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Implications of genome-wide association studies in novel therapeutics in primary biliary cirrhosis

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Abstract

Genome-wide association studies (GWAS) have revolutionized the search for genetic influences on complex disorders, such as primary biliary cirrhosis (PBC). Recent GWAS have identified many disease-associated genetic variants. These, overall, highlighted the remarkable contribution of key immunological pathways in PBC that may be involved in the initial mechanisms of loss of tolerance and the subsequent inflammatory response and chronic bile duct damage. Results from GWAS have the potential to be translated in biological knowledge and, hopefully, clinical application. There are a number of immune pathways highlighted in GWAS that may have therapeutic implications in PBC and in other autoimmune diseases, such as the anti-interleukin-12/interleukin-23, nuclear factor-kb, tumor necrosis factor, phosphatidylinositol signaling and hedgehog signaling pathways. Further areas in which GWAS findings are leading to clinical applications either in PBC or in other autoimmune conditions, include disease classification, risk prediction and drug development. In this review we outline the possible next steps that may help accelerate progress from genetic studies to the biological knowledge that would guide the development of predictive, preventive, or therapeutic measures in PBC.

Introduction

Primary biliary cirrhosis (PBC) is the most common autoimmune liver disease and is considered a model of organ-specific autoimmune diseases [1]. It is characterized by loss of tolerance, production of a multilineage immune response to mitochondrial auto-antigens, inflammation of small bile ducts, and in some patients, the development of fibrosis and

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cirrhosis. Patients with PBC may present with symptoms as fatigue, pruritus and/or jaundice, but the majority of them are asymptomatic at diagnosis. A diagnosis of PBC can be made with confidence in adult patients with otherwise unexplained elevation of alkaline phosphatase and presence of anti-mitochondrial antibodies (AMA) at a titre of ≥1:40 and/or AMA type M2. A liver biopsy is not essential for the diagnosis of PBC in these patients, but allows activity and stage of the disease to be assessed. Progression of disease in PBC is variable with a substantial proportion of patients eventually developing cirrhosis and liver failure. The only licensed therapy for PBC is ursodeoxycholic acid (UDCA) which has been demonstrated to exert anticholestatic effects in various cholestatic disorders. Several potential mechanisms and sites of action of UDCA have been unraveled in clinical and experimental studies which might explain its beneficial effects. These include protection of injured cholangiocytes against the toxic effects of bile acids, particularly at an early stage; stimulation of impaired hepatocellular secretion by mainly post-transcriptional mechanisms, including stimulation of synthesis, targeting and apical membrane insertion of key transporters, more relevant in the advanced cholestasis; stimulation of ductular alkaline choleresis and inhibition of bile acid-induced hepatocyte and cholangiocyte apoptosis.

Many aspects of the basic biology of PBC, including, rigorous definitions of the signature AMA, disease-specific anti-nuclear autoantibodies, the definition of autoreactive $CD4^+$ and $CD8^+$ T-cell responses and the association with some immunological pathways, such as IL-12, NF-κB and TNF, have been elucidated through the development of animal models of PBC, including models that develop fibrosis [2, 3]; and large scale epidemiologic studies, including a number of genome-wide association studies (GWAS) (reviewed in [1, 4–5]). Despite this knowledge, an enormous gap still exists between our knowledge of the etiopathogenesis of PBC and new therapeutic approaches for patients. There has not been a new drug approved for PBC for more than 2 decades and indeed newer biologics merits further investigation to show their safety and efficacy [6]. Since there are a significant number of patients with PBC who do not respond to UDCA [19], there is a strong need for new therapies.

The advent of genome-wide association technology has transformed the landscape of human genetics research. Thanks to GWAS, common genetic variants associated with well-phenotyped diseases, such as inflammatory bowel disease [7] and diabetes [8], have been identified in a non-biased fashion. Such studies are conducted based on the assumption that at least some of the genetic influences on many common diseases are attributable to a limited number of common allelic variants that are present in more than 5% of the population [9] The best-known examples of common disease genes include the ApoE ε4 allele in Alzheimer's disease [10], Factor V (C→A at 1691) allele in deep-venous thrombosis [11], and CKR5Δ32 in resistance to human immunodeficiency virus infection [12].

GWAS typically involve the analysis of hundreds of thousands of common single nucleotide polymorphisms (SNPs) and are not limited to known genes or regulatory regions. These studies require a large sample size not only in order to detect robust associations as false-positive findings arise due to chance alone, but also because of the low effect size of most disease variants detected in GWAS (odds ratios = 1.1−1.4) [13]. The landmark Wellcome

Trust Case Control Consortium (WTCCC) study included 2000 cases of each of seven common diseases and 3000 shared controls [14]. It is also mandatory for any GWAS protocol to include a replication of associations claimed to be genuine, in at least one independent case-control panel.

GWAS provide starting points for further biological studies of the affected pathways. Strategies for translating the genetic findings into an applicable understanding of disease pathogenesis are a work in progress. Despite the advent of newer technologies for genetic analysis, in particular sequencing-based methods for identifying disease-associated variants, GWAS-based findings will remain essential, for some time, for designing effective clinical applications. This is in part because the mass of GWAS data that have already been generated continues to be mined for additional trait associations, and because of the unflagging pace with which new GWAS findings are still being published. On the other hand, the prolific associative findings of GWAS are creating a substantial need for downstream genetic, biochemical and cell biological assays to confirm the biological relevance of genotype-phenotype associations and to elucidate the underlying mechanisms of disease.

Recent highlights from high-throughput genetic studies of PBC

In the past decade various GWAS have revealed dozens of disease-associated loci and have provided insights into the allelic architecture of many complex disorders, such as PBC [6–9, 69,70,75]. Large, well-characterized patient cohorts for high-throughput genetic studies of PBC have been established in Europe, North America and Japan; and four GWAS [15–18] and two iCHIP-association [19, 20] studies of PBC have been published.

Similar to the risk alleles identified by GWAS findings for other immune-related conditions, such as rheumatoid arthritis (RA), Crohn's disease (CD) and multiple sclerosis (MS), many of the risk alleles identified in PBC by GWAS are found in conjunction with genes related to immune function, both within and outside the human leukocyte antigen (*HLA*) [21]. Overall, the data suggest important contributions from a number of immune pathways to the development of PBC (Table 1): from the differentiation of the myeloid cell compartment (*SPIB, IRF5, IRF8* and *IL-7R*) to antigen presentation and T-cell differentiation (*IL7R*, class II *HLA, CD80, IL12, IL12R, TYK2, STAT4, SOCS1*) up to B-cell function (*SPIB, IRF8, PLC-L2, SPIB, PLC-L2, IKZF3, CXCR5*) [22]. Importantly, most of these genes play important roles in many different immune pathways and are not specifically involved in a single, unique function. For instance, IL-7R is induced upon T-cell positive selection and controls thymic CD8 lineage specification and peripheral naive T-cell homeostasis [23] while also having a role in myeloid cell differentiation [24]. Along the same lines, IRF8 is widely involved in immune functions in both innate and adaptive immunity, including B-cell differentiation [25, 26], antigen presentation [27], and homeostasis of the myeloid cell compartment [28].

For most associated loci, there is a substantial lack of understanding regarding the mechanisms by which a genetic variation could influence a phenotype: the identity of the gene(s) affected by the susceptibility variant(s) at each locus is often uncertain, and the

mechanisms by which the causal variants (also often unknown) influence phenotype is usually unclear. This uncertainty is a substantial impediment to the understanding needed to make progress towards new therapies or preventive measures. This obstacle highlights the need to pinpoint the causal variants and the genes affected by those variants, as well as the need for informative functional and computational studies to move from gene identification to possible mechanisms that could guide translational progress. As with the translation of many genomic discoveries, translating this work into direct health benefits will require interaction among a wide array of biomedical disciplines, including genomics, molecular biology, clinical medicine, pharmacology and bioinformatics.

Treatment of patients with PBC is not disease-specific due to the lack of knowledge of pathogenic mechanisms. The standard of care is therapy with the secondary bile acid UDCA [29, 30]. The clinical consensus is that a biochemical response to UDCA delays the progression of disease in most patients [31–34]. However, there is a sub-population of patients who do not respond to UDCA and progress more rapidly to liver failure. An inadequate response to UDCA represents a major unmet clinical need in hepatology and GWAS may represent the way forward to address this need.

Areas in which GWAS findings are leading to clinical and therapeutic applications in many diseases include drug development, drug-response studies [35], and risk prediction which can allow patient stratification [62]. Illustrative examples that highlight the potential application of GWAS discoveries in PBC are discussed in some detail below, and other examples on the horizon are briefly listed thereafter.

Possible therapeutic targets highlighted by GWAS

One of the major goals of GWAS findings has been to flag relevant pathways, not previously implicated, in the pathogenesis of complex disorders that could reveal novel therapeutic targets. Explicative findings are the complement pathway in macular degeneration [36] and the autophagy pathway in inflammatory bowel disease [37].

An emerging theme in the genetics of complex disorders is the considerable overlap of genetic susceptibility factors between related diseases. This has been highlighted in the recent primary sclerosing cholangitis (PSC) iCHIP study [38], in which 44 non-HLA loci were correlated in a GWAS of seven clinically associated autoimmune disorders, including ulcerative colitis (UC), CD, T1DM, coeliac disease (CeD), psoriasis, RA and sarcoidosis. In this study, eleven loci of significance were associated at genome-wide level and 33 loci achieved suggestive significance ($P<5\times10^{-5}$) [38]. This suggested a close similarity in the genetic architecture of PSC and each of the other autoimmune conditions. Functional network analysis showed that candidate genes at pleiotropic loci are related in terms of their function, highlighting common pathways involved in the pathogenesis of PSC and other clinically associated disorders. These observations suggest there might be distinct mechanisms by which autoimmunity occurs, each mechanism predisposing to a particular phenotype or set of phenotypes. This might also suggest that there are unique immunologic pathways that should be focussed on for therapeutic intervention. Likewise, in PBC, many of the disease-related variants have been identified in other GWAS of immune-related

diseases, with a different mosaic of disease-specific risks contributing to the pathogenesis of PBC. Overall, the data suggest important contributions from a number of immune pathways to the development of PBC. Some of the pathways that may have therapeutic implications in PBC are reported below and in Table 2.

Anti-interleukin 12 (IL-12)/IL-23

Loci identified in GWAS in PBC suggest a role for T-lymphocyte differentiation in the development of the disease [6–8]. Th1 immune responses have been implicated in many autoimmune diseases [39] and may be involved in the development of autoreactive T cells, consistent with the putative role of the pyruvate dehydrogenase complex (PDC)-specific autoreactive Th1 cells in the pathogenesis of human PBC [40]. Anti-IL-12 signaling promotes Th1-type immune responses by driving differentiation of activated, naïve T cells to Th1 cells. This, together with the IL-12-driven interferon-γ (IFN-γ) production, contributes to loss of tolerance in several models of autoimmunity [41]. Three loci containing genes involved in IL-12 signaling have been identified in GWAS of PBC: the genes *IL12A*, *IL12RB2* [15–17] and *STAT4* [17] codifying the subunit p35 of the IL-12, the chain IL12Rβ2 of the IL-12 receptor, and the signal transducer and activator of transcription (STAT4) respectively [42]. Studies conducted in an animal model of PBC have strongly suggested a role for the IL-12 pathway in PBC [43]. Currently, multiple clinical trials have been initiated to test whether monoclonal antibody or transcription-inhibitors of p40 (a subunit of the IL-12 receptor) is of therapeutic benefit in psoriasis [44] and CD [45, 46]. Of note, the p40 subunit of IL-12 is also a component of the dimeric cytokine IL-23, which is essential for the differentiation of Th17 cells. 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).

Additional studies are nevertheless required: specifically, genetic association studies and sequencing studies to enable the definition of the specific *IL12A* and *IL12RB2* alleles conferring risk for PBC; molecular analyses that clarify the crosstalk between IL-12 and IL-23 signaling pathways; and in vivo experiments that elucidate the relative contributions of Th17, Treg cells, and other immune cellular subpopulations to PBC. A role for IL-35 is also worthy of investigation, given the subunit nature of the cytokine IL-35 and its receptor, which includes IL-12 p35 and IL-12Rβ2, respectively. Findings from these investigative approaches should then be translated into novel therapy and better outcomes for patients with PBC and other associated autoimmune diseases.

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)

Two GWAS in PBC [17, 18] identified loci containing genes involved in activation of nuclear factor κB (NF-κB), a transcription factor which regulates expression of many genes involved in the immune response; NF-κB is also highly activated in other autoimmune disorders such as RA, MS, and asthma [47]. The loci identified in PBC contain the *NFKB1* gene itself, and genes in pathways leading to NF-κB activation such as *TNFRSF1A*, *CD80* and *RPS6KA4*.

Accumulating evidence indicates that coinhibitory molecules, such as CD80, are key in the prevention of autoimmune diseases. The CD80/CD86:CD28/CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) pathway is the best-characterized inhibitory pathway for T-cell activation [48, 49]. CD28 is constitutively expressed on naïve and activated T cells. CD80 is expressed at low levels on resting antigen-presenting cells (APCs) and is upregulated with prolonged interaction with T-cells, whereas CD86 is constitutively expressed and rapidly upregulated on APCs. Thus, CD86 is likely to be mainly involved in mediating initial T-cell activation, while CD80 may play an important role in propagating the immune responses. After activation, T cells express CTLA-4 (CD152). Engagement of CTLA-4 delivers negative signal into T cells, resulting in inhibition and/or termination of T-cell responses.

Taking advantage of the fact that CTLA-4 binds CD80 and CD86 with much higher affinity than CD28 does, a fusion protein consisting of the extracellular domain of CTLA-4 and the constant region of IgG (CTLA-Ig) has been developed to block the interaction between CD80–CD86 and CD28 and thereby inhibit T-cell activation [34]. Such a fusion protein would preferentially inhibit lymphocytes that are in the process of responding to self-antigens without affecting resting T cells that recognize other antigens.

After the encouraging results of in vivo studies in animal models, including PBC models [50], the efficacy of the CTLA-4 Ig (Abatacept) has been examined in patients with autoimmune diseases. Abatacept has shown efficacy in a broad spectrum of RA patients from early stage to refractory diseases that are resistant to TNF blockers [51, 52] and in patients with psoriasis in a phase I trial [53]. Blockade of costimulation between T cells and antigen-presenting cells through CD80 could represent an important therapeutic approach for the treatment of refractory PBC.

Tumor necrosis factor alpha (TNF-α)

TNF-α is an activating factor for a number of intracellular pathways that determine the fate of hepatocytes, and thus plays a key role in liver homeostasis [54]. Interactions between specific members of the TNF pathway lead to the induction of apoptosis as well as the activation of NF-kB signaling, which is anti-apoptotic and pro-inflammatory [55]. GWAS in PBC identified three loci containing genes in TNF-α signaling pathways: *TNFRSF1A*, *DENND1B* [17], and *TNFAIP2* [17, 18]. TNFRSF1A is one of two receptors for TNF-α; TNFRSF1A$^{-/-}$ mice show attenuated liver fibrosis when compared with wild-type mice after administration of a potent hepatotoxin [56]. DENND1B interacts directly with TNFRSF1A [57] and has previously been associated with asthma [58]. TNF-α signaling also directly induces TNFAIP2 expression [59]. Macrophages from PBC patients, when stimulated with apoptotic bodies from cholangiocytes, produce high levels of TNF-α [60]. Furthermore, serum levels of TNF-α reflect the severity of morphological liver changes in PBC [61]. The TNF signaling pathway was also identified in the pathway analysis of the Italian GWAS cohort [22] where a `self-contained' GWAS pathway analysis method (the linear combination test (LCT)), was applied.

Overall, these findings sustain a prominent role for TNF-α in the pathogenesis of PBC, suggesting that anti-TNF-α treatment, currently used for most inflammatory rheumatic

conditions, such as RA, ankylosing spondylitis (AS) and CD, may also represent a promising agent in PBC.

Phosphatidylinositol signaling and hedgehog signaling pathways

Pathway analysis of both the Italian and Canadian GWAS PBC cohorts have highlighted the phosphatidylinositol signaling system pathway, which is an integral component of the adaptive immune response and is essential for the maintenance of self-tolerance [62]. Possible involvement of the phosphatidylinositol pathway in PBC appears to fit well with the TNF hypothesis as this signaling system has been shown to mediate the effects of TNF-α on NF-kB activation [63, 64].

The same pathway analysis also identified the hedgehog (Hh) signaling system, suggesting its involvement in PBC genetic susceptibility. Hh proteins comprise a group of secreted proteins that are involved in organogenesis, and have been shown to promote adult stem cell proliferation [65–67]. Hh signaling has been widely described in PBC. It is involved in the ductular response to cholestatic damage in PBC, characterized by periportal accumulation of proliferating bile ductular cells and associated stromal elements, including myofibroblastic cells and fibrous matrix [68]. Hh signaling was found to be increased in a murine model of bile duct ligation in periportal epithelial cells expressing pan-cytokeratin, representing potential liver progenitor cell populations [53]. Hh signaling has also been shown to be able to promote the survival of biliary epithelial cells, possibly mediated through the inhibition of caspase activity [69]. Lastly, Hh signaling pathway activation has been associated with upregulation of ductular cell expression of genes that promote inflammatory response, such as the gene producing Cxcl16; Hh dependent induction of Cxcl16, demonstrated in both bile duct ligated rats and humans with PBC, resulted in Natural Killer T (NKT) cell chemotaxis towards cholangiocytes *in vitro* [70].

Hh signaling may represent an important protective factor within the damaged liver, promoting the survival of small periportal epithelial cells representing potential hepatic progenitor cells. Despite the preliminary nature of these studies, the Hh signaling pathway may represent a new therapeutic target to protect or promote cell proliferation and tissue repair within the chronically damaged liver in PBC and other chronic liver diseases.

GWAS in pharmacogenetics

Some scientists believe that, as humans did not evolve in an environment of drug therapies, there is no evolutionary pressure on responses to recently developed pharmacologic agents. Therefore the search for common genetic variants affecting response to pharmacotherapy is likely to be successful because the genetic effect size is likely to be larger than that for disease susceptibility. Since 2007, GWAS have increasingly been applied to pharmacogeneticsto identify loci that affect either drug response or susceptibility to adverse drug reactions. These studies have shown the value of this approach in many fields [71–77].

However, there are limitations in conducting GWAS in pharmacogenetics. First, the variation in drug response is likely to be multifactorial, with many genes working in conjunction with the environment. Second, current GWAS are targeted at elucidating the

independent effects of single genes, and may miss interactive or synergistic effects. Furthermore, the challenges in performing adequate replication studies have to be considered for GWAS in pharmacogenetics, particularly when evaluating small cohorts, such as non-responders to UDCA in PBC.

UDCA, which is currently the only available drug in PBC, is thought to work on the downstream events of the pathogenic mechanism of the disease, through reducing the toxicity of bile and reducing bile duct cell apoptosis [78]. There are ongoing studies, focused on exploring, with a GWA approach, the mechanism(s) beyond the lack of biochemical response to UDCA treatment. A major aim of this ongoing project is to identify potential sites for therapeutic intervention in non-responsive patients. New therapeutic targets that may be highlighted by GWAS, as applied to pharmacogenetics, can be localized either in the upstream or downstream processes of PBC pathogenesis; from the mechanisms that lead to loss of tolerance to the fibrotic phase secondary to cholestasis. Furthermore, improved knowledge of the genetic basis of the lack of response to UDCA will allow to identify non-responders at an early stage and to select them for next-generation drug trials.

Risk prediction

Attempting to predict the onset and progression of disease is one of the cornerstones of epidemiology. GWAS show significant potential to identify molecular factors that enable patient stratification and might prove useful in personalized medicine. Accurate risk prediction can enable targeted preventative treatments or more intensive follow-up, particularly for patients at high risk of progression.

The success of recent GWAS has rapidly changed the outlook for genetic risk prediction. These studies have unlocked thousands of clearly validated genetic associations to complex diseases, but their generally weak effects have left their predictive value and clinical utility subject to hot debate. GWAS data might find ready application in risk prediction in PBC in those patients identified at an early stage of the disease. Risk stratification at an early stage may be important from the perspective of developing treatments that either prevent disease entirely or that improve the outcome when instituted before biliary fibrosis and cirrhosis develop.

Progression of disease in PBC is variable with a substantial proportion of patients eventually developing cirrhosis and liver failure, while other patients remain well and never develop liver-related problems. Progression of disease may represent a complex trait with genetics factors and environmental factors playing together. Genetic variants associated with disease progression detected with GWAS can allow identifying patients at high risk of progressive disease for whom second-line “targeted” therapies would be a valuable therapeutic option. Studies aiming to identify common genetic variants associated with disease progression in PBC at genome-wide level of significance, are currently in progress. It is unlikely that genetic variants associated with disease progression are similar to those associated with susceptibility to PBC. More likely, these studies will identify genetic variants associated with fibrosis progression, which may be then extrapolated for other liver diseases and translated into clinical practice.

Predictive accuracy from genetic models varies greatly across diseases, but the range is similar to that of non-genetic risk-prediction models. A significant improvement in reclassification statistics compared to established clinical risk factors alone is possible. In a cohort that had been classified for risk of cardiovascular events, a combination of genetic variants associated with cholesterol levels was used to develop a genotype score for reclassification [79]. As a result, of the 26% of the study cohort that had been initially estimated to be at intermediate risk, 35% (9% of the total cohort) were reclassified into low- or high-risk categories [79]. For PBC, where non-genetic prediction of outcome has already been explored in preliminary studies with the use of the liver function tests at presentation, it is important to evaluate the information added by genetic loci. Clearly, if classical prediction is strong and genetic prediction is weak, little additional value is added. Furthermore, GWAS risk factors are not necessarily independent of the classical predictors.

There are a number of benefits of such genetic prediction over classical alternatives. For instance, unlike classical clinical risk prediction, genetic risk prediction is highly stable over time, as a person's genetic sequence is essentially constant throughout their life. Such stable risk stratification could be especially important when the proposed interventions are more effective if started at an early age, or continued over a long time period. The utility of genetic risk prediction is dependent not just on predictive accuracy, but also on cost and the ability of clinicians and patients to effectively use this information. The falling cost of whole-genome sequencing will drive the marginal cost of prediction lower, but further progress in gene-mapping research, infrastructure and medical practice will be needed to take full advantage of genetic risk prediction.

Conclusion

During the past decade, there have been significant advances in our understanding of the immunobiology of PBC and, in particular, a rigorous dissection of not only the serologic abnormalities, including AMA, but also the definition of autoreactive $CD4^+$ and $CD8^+$ T cells. Further, there is increasing evidence for the interplay of genetic and environmental factors in individual host susceptibility. Prior to the advent of GWAS, only class II HLA loci had been reproducibly shown to associate with disease [21]. Non-HLA loci were suggested for several genes (e.g., CTLA-4, MDR3), but often inconclusively replicated. With the application of genome-wide technology, HLA was confirmed as the strongest association and many other risk loci have been identified, with equivalent effect size to HLA, including IL12A, IL12RB2, STAT4, IRF5-TNPO3, 17q12.21, MMEL1, SPIB, and CTLA-4. Pathways such as TNF signaling, antigen processing and presentation, and apoptosis, each of which is an established contributor to genetic predisposition to PBC, are among the top pathways identified trough GWAS. These studies highlight the interplay between innate and acquired immunity in PBC. Elucidating the effects of these pathways in PBC is complicated, and it will require additional studies that clarify the effector mechanisms involved; indeed response to therapy, clinical progression, and symptoms remain additional areas for further dedicated studies, and in which different genetic risk factors may be relevant. Nowadays, identification of risk loci associated with disease is leading to the development of rational, disease-specific, therapies for the future.

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Table 1

Non-HLA risk loci for primary biliary cirrhosis[a)]

Locus	SNP	Odd ratio	P-value	Candidate gene	Diseases sharing risk loci with PBC[b),c)]
1p36 [85]	rs3748816	1.33	3.15E-08	MMEL1	-
1p31.3 [38]	rs72678531	1.61	2.47E-38	IL12RB2	-
1q31.3 [38]	rs2488393	1.28	4.29E-12	DENND1B	CD
2q32.2 [38]	rs3024921	1.62	2.59E-18	STAT4	CeD, Ra, T1DM, SLE, SSc
3p24.3 [38]	rs1372072	1.2	2.28E-08	PLCL2	MS
3q13.3 [38]	rs2293370	1.39	6.84E-16	CD80	MS, CeD, Vit
3q25.33 [38]	rs2366643	1.35	3.92E-22	IL12A	MS, CeD
4q24 [38]	rs7665090	1.26	8.48E-14	NFKB1	MS,UC
5p13 [38]	rs6871748	1.3	2.26E-13	IL7R	MS, UC
7p14.1 [38]	rs6974491	1.57	4.44E-08	ELMO1	MS, CeD, PS
7q32 [38]	rs35188261	1.52	6.52E-22	IRF5	UC, RA, SLE, SSc,
9q32 [18]	rs4979462	1.57	1.85E-14	TNFSF15	UC, CD
11q13 [17]	rs538147	1.23	2.06E-10	RPS6KA4	MS, CD, PS, SARC
11q23.3 [38]	rs80065107	1.39	7.20E-16	CXCR5	MS, CeD, SLE, VIT
11q23 [38]	rs4938534	1.38	3.27E-08	POU2AF1	CeD
12p13.2 [38]	rs1800693	1.27	1.18E-14	TNFRSF1A	MS CeD, RA, T1DM, VIT, AITD,
12q24 [38]	rs11065979	1.2	2.87E-09	SH2B3	PSC
13q14 [10]	rs3862738	1.33	2.18E-08	TNFSF11	CD
14q24 [38]	rs911263	1.26	9.95E-11	RAD51B	-
14q32 [8]	rs8017161	1.22	2.61E-13	TNFAIP2	-
16p13.13 [38]	rs12708715	1.29	2.19E-13	CLEC16A	MS, UC, T1DM
16q24.1 [38]	rs11117433	1.26	1.41E-09	IRF8	MS, UC, RA, SSc
17q12 [38]	rs17564829	1.26	6.05E-14	IKZF3	UC, CD, RA, T1DM
17q21.1 [38]	rs17564829	1.25	2.15E-09	CRHR1	-
19p13.2 [38]	rs34536443	1.91	1.23E-12	TYK2	MS, CD, RA, T1DM, SLE, PS
19q13.3 [38]	rs3745516	1.46	7.97E-11	SPIB	-
22q13.1 [38]	rs2267407	1.29	1.29E-13	MAP3K7IP1	CD

[a)] This table reports risk loci identified at genome-wide level of significance in at least one genome-wide association study or iCHIP study of primary biliary cirrhosis. For each locus, results are from the study with strongest evidence of association.

[b)] Phenotypically-associated disorders that share risk loci with PBC are listed.

[c)] AITD, autoimmune thyroid disease; CD, Crohn's disease; CeD, celiac disease; T1DM, diabetes mellitus type 1; MS, multiple sclerosis; PS, psoriasis; RA, rheumatoid arthritis; SARC, sarcoidosis; SLE, systemic lupus erythematosis; SSc, systemic sclerosis; UC, ulcerative colitis; VIT, vitiligo.

Table 2

Molecular pathways highlighted in GWAS of PBC that may be considered for therapeutic intervention.

Pathway	Mechanism	Drugs	Autoimmune diseases where modulators (inhibitors) of these pathways have been used in clinical practice
IL-12/IL-23	IL-12 promotes Th1-type immune response by driving differentiation of activated, naïve T-cells to Th1 cells. This, together with the interferon-γ (IFN-γ) production IL-12 driven, contributes to loss of tolerance in several models of autoimmunity; IL-23 (which share the p40 subunit of IL-12) is essential for the differentiation of Th17 cells, implicated in the breakdown of immune self-tolerance in PBC	Anti-IL-12/IL-23 (*Ustekinumab*)	Psoriasis [44] and Crohn's disease [45, 46]
NF-κB	Transcription factor which regulates expression of many genes involved in the immune response. NF-κB coinhibitory molecules are key in the prevention of autoimmune diseases. The CD80/CD86-CTLA-4 pathway is the best-characterized inhibitory pathway for T-cell activation	Anti-CD80 (*Abatacepi*)	Rheumatoid arthritis [35,36] and psoriasis [37]
TNF-α	Deregulated TNF production characterizes many autoimmune diseases. Recent evidence supports a dualistic, proinflammatory and immune- or diseasesuppressive role for TNF in these conditions	Anti-TNF (*Infliximab, Etanercept, Adalimumab, Certolizumab, Golimumab*)	ankylosing spondylitis [80], Crohn's disease [81], psosiasis [82], rheumatoid arthritis [83].
Phosphatidylinositol signaling	Key pathway for the maintenance of self-tolerance	—	—
Hedgehog signaling	Pathway involved in the inflammatory and ductular response to cholestatic damage	Hedgehog-inhibitor (*Cyclopamine*)	Psoriasis [84]