesis-free generated data complement animal models (Table 2) and thereby focus research on teasing out the intricate balance of immunoregulatory triggers of disease.

With regard to IL-12 signaling, the involvement is consistent with the major immunoregulatory roles of the gene products IL-12 p35 and IL-12 receptor $\beta 2$, which associate with the IL-12 p40 and IL-12 receptor $\beta 1$ chains, respectively, to generate the complex of IL-12 and its receptor. The IL-12 pathway has been implicated in other immune-mediated diseases,³² and it is central to generating Th1 immune responses directed toward clearance of intracellular pathogens.³⁸ The engagement of IL-12 to its receptor is believed to modulate immune responses by evoking interferon gamma production, which in turn inhibits IL-23–driven induction of IL-17–producing helper T lymphocytes. A role for IL-35 is also worthy of investigation, given the subunit nature of the cytokine IL-35 and its receptor, that includes IL-12 p35 and IL-12R $\beta 2$, respectively.³⁹ It is also notable that the regulation of *IL12RB2* expression in T regulatory cells appears to be important in determining Th cell lineage effects,⁴⁰ because impaired expression of IL-12R $\beta 2$ has been shown to help maintain T regulatory cell–suppressive function in the context of inflammatory Th1 cell responses.

A bidirectional dynamic interaction of T cells and biliary epithelial cells is likely relevant, with the “secretome” of the injured biliary cell having the potential to also contribute to the fate of the infiltrating lymphocytes. Many of the loci associated with PBC, such as *IRF5*, *SOCS 1*, and *NF- κ B*, further suggest that Toll-like receptor signaling upstream to the IL-12 production may be relevant to disease (Figure 2). For example, IRF5 interacts with nuclear factor κ B, which consequently causes expression of a number of potentially relevant Th1 cytokines, including IL-12. A putative role for IRF5 in macrophage polarization is also relevant and highlighted in the pathogenesis of systemic lupus erythematosus, a complex autoimmune disease also associated genetically with *IRF5* variants.⁴¹ Additionally, IRF8 is a transcription factor that binds to the IL-12 promoter and directly modulates IL-12 and interferon gamma production. Downstream, engagement of IL-12 with the IL-12 receptor leads to production of another candidate gene in PBC, the STAT4 transcription factor, that leads to activation of Th1 cytokines (Figure 2).

It is relevant to reflect on the fact that genetic risk varies globally, such that a Japanese GWAS identifies a different panel of immunoregulatory pathways. This supports the concept of many pathways leading to disease. Genetic data ferment more precise biologic experimentation to refine understanding of the molecular immunology at play, whether through pathways associated with sensing of microbes or xenobiotics and antimicrobial responses. This is needed because we still lack sufficient clinical and experimental data concerning the specific role of IL-12 signaling, for instance, in PBC and animal models. In this regard, a case report documents the early onset of biliary cirrhosis in an IL-12–deficient child, although the immediate relevance to PBC is unclear.⁴² Collectively, more data will ensure we are better placed to guide future management strategies that seek to modulate IL-12 signaling and other relevant pathways. Pilot studies are under way to test the efficacy and safety of the human monoclonal anti-IL-12/IL-23 ustekinumab in patients with PBC (ClinicalTrials.gov identifier: NCT01389973).

GWAS in PSC

Several reports have documented results from GWAS including patients with PSC and controls mainly from northern Europe.^{15,43,44} The fibrotic strictures and intervening dilatations of the intrahepatic and extrahepatic bile ducts define the condition that results in the development of cholestatic cirrhosis in most cases.⁴⁵ In 10% to 20% of patients, development of cholangiocarcinoma represents an important addition to the hepatobiliary phenotype. Up to 80% of patients with PSC from northern Europe have concurrent IBD, whereas the frequency of IBD in Southern Europe and Asia is approximately 30% to 50%.⁴⁵ To date, most of the genetic studies in PSC have been performed in patients from northern Europe; therefore, the genetic data generated should be considered representative of the PSC and IBD phenotype. It is also important to note that 25% of patients with PSC of northern European origin with IBD have additional immune-mediated diseases.⁴⁶ Therefore, the phenotypic expression of PSC can be divided into the hepatobiliary disorder and the associated extrahepatic disease involving other organ systems.⁴⁷

The overlap between PSC and IBD as well as prototypical autoimmune diseases is reflected in the causal variants that have been detected to date (Figure 2).^{15,44,47} Similar to type 1 diabetes, celiac disease, and multiple sclerosis, the predominant attributable risk factors are found within the MHC on chromosome 6p21 and weaker associations are detected for multiple IBD-related and autoimmunity loci outside this region (Table 1). The overlap with IBD is far from complete, and even taking into account statistical power directly related to the number of cases of PSC available in the genetic studies, the majority of both ulcerative colitis and Crohn's disease risk loci do not confirm associations with PSC.^{45–49} This mirrors the clinical impression of IBD in PSC, which shows several distinguishing features when compared with regular UC, such as rectal sparing or backwash ileitis.⁵⁰

Of note, shared risk loci for PSC and PBC have recently been confirmed with *MMEL1*/*TNFRS14* on chromosome 1p36 and *CLEC16A* on chromosome 16p14. However, these risk loci are not specific to biliary disease (Table 1). Of interest, it appears that the *MMEL1*/*TNFRS14* allele may confer differential risk, because it has opposite effects in PSC as compared with PBC.^{33,43} *MMEL1* encodes a membrane metalloendopeptidase-like protein of unknown function located within the biliary tree; these proteins have diverse roles in metabolizing peptide hormones and β -amyloid, among others. More is known about *TNFRS14*, which encodes a receptor for cytokines and membrane-bound ligands, including B and T lymphocyte attenuator.⁵¹ The receptor acts as a molecular switch to provide positive costimulation to lymphocytes with certain ligands and negative signals with others, such as B and T lymphocyte attenuator. Notably, the gene product of *TNFRS14* also functions as an entry receptor for several herpes viruses, and in turn this engagement modulates immune responses to viral infection.⁵¹ Although the biology of this causal variant with PSC remains unexplored, it may be relevant that mice lacking B and T lymphocyte attenuator develop spontaneous autoimmune responses, cholangitis, irregular bile ducts, and histologic features comparable to PSC.⁵²

Other causal variants have been identified in PSC GWAS that potentially interact directly with infectious agents. Specifically, the risk of PSC has recently been associated with the *FUT2* gene encoding fucosyltransferase 2 (Table 1). The enzyme regulates expression of the ABO blood group antigens on the surface of epithelial cells and as a result determines the secretor status of the individual. Importantly, specific genotypes of *FUT2* have been linked with both bacterial and viral infections. For example, subjects homozygous for the *FUT2* alleles are nonsecretors who cannot express the H type 1 oligosaccharide ligand required for Norwalk virus binding⁵³; these individuals are protected from viral infection. Similarly, expression of the secretor status has been shown to influence binding of bacteria as well,

such as *Helicobacter pylori*.⁵⁴ The direct implications for patients with PSC are unknown, but it is interesting to note that the nonsecretor genotype found in patients with PSC has been associated with differences in the biliary microbiome with increased *Firmicutes* and diminished *Proteobacteria* in the bile.⁴³ An interesting parallel has also been observed in the gut microbiome in both healthy individuals and patients with Crohn's disease, where the nonsecretors also display a predominance of *Firmicutes* compared with *Proteobacteria* in the gut.⁵⁵

Similar to PBC, mechanistic studies addressing PSC-specific aspects of the risk loci are lacking. A common theme from genetic association studies is that the genetic predisposition in PSC is likely to resemble that of other immune-mediated diseases. However, it remains unclear how this perception aligns with the general lack of efficiency of trials of immunosuppressive medication in PSC. Potentially, the diagnosis of PSC needs to be made at a preclinical, prefibrotic stage of bile duct injury for such a treatment to be efficient, but this has never been shown clinically. Moreover, there is the possibility that essential pathogenic mechanisms may also be relatively inert to drugs tested so far. The observation that causal variants influence functional receptors for microbes also requires further study. There is a need to explore the biliary and intestinal microbiome in the context of genetic findings for gene-microbial interactions.^{55,56} Because the genetics of PSC seem to put the disease somewhat at the intersection of prototypical autoimmunity and IBD (Table 1), queries into such aspects in PSC may even provide clues as to the role of gut microbial community composition in these disease groups. In turn, these studies may lead to a better understanding of the environmental factors that affect PSC.

GWAS in Gallstone Disease

The first GWAS for gallstones found a highly significant association with the p.D19H variant of *ABCG8* (odds ratio, 2.2; population attributable fraction, 11%),⁵⁷ which had been previously identified as a risk factor in animal models.⁵⁸ This risk factor was confirmed globally.^{57,59–66} The *ABCG5/G8* hepatocanalicular heterodimeric transporter for cholesterol is a member of the adenosine triphosphate-binding cassette (ABC) transporters. Three of these genes, *ABCB4*, *ABCB11*, and *ABCG5/G8*, are responsible for the biliary secretion of phosphatidylcholine, bile salts, and cholesterol, respectively (Figure 4). Accordingly, their secretory capacities determine the composition of mixed micelles and multilamellar vesicles, which form the “liquid crystals” where cholesterol precipitates in bile.

Subsequent studies showed that obese women older than 60 years of age homozygous with the p.D19H risk allele had a 13% absolute 10-year risk of symptomatic gallstone disease compared with a range of 2% to 4% in noncarriers in the same risk stratum.⁶⁶ It is likely that decreased intestinal cholesterol absorption and increased hepatic cholesterol synthesis represent the primary defect intensifying the susceptibility for the development of stones.⁶⁷ This indicates that the lithogenic *ABCG8* p.D19H variant confers decreased cholesterol absorption, higher cholesterol synthesis, and a trend toward lower serum cholesterol levels as well as a better response to statins. Accordingly, *ABCG8* p.D19H might represent a “gain-of-function” mutation that increases clearance of sterol from the body.^{68–70} As a corollary, this genotype has also been shown to be protective against the development of atheromatous vascular disease by lowering cholesterol levels.^{71,72}

Preliminary data from 2 other GWAS for gallstone disease have identified additional lithogenic candidate genes (Table 1).^{59,60} Data from a genome-wide association study of 4300 Sardinian subjects with increased serum bilirubin levels was used to identify the Gilbert syndrome variant in the promoter of the *UGT1A1* gene as a candidate gene for gallstone disease⁷³ that was verified in 2800 German and Chilean patients with gallstones.⁷⁴

The link of diminished bilirubin conjugation with gallstone formation reinforces the concept of nucleation in supersaturated bile, because cholesterol gallstones usually contain small amounts of bilirubin pigment. The population-attributable fraction of the common *ABCG8* and *UGT1A1* variants in European subjects is 15% to 20%, approximating the total genetic gallstone risk stated in the preceding text (Figure 1B).

Other low-frequency variants with intermediate effects and rare Mendelian mutations have been identified in individual families with hereditary gallstones that show a geographic variance in prevalence (Figure 1B). These include *ABCB11* associated with reduced bile salt secretion, *APOB* linked with increased biliary cholesterol secretion, farnesoid X receptor *FXR* regulating bile salt excretion, cholecystokinin-1 receptor *CCKAR* linked with gallbladder motility, and *CYP7A1* linked with impaired cholesterol catabolism into bile salts (Table 1).^{22,75} Case reports and family linkage studies found that mutations of the *ABCB4* gene might underlie a peculiar type of hereditary gallstone disease, referred to as low phospholipid-associated cholelithiasis (LPAC). Patients with LPAC usually present with symptoms before the age of 40 years and have cholesterol gallbladder stones and intrahepatic sludge or microlithiasis. As a result, biliary colic often recurs after cholecystectomy. These patients also frequently present with obstetric or mild chronic cholestasis (Figure 1). Hence, gallstones are part of the phenotypic spectrum of progressive familial intrahepatic cholestasis (PFIC) type 2 associated with low GGT levels and PFIC type 3, which are caused by *ABCB11* and *ABCB4* mutations, respectively. The genetic diagnosis is hampered by the genotype-to-phenotype challenge in as much as a large number of gene variants await extensive functional studies.⁷⁶

Animal Models of Cholestasis Parallel Genomic Efforts

After initial experimental studies in prairie dogs, hamsters, and guinea pigs, susceptible and resistant in-bred strains of mice played an important role in defining the heritability for gallstone disease before GWAS. Following challenge with a lithogenic diet of 15% fat, 1% cholesterol, and 0.5% cholic acid, lithogenic (*Lith*) loci were identified as quantitative trait regions within the mouse genome that were subsequently characterized as risk factors for gallstones (Table 2).⁷⁵ Initially the *Lith9* candidate was found to harbor the *Abcg5/g8* genes, and subsequently other *Lith* loci in mice have been recognized that are relevant to human gallstone disease (Table 1).⁵⁸

In contrast, animal models have played a minor role in identifying the heritability of PBC and PSC but have provided other insights into disease.²¹ For PSC, histologic evidence of cholangitis or large duct disease has been induced by chemical cholangitis^{77–80} or targeting of specific genes that alter the composition of bile.^{81–86} The resultant pathology of genetic manipulation usually exemplifies other biliary processes, however, such as vanishing bile syndrome (Table 2). PSC models have been generated by bacterial products^{87,88} and *Cryptosporidium* infection in immune-deficient strains.^{89,90} The latter provides close parallels with acquired immunodeficiency syndrome cholangiopathy⁹¹ and supports a model whereby biliary strictures occur at the intersection of infection and immunodeficiency. On a more global level, it is notable that some of the candidate genes for PSC and PBC are linked with primary immunodeficiency syndromes, as recently reported for patients with IBD³⁰ (Figure 2).

In a similar vein, most of the mouse models of PBC that develop spontaneous antimitochondrial antibodies (AMA) and hepatic inflammation can be considered immune deficient (Table 2). These models include the NOD.c3c4 that develops granulomatous cholangitis^{92,93} as well as the T-cell transforming growth factor β receptor II dominant-negative (dnTGFBRII),⁹⁴ IL-2R $\alpha^{-/-}$,⁹⁵ and the Scurfy mouse lacking T-regulatory cells⁹⁶

that all die at a young age of diffuse extrahepatic inflammatory disease (Table 2). In the setting of immunodeficiency, these mice express mouse mammary tumor virus related to the betaretrovirus characterized in PBC; these viral proteins are located in biliary epithelium or lymphoid tissues that also display aberrant mitochondrial PDC-E2 expression, providing a mechanism for production of AMA.^{97–99} Other stimuli, including xenobiotics¹⁰⁰ and bacteria,¹⁰¹ have also been shown to trigger AMA production in mouse models. With regard to the known genetic risk factors for PBC, conflicting results have been derived in a spontaneous mouse model of PBC, where mice lacking IL-12 p35 or IL-12 p40 developed either increased hepatic fibrosis or diminished autoimmune cholangitis, respectively.^{102,103} One exception of the spontaneous AMA-producing mice is the anion exchanger deficient AE2_{a,b}^{−/−} model, which provides an example of altered bile composition driving small duct disease (Figure 4).¹⁰⁴ Collectively, these mouse models provide important information concerning immune, environmental, and genetic factors for disease.

Where Is the Missing Heritability?

Although GWAS have provided important genetic insights, concerns have been voiced that only a small proportion of the expected heritability has been uncovered by reported findings, referred to as the “missing heritability” of disease. Some have even gone as far as to state that the general hypothesis is incorrect and common variants do not explain the vast majority of genetic heritability of any disease.¹⁰⁵ This of course assumes that our previous estimates based on family studies of heritability for different diseases are correct; however, familial disease may be triggered by environmental and nongenetic elements, including the various microbial compartments.¹⁰⁶ In type 2 diabetes, for instance, a limited selection of bacterial genes within the gut microbiome¹⁰⁷ has recently been found to be more predictive of developing disease than accumulated GWAS data^{108,109} (Figure 1C).

Others have argued that statistical approaches are incomplete and Bayesian inference analyses have the power to identify an additional 20% of heritability.¹¹⁰ It is also possible that a smaller number of undetected loci encompass rare causal variants exerting relatively high risk. Another reason for missing heritability may lie with nonadditive genetic effects such as epistasis, which is the effect of the interaction of 2 or more genes on the phenotype. Although a proportion of additive genetic variation can be shown when all polymorphisms are considered simultaneously,¹¹¹ the extent of epistasis is difficult to determine using current methods. Suggestive evidence for epistasis arose from one study of PBC, for example, with demonstrated interaction between *IL12RB2* and *IRF5*.¹¹²

The difficulty with detection of missing heritability may also be related to the structural intricacy of our genomes. GWAS are powered to examine common genomic variants in single nucleotide polymorphisms with minor allele frequency of 5% or greater. Therefore, rare genetic variants that may cause illness or contribute to disease escape detection because we simply lack sufficient numbers of patients.¹⁰⁵ Also, copy number variants with insertions and deletions as well as inversions and translocations are difficult to detect using data from GWAS. Furthermore, GWAS are unable to discover de novo mutations occurring in the germline that have been uncovered by resequencing candidate genes associated with common disorders such as autism.¹¹³

Missing heritability may also result from epigenetic effects such as methylation of CpG islands that regulate gene expression. Studies of methylation status in twins with multiple sclerosis, for example, have been somewhat disappointing, largely because the differences between discordant twins with multiple sclerosis were found to be far less than in unrelated individuals.¹¹⁴ Another example of epigenetic modification involves the interesting phenomena of X chromosomal inactivation that ensures that all cells in male and female

subjects have one active X chromosome. Enhanced X chromosome monosomy rates have been observed in patients with PBC,¹¹⁵ other autoimmune diseases such as systemic lupus erythematosus, and some hormone-responsive cancers.¹¹⁶ There is also the recent findings of increased Y chromosome loss in male subjects with PBC.¹¹⁷ The implications of chromosomal inactivation remain a mystery in chronic disease, and GWAS in PBC have not pinpointed sex chromosomal changes as relevant to date.

Overall, the accumulated data suggest that approximately 10% to 20% of the heritability of complex immune-mediated diseases have been uncovered by GWAS, which is comparable to that seen with other complex diseases such as cancer.¹¹⁸ Therefore, it is likely that some of the missing risk will be accounted for by environmental agents, and some have gone as far to suggest that the major contributors to many complex diseases are nonhereditary.¹¹⁹

Beyond GWAS to the Microbiome

On a simplistic level, we cohabit a microecosystem contingent on an interaction with a staggeringly diverse microbiologic environment that has influenced our genetic variation over time. As a result, the application of genetics should reach beyond the host genome to so-called “meta-genetics” by investigating all the genes in the metagenome.¹²⁰ Although we have already commented on the differences in the microbiome of patients with Crohn's disease and PSC associated with the *FUT2* risk loci,^{43,55} these studies lack the design to directly implicate the changes in the microbiome with the disease process and animal models are often required to show the importance of the microbiome. One staggering example was recently described in a nonobese diabetic mice model, whereby the exchange of gut microflora from male to female mice transformed the disease to the male phenotype and delayed the onset of diabetes.¹²¹

Other elegant animal models have shown the interaction of susceptibility genes with the microbiome to promote a disease-specific phenotype.¹²² For instance, mice missing key inflammasome genes fed a methionine-choline-deficient diet develop progressive nonalcoholic steatohepatitis.¹²³ In this model, changes in the gut microbiome were associated with modulation of cytokine expression and development of the metabolic syndrome. Indeed, a direct role for the microbiota was inferred by cohousing the inflammasome-deficient mice with wildtype mice that subsequently developed increased obesity and hepatic steatosis as well.¹²³ A similar study linking microbiome and host susceptibility genes has been conducted using a mouse model with a hypomorphic mutation in *ATG16L1*, a Crohn's disease susceptibility gene involved in autophagy. After infection with murine norovirus, the mice developed intestinal pathology comparable to IBD, and intriguingly the disease was preventable by administration of broad-spectrum antibiotics.¹²⁴ This metagenetic process illustrates how the interaction of viral infection and Crohn's disease susceptibility genes disrupted the commensal flora to generate an IBD phenotype.

Another well-recognized role of the microbiome is prevention of pathogen invasion by organisms. One case in point is *Clostridium difficile* infection in patients receiving antibiotics who lose their ability to provide “colony resistance.” Similarly, the microbiome is altered in patients with global immunodeficiency to provide diminished protection against common pathogens.¹²⁵ In these circumstances, complex infections with diverse organisms may arise that cause phenotypes resembling immune-mediated inflammatory diseases. One example is the various combinations of microbial infection with human immunodeficiency virus, cytomegalovirus, *Cryptosporidium*, and other pathogens that have been associated with the development of acquired immunodeficiency syndrome cholangiopathy.⁹¹ The study of bile microbiota is relatively unexplored as compared with other bodily compartments, and

an unbiased metagenomic survey would seem to be an attractive approach to investigate the microbiome associated with cholestatic diseases.

Effect on Clinical Practice

With the falling costs of next-generation sequencing, whole exome and genome sequencing is starting to move into the clinic. In one remarkable report, the genome of a 4-year-old boy with severe inflammatory bowel disease was sequenced to reveal a loss-of-function polymorphism in the *XIAP* gene encoding the X-linked inhibitor of apoptosis protein.¹²⁶ XIAP plays an important role in regulating immune responses to mucosal flora and directly interacts with the intracellular pattern recognition receptor encoded by *NOD2*, the first susceptibility gene found for Crohn's disease.¹¹¹ The findings had translational implications and a bone marrow transplant corrected the pathology, presumably in part by restoring XIAP function.¹²⁶ Indeed, the investigation of infants with very early-onset IBD has uncovered other novel candidate genes such as those encoding the IL-10 receptor or neutrophil cytosolic factor 2 in the reduced nicotinamide adenine dinucleotide phosphate oxidase complex that intersect along pathways previously uncovered by GWAS.^{127,128} Accordingly, the study of familial cases or highly characterized phenotypes of PBC, PSC, or gallstone disease might lead to the identification of rare, high-penetrant alleles at risk loci that shed light on pathogenesis. Phenotypes might include the biochemical response to ursodeoxycholic acid or the presence of pruritus.

With regard to genetic tests for gallstone disease, diagnostic tests in familial gallstone disease comprise resequencing of the *ABCB4* and/or *ABCB11* genes in patients who present with PFIC or LPAC phenotypes (Figure 1) and genotyping of the *UGT1A1* promoter variant, which predisposes to unconjugated hyperbilirubinemia and gallstones¹²⁹ but is also associated with decreased cardiovascular risk.¹³⁰ In the future, it is possible that polygenic disease predisposition for gallstones and cholestasis might be assessed by gene tests. It is tempting to speculate that stratified polygenic risk scores¹³¹ based on genetic factors, including variants of *ABCG8*, *UGT1A1*, and *ABCB4*, and environmental factors, such as overeating, physical inactivity, and infections, might assist in defining normal and high-risk groups. Whereas lifestyle modifications with weight reduction, increased physical activity, and dietary alterations are appropriate for the normal-risk group, high-risk individuals might benefit from early prevention and diagnosis as well as targeted therapy with ursodeoxycholic acid, statins, ezetimibe, and/or modulators of bile salt signaling pathways.¹³² A similar process may be relevant in patients with PBC and PSC, with personal stratification of clinical events informed by genetic analysis of risk variants. Indeed, preliminary attempts are being made in regard to recurrence of PBC in liver allografts.¹³³

Despite the optimism, there is a huge gap between the genetic information collected to date and the surprisingly small influence this knowledge has had on the clinical management of patients. Translation into real life is still pending, a fate that cholestatic liver diseases share with other complex diseases studied on a genome-wide level. Nevertheless, overall, the advances to date in understanding the genetics of cholestasis speak broadly to the ultimate goal of all such studies to guide personalized care that is rational and mechanism driven.

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Appendix

Concepts and Definitions Relevant to Genetic Studies in Cholestasis^{76,120,134}

Allele: A particular nucleotide (or nucleotide sequence) at any type of polymorphism

Causal variant: A genetic variant that has a direct functional effect on disease risk or that causes the observed association signal (difficult to prove causality beyond reasonable doubt)

Epistasis: The phenomenon in which the effects of one genetic variant are modified by one or several other genetic variants

Exome: Protein coding regions (exons) of the genome

Genotype: The combination of alleles on the 2 chromosomes of an individual

Haplotype: A distinct combination of 2 or more alleles of polymorphisms that occur together on the same chromosome

Heritability (h^2): The phenotypic variation in a population that is contributed by genetic variation

HLA: Antigen-presenting molecules encoded by genes within the major histocompatibility complex located on human chromosome 6p21

Linkage disequilibrium: Occurs when 2 alleles of polymorphisms on the same chromosome occur more frequently together than would be expected from their respective population frequencies

Meta-genetics: Genetic and genomic studies considering all of the genes in symbiotic organisms (the metagenome), for example, as opposed to considering host genes or microbial genes in isolation

Microbiome: The sum of all microbial genes in a given host compartment (eg, the gut microbiome)

Microbiota: The sum of all microbial species in a given host compartment (eg, the gut microbiota)

Polymorphisms: Genetic variants arising from mutational events in DNA; the variant should occur at a frequency >1% in the general population

Population attributable fraction: The proportional reduction in average disease risk over a specified time interval that would be achieved by eliminating the exposures of interest from the population while the distribution of other risk factors within the population remains unchanged

Single nucleotide polymorphisms: DNA polymorphisms that differ at only a single base

Abbreviations used in this paper

AH	ancestral haplotype
AMA	antimitochondrial antibodies
GGT	γ -glutamyltransferase
GWAS	genome-wide association studies
IBD	inflammatory bowel disease
IL	interleukin
LPAC	low phospholipid-associated cholelithiasis
MHC	major histocompatibility complex
PBC	primary biliary cirrhosis
PDC-E2	pyruvate dehydrogenase complex E2
PFIC	progressive familial intrahepatic cholestasis
PSC	primary sclerosing cholangitis
Th	T-helper
TNF	tumor necrosis factor

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Figure 1.

Interaction of genes and environment in complex cholestatic disease. (A) Variations in severity of disease manifestation are observed with different genotypic variants in the *ABCB4* encoding the biliary phosphatidylcholine transporter. Heterozygous *ABCB4* variants encompass mild phenotypes, whereas homozygous deficiency leads to more severe diseases (ie, biliary cirrhosis and chronic liver failure). Specific genotypes might also contribute to chronic cholestasis and/or modify disease progression in patients with PBC and PSC. (B) Venn diagram illustrating variants that have been confirmed in replication studies. The size of each circle reflects the estimated number of adults with gallstones in each continent. Previous studies showed that Latin American populations have the highest (~30%) incidence of gallstone disease, with intermediate frequency of the disease in Europe (15%–20%) and lowest relative frequency in Asia (5%–6%). (C) The gut microbiome is more predictive of type 2 diabetes than current candidate loci derived by GWAS. A comparison of data derived from candidate type 2 diabetes loci^{108,109} and a selection of genes derived from metagenomic analyses of the gut microbiome from individuals with type 2 diabetes¹⁰⁷ shows a superior correlation with the microbiome (based on the original cited data). ICP, intrahepatic cholestasis of pregnancy.

Figure 2. Circus plot of associations of cholestasis candidate genes with other diseases. Many of the candidate genes associated with PBC and PSC have previously been linked with other immune-mediated disorders and few, if any, specifically associated with biliary disease. Candidate loci found in patients with gallstone disease are predominantly associated with bile contents, lipid metabolism, and risk of coronary artery disease. Each *radial line* represents a PBC, PSC, or gallstone locus, ordered by genomic position and labeled around the rim, and each *circular line* represents a phenotype, with all points on a line colored according to the phenotype key given. Points sit at the intersection of radial and circular lines and represent sharing of a PBC, PSC, or gallstone locus with a given phenotype. The

location of each locus is recorded in Table 1 and is shown as shapes in this figure, with *triangles* indicating PBC-specific disease, *squares* indicating PSC-specific disease, and *circles* indicating gallstone disease. AnkS, ankylosing spondylitis; CAD, coronary artery disease; CeID, celiac disease; CholM, cholesterol metabolism; CroD, Crohn's disease; GD, Graves' disease; GS, gallstone disease; IBD, inflammatory bowel disease; MS, multiple sclerosis; PID, primary immunodeficiency syndromes; Ps, psoriasis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SS, systemic sclerosis; T1D, type 1 diabetes; TrigM, triglyceride metabolism; UC, ulcerative colitis; Viti, vitiligo.

Figure 3.

The putative role of IL-12 signaling in risk of PBC. IL-12 is a heterodimeric cytokine encoded by *IL-12A* and *IL-12B*, produced mainly by monocytes and macrophages, dendritic cells, and neutrophils. A strong role for IL-12 and related cytokines is implied in the pathogenesis of PBC by the identification of upstream and downstream mediators of IL-12 signaling as susceptibility loci in the PBC GWAS. CTLA-4, cytotoxic T-lymphocyte antigen 4; Foxp3, forkhead box P3; IFN- γ , interferon gamma; I κ B, inhibitory κ B; IRF-5, interferon regulatory factor 5; JAK, Janus kinase; NF- κ B, nuclear factor κ B; SOCS-1, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; TLR, toll-like receptor; TNFRSF, tumor necrosis factor receptor super-family; TYK2, tyrosine kinase 2.

Figure 4.

Modeling of genetic influence in cholestatic liver diseases. The liver compartment comprises genes involved in hereditary cholestatic syndromes and gallstone disease (highlighted in red). The same loci and loci in the biliary compartment may also serve as modifiers in immune-mediated injury, as exemplified by the immunologic compartment. Susceptibility to cholestatic liver disease has also recently been shown to be associated with genetic factors influencing microbial community composition, as illustrated by the gut compartment that also involves the enterohepatic circulation of bile acids. Cyp7A1, cytochrome P450, family 7, subfamily A, polypeptide 1; NTCP, Na/taurocholate cotransporting polypeptide; OATP, organic anion-transporting polypeptide; BSEP, bile salt export pump; MRP2, multidrug resistance-associated protein 2; MDR3, P-glycoprotein-3/multiple drug resistance-3; ABCG5/8, adenosine triphosphate-binding cassette subfamily G member 5/8 heterodimer; ATP8B1, adenosine triphosphatase, class I, type 8B, member 1; CYP3A, cytochrome P450, family 3, subfamily A; UGT, uridine diphosphate glucosyltransferase; SULT, sulfotransferase; GST, glutathione S-transferase; MRP3/4, multidrug resistance-associated protein 3/4; OST, organic solute transporter; F, FXR, farnesoid X receptor; S, SXR/PXR, steroid and xenobiotic receptor/pregnane X receptor; C, CAR, constitutive androstane receptor; CFTR, cystic fibrosis transmembrane conductance regulator; TGR5, G protein-coupled bile acid receptor 1; ASBT, apical sodium-dependent bile acid transporter; AE2, anion exchange protein 2; TCR, T-cell receptor; ANCA, anti-neutrophil cytoplasmic antibodies; FUT2, fucosyltransferase 2; IBABP, ileal bile acid binding protein; FGF19, fibroblast growth factor 19.

Table 1

Current Non-HLA Associations Recognized for PBC, PSC, and Gallstone Disease

Chromosome (Position Mb)	Probable gene association	Primary cholestatic disease associations	Other disease associations from GWAS
1 (2.5)	<i>MMEL1, TNFRSF14</i>	PBC, PSC	Celiac disease, multiple sclerosis, RA, UC
1 (67.7)	<i>IL12RB2</i>	PBC	Systemic sclerosis
1 (197.5)	<i>DENND1B</i>	PBC	Asthma, Crohn's disease
2 (3.7)	<i>ALLC</i>	PSC	
2 (21.2)	<i>APOB</i>	GS	Cholesterol metabolism, coronary artery disease, familial hypercholesterolemia, triglyceride metabolism
2 (44.1)	<i>ABCG8</i>	GS	GS, cholesterol metabolism, coronary artery disease
2 (61.1)	<i>REL</i>	PSC	Celiac disease, IBD, lymphoma, psoriasis, SLE, RA
2 (111.9)	<i>BCL2L11</i>	PSC	Lymphoma
2 (169.8)	<i>ABCB11</i>	GS	Benign recurrent intrahepatic cholestasis 2, drug-induced liver injury, intrahepatic cholestasis of pregnancy, neonatal giant cell hepatitis, PFIC 2, hepatocellular carcinoma
2 (191.8)	<i>STAT1, STAT4</i>	PBC	Celiac disease, RA, systemic sclerosis, SLE
2 (204.5)	<i>CD28</i>	PSC	Celiac disease, chronic lymphocytic leukemia, Graves' disease
2 (219.1)	<i>GPBAR1</i>	PSC	UC
2 (228.7)	<i>CCL20</i>	PBC	Hypertension
2 (234.7)	<i>UGT1A1</i>	GS	Bladder cancer, Gilbert's syndrome, irinotecan toxicity, neonatal jaundice
2 (241.5)	<i>GPR35</i>	PSC	UC
3 (17.0)	<i>PLCL2</i>	PBC	
3 (49.7)	<i>MST1</i>	PSC	IBD
3 (119.2)	<i>CD80</i>	PBC	Celiac disease
3 (149.2)	<i>TM4SF4</i>	GS	
3 (159.7)	<i>IL12A, SCHIP1</i>	PBC	Celiac disease, multiple sclerosis
4 (3.5)	<i>LRPAP1</i>	GS	
4 (26.5)	<i>CCKAR</i>	GS	
4 (38.7)	<i>KLF3</i>	GS	
4 (103.4)	<i>NFKB1</i>	PBC	Schizophrenia
4 (123.4)	<i>IL2, IL21</i>	PSC	Celiac disease, Graves' disease, psoriasis arthritis, primary immunodeficiency syndromes, RA, Sjögren's syndrome, SLE, type 1 diabetes, UC
4 (169.4)	<i>PALLD</i>	GS	Nonalcoholic fatty liver disease, pancreatic cancer
5 (35.9)	<i>IL7R</i>	PBC	UC, multiple sclerosis, type 1 diabetes
5 (158.7)	<i>IL12B</i>	PBC	Ankylosing spondylitis, Crohn's disease, multiple sclerosis, psoriasis, primary immunodeficiency syndromes, UC
6 (90.6)	<i>BACH2</i>	PSC	Celiac disease, Crohn's disease, multiple sclerosis, type 1 diabetes
6 (137.5)	<i>IL22RA2</i>	PBC	Multiple sclerosis

Chromosome (Position Mb)	Probable gene association	Primary cholestatic disease associations	Other disease associations from GWAS
7 (36.9)	<i>ELMO1</i>	PBC	Celiac disease
7 (87.0)	<i>ABCB4</i>	GS	Intrahepatic cholestasis of pregnancy, LPAC, PFIC 3, hepatocellular carcinoma
7 (128.6)	<i>IRF5, TNPO3</i>	PBC	Systemic sclerosis, SLE, RA, UC
8 (59.4)	<i>CYP7A1</i>	GS	Cholesterol metabolism, hypertension, neuromyelitis optica
9 (117.5)	<i>TNFSF15</i>	PBC	Crohn's disease, UC
9 (139.2)	<i>CARD9</i>	PSC	Ankylosing spondylitis, Crohn's disease, UC
10 (6.1)	<i>IL2RA</i>	PSC	Alopecia areata, Crohn's disease, multiple sclerosis, RA, primary immunodeficiency syndromes, SLE, type 1 diabetes, vitiligo
10 (26.5)	<i>GAD2</i>	GS	Obesity
11 (64.1)	<i>RPS6KA4</i>	PBC	
11 (111.2)	<i>POU2AF1</i>	PBC	
11 (111.5)	<i>SIK2</i>	PSC	
11 (116.7)	<i>APOA1</i>	GS	Cholesterol metabolism, triglyceride metabolism
11 (118.8)	<i>CXCR5, DDX6</i>	PBC	Multiple sclerosis
12 (6.4)	<i>TNFRSF1A</i>	PBC	Multiple sclerosis, primary immunodeficiency syndromes
12 (21.2)	<i>SLCO1B1</i>	PSC, GS	Hyperbilirubinemia, drug-induced liver injury, statin-induced myopathy
12 (48.2)	<i>HDAC7</i>	PSC	IBD
12 (100.9)	<i>NR1H4</i>	GS	
12 (111.8)	<i>SH2B3, ATXN2</i>	PBC, PSC	Celiac disease, cholesterol metabolism, coronary artery disease, RA, type 1 diabetes
12 (121.4)	<i>HNFI1alpha</i>	GS	Cholesterol metabolism, coronary artery disease, GGT levels, type 2 diabetes
13 (43.1)	<i>TNFSF11 (RANKL)</i>	PBC	Crohn's disease
13 (93.5)	<i>GPC5, GPC6</i>	PSC	Multiple sclerosis
13 (103.7)	<i>SLC10A2</i>	GS	Bile salt malabsorption, Crohn's disease, triglyceride metabolism, prostate cancer
14 (64.7)	<i>ESR2</i>	GS	Gallbladder cancer
14 (68.2)	<i>RAD51L1</i>	PBC	
14 (103.6)	<i>TNFAIP2</i>	PBC	
16 (11.3)	<i>CLEC16A, SOCS1</i>	PBC, PSC	Celiac disease, immunoglobulin A deficiency, multiple sclerosis, SLE, type 1 diabetes, UC
16 (57.0)	<i>CETP</i>	GS	Cholesterol metabolism, coronary artery disease, macular degeneration, triglyceride metabolism
16 (85.9)	<i>IRF8 - FOXF1</i>	PBC	SLE, UC
17 (38.1)	<i>IKZF3, ZBP2, GSDMB, ORMDL3</i>	PBC	Asthma, Crohn's disease, RA, type 1 diabetes, UC
17 (44.0)	<i>MAPT</i>	PBC	Parkinson's disease
18 (52.9)	<i>TCF4</i>	PSC	Corneal dystrophy, diabetic retinopathy, schizophrenia
18 (55.3)	<i>ATP8B1</i>	GS	Benign recurrent intrahepatic cholestasis 1, PFIC 1, vitiligo

Chromosome (Position Mb)	Probable gene association	Primary cholestatic disease associations	Other disease associations from GWAS
18 (67.5)	<i>CD226</i>	PSC	Mean platelet volume, type 1 diabetes
19 (10.5)	<i>TYK2</i>	PBC	Crohn's disease, primary immunodeficiency syndromes, psoriasis, type 1 diabetes
19 (45.4)	<i>APOC1</i>	GS	Alzheimer's disease, cholesterol metabolism, triglyceride metabolism
19 (47.2)	<i>FUT2, PRKD2, STRN4</i>	PSC	Alkaline phosphatase levels, bipolar disorder, chronic lymphocytic leukemia, Crohn's disease, folate and vitamin B ₁₂ levels, multiple sclerosis, type 1 diabetes
19 (50.9)	<i>SPIB</i>	PBC	
21 (43.8)	<i>UBASH3A, PSMG1</i>	PSC	Ankylosing spondylitis, celiac disease, IBD, RA, type 1 diabetes, vitiligo
21 (45.6)	<i>ICOSLG</i>	PBC	Celiac disease, UC
22 (25.0)	<i>GGT1</i>	PSC	
22 (39.8)	<i>MAP3K7IP1, SYNGR1</i>	PBC	Crohn's disease
22 (48.8)	<i>IL17REL</i>	PSC	UC
X (66.8)	<i>AR</i>	GS	Cholesterol metabolism, male pattern baldness, prostate cancer

NOTE. For gene name abbreviations, see <http://www.ncbi.nlm.nih.gov/gene>.

RA, rheumatoid arthritis; UC, ulcerative colitis; GS, gallstone disease; SLE, systemic lupus erythematosus.

Animal Models of Cholestasis

Table 2

Human disease (reference)	Animal model			Phenotype				
	Genotype background	Spontaneous versus inducible	Pathology	Portal inflammation	AMA production	Gallstone formation	Biliary strictures	Colitis
PBC ^{92,93}	NOD.c3e4 mouse NOD strain with multiple innate and adaptive immune deficiencies	Spontaneous Immunodeficient	50% liver mortality from 9 to 11 mo	++	50%–60%	–	Cystic dilatation of bile ducts	–
PBC ⁹⁴	dnTGFβRII mouse C57Bl dominant negative TGFβ receptor II in lymphocytes	Spontaneous Immunodeficient	Inflammatory disease in lung, kidneys and intestinal tract Death ~25 wk	+++	100%	–	ND	++
PBC ⁹⁵	IL-2Rα ^{−/−} mouse C57Bl knockout of IL-2 receptor α	Spontaneous Immunodeficient	Inflammatory bowel disease within 12 wk Death ~25 wk	+++	100%	–	ND	++
PBC ⁹⁶	Scurfy mouse C57Bl knockout of FoxP3	Spontaneous Immunodeficient	Multioorgan inflammation Death ~4 weeks	+++	100%	–	NA	+
PBC ¹⁰⁴	Ae2 _{ab} ^{−/−} mouse FVB/N, 129/Sv knockout of AE2 Cl/HCO ₃ anion exchanger	Spontaneous Altered bile composition	Slow onset of AMA over 52 wk with biliary inflammation	+	40%–80%	–	ND	–
PBC ¹⁰⁰	2-Octynoic acid-treated mouse C57Bl, NOD 1101	Inducible Xenobiotic with Freund's adjuvant	Portal inflammation with granuloma formation	+	100%	–	ND	–
PBC ¹⁰¹	<i>Novosphingobium aromaticivorans</i> infection NOD 1101 mouse	Inducible Bacterial infection	Portal inflammation, cholangitis with granuloma formation	++	80%–100%	–	ND	–
PSC ⁷⁸	Retrograde biliary TNBS Sprague–Dawley rats	Inducible Chemical cholangitis	Pericholangitis and periductal onion skin fibrosis	+	ND	–	Ecatic extrahepatic ducts and beading intrahepatic ducts	–
PSC ⁷⁹	Retrograde biliary TNBS alcohol and incomplete BD ligation Lewis rats	Inducible Chemical cholangitis	Pericholangitis and biliary fibrosis	+	ND	–	Strictures in intrahepatic and extrahepatic ducts	–
PSC ⁷⁷	Lithocholic acid feeding Swiss albino mice	Inducible Chemical cholangitis	Bile infarcts, fibrosis, destructive cholangitis	+	ND	–	ND	–
PSC ⁸⁸	<i>Helicobacter</i> sp. intraperitoneal injection A/JCr, C3H/Hen, C57Bl/6	Inducible Bacterial infection	Mild hepatitis, pericholangitis, and ductal proliferation	+	ND	–	ND	+
PSC ^{89,90}	<i>Cryptosporidium parvum</i> gavage BALB/c Nu/Nu, NHI-III Nu/Nu	Inducible Bacterial infection	Severe cholangitis, pericholangitis, biliary fibrosis, and hepatic necrosis	+++	ND	–	ND	+
PSC ⁸⁷	Small bowel bacterial overgrowth following blind loop construction Lewis and Wistar rats	Inducible Bacterial products	Cholangitis, pericholangitis, bile duct proliferation, and onion skin fibrosis	+	ND	–	Ecatic extrahepatic with irregular intrahepatic ducts	+
PSC ⁸⁰	Rectal administration of fMLT ^{+/−} acetic acid Lewis rats	Inducible Bacterial products	Cholangitis and pericholangitis	+	ND	–	Irregular, intrahepatic ducts	+

Human disease (reference)	Animal model			Phenotype				
	Genotype background	Spontaneous versus inducible	Pathology	Portal inflammation	AMA production	Gallstone formation	Biliary strictures	Colitis
PSC, gallstones ^{82,84,86}	<i>Abcb4</i> ^{-/-} mouse <i>Mdr2</i> knockout mice on 129, FVB/N, or BALB/c backgrounds	Spontaneous Altered bile composition	Cholangitis, periductal onion skin fibrosis, vanishing bile ducts, intrahepatic and extrahepatic gallstones	+	ND	+	+	-
PSC, gallstones ^{81,83}	<i>CFTR</i> mouse models (KO, DF508) on 129/C57BL/6 background	Spontaneous Altered bile composition	Cholangitis, periductal onion skin and fibrosis, black pigment stones	+	ND	+	ND	-
PSC ⁸⁵	Ferrochelatase-deficient mouse BALB/c <i>fch/fch</i> model of erythropoietic protoporphyria	Spontaneous Altered bile composition	Cholangitis, periductal onion skin and fibrosis	+	ND	-	ND	-
Gallstones ^{135,136}	Syrian hamster	Inducible EFA-deficient diet (20% casein, 40%–74% sucrose/glucose) + 10% butter fat or 25% fiber	Cholesterol or black pigment gallbladder stones; ductular proliferation and portal fibrosis	+	ND	+	ND	-
Gallstones ^{137,138}	Prairie dog	Inducible Cholesterol diet (0.3%–1.2% cholesterol)	Cholesterol gallbladder stones and mucin hypersecretion, polypoid hyperplasia of gallbladder mucosa and inflammation; microvesicular steatosis, ductular proliferation, and portal fibrosis	+	ND	+	ND	-
Gallstones ¹³⁹	Inbred mouse strain C57L/J and others	Inducible Lithogenic diet (1% cholesterol, 0.5% cholic acid, 15% butter fat)	Cholesterol gallbladder stones, gallbladder wall thickening with stromal granulocyte infiltration, fibrosis, and epithelial cell indentation		ND	+	ND	-
Gallstones ¹⁴⁰	Guinea pig	Inducible Chow supplemented with 0.5% cholesterol	Calcium bilirubinate precipitates in enlarged gallbladders, fatty liver	+	ND	+	ND	-

ND, not done; NA, not available; TNBS, trinitrobenzene sulfonic acid; fMLT, *N*-formyl L-methionine L-leucine L-tyrosine.