Controlling Autophagy: A New Concept for Clearing Liver Disease


Abstract

In the classical form of alpha1-antitrypsin (AT) deficiency, a point mutation in AT alters the folding of a liver-derived secretory glycoprotein and renders it aggregation-prone. In addition to decreased serum concentrations of AT, the disorder is characterized by accumulation of the mutant alpha1-antitrypsin Z (ATZ) variant inside cells, causing hepatic fibrosis and/or carcinogenesis by a gain-of-toxic function mechanism. The proteasomal and autophagic pathways are known to mediate degradation of ATZ. Here we show that the autophagy-enhancing drug carbamazepine (CBZ) decreased the hepatic load of ATZ and hepatic fibrosis in a mouse model of AT deficiency-associated liver disease. These results provide a basis for testing CBZ, which has an extensive clinical safety profile, in patients with AT deficiency and also provide a proof of principle for therapeutic use of autophagy enhancers.

Comment

With an expected prevalence of 0.02%, alpha-1-antitrypsin (AAT) deficiency is one of the most common genetic origins of liver disease in childhood and an important hereditary cause of cirrhosis and hepatocellular carcinoma in adulthood. AAT is an important serine protease inhibitor that is synthesized in the liver (normally as the protease inhibitor M [PiMM] protein), is found in the circulation at substantial levels (>0.8 g/L in serum), and inhibits proteolytic enzymes such as elastase released by neutrophils and macrophages (Fig. 1A). The most common initial clinical presentation of AAT deficiency is chronic obstructive pulmonary disease with typically severe, early-onset panacinar emphysema with a basilar predominance in adults. Emphysema in patients with AAT deficiency is thought to result from increased activity of neutrophil elastase in the lungs, which destroys alveolar septa and other components of the lung interstitium because of the lack of sufficient elastase inhibition by circulating AAT (Fig. 1B). The classic form of AAT deficiency is caused by a glutamate-to-lysine exchange at position 342 in the serpin peptidase inhibitor A1 gene [called the protease inhibitor ZZ (PiZZ) genotype], which leads to hepatic synthesis of mutant alpha1-antitrypsin Z (ATZ) proteins. These mutant ATZ proteins are prone to polymerization and form polymers between the mutated reactive center loop and the beta sheet of the next molecule within the endoplasmatic reticulum (ER) of the hepatocytes. The massive formation of insoluble aggregates of mutant ATZ proteins in the hepatocytic ER results in apoptosis, hepatic inflammation, and fibrosis/cirrhosis and strongly predisposes patients to hepatocellular carcinoma (Fig. 1B). The diagnosis of AAT deficiency is established by low serum AAT levels, which are measured for the screening of suspected patients; this is followed by genotyping (with PiZZ-specific polymerase chain reaction) and protein phenotyping (with isoelectric focusing gel) as verification tests. In liver histology, periodic acid-Schiff–positive, diastase-resistant globules containing ATZ protein polymers in hepatocytes are typically seen with AAT deficiency.

Therapeutic options for AAT deficiency are limited at present. Patients with pulmonary manifestations are treated with standard chronic obstructive pulmonary disease drugs. In addition, augmentation therapy with regular intravenous administrations of partially purified plasma preparations highly enriched with AAT is available (Prolastin, Zemaira, and Aralast), but this therapy is expensive (ca. $60,000-$150,000 per year), and data on its effectiveness are less robust. Clinical trials with augmentation therapy have indicated that emphysema progression might be moderately reduced, but large studies with mortality as an endpoint are lacking at present. There is currently no therapeutic medical option available for treating liver diseases associated with AAT deficiency. Ultimately, liver transplantation is a causative therapy for AAT deficiency because it reverts the peripheral AAT deficiency and hepatic disease manifestation. Graft and patient survival rates after liver transplantation due to AAT deficiency are similar to those for other etiologies of cirrhosis.

Several new therapeutic strategies for AAT deficiency have been proposed and investigated in the past. For instance, intravenous augmentation therapy might be replaced by intranasal drug formulations in the future.
and in experimental settings, protective AAT serum levels may also be reached with gene therapy approaches (e.g., viral gene transfer into muscle cells). Targeting AAT deficiency–related liver disease has turned out to be more complex. Efficient inhibition of mutant protein polymerization is feasible in vitro but is difficult to translate into nontoxic, liver-specific drugs. An initial clinical trial with phenylbutyric acid as a chemical chaperone that enhanced AAT secretion in cell culture and mouse models failed because of a lack of efficacy and severe side effects in patients. David Perlmutter’s group investigated an alternative strategy: enhancing the cellular pathways responsible for the degradation of these aberrant molecules (Fig. 1C). Therapeutic intervention with the well-known anticonvulsant carbamazepine promotes proteolytic elimination of misfolded ATZ proteins by activating the autophagosomal degradation pathway and, to a lesser extent, the proteasomal degradation pathway in vitro and in a mouse model of AAT deficiency.

Fig. 1. Pathogenesis of AAT deficiency and novel therapeutic strategies for AAT deficiency–related liver disease. (A) AAT is a serine protease inhibitor. In healthy individuals, AAT is synthesized in the liver, is released into the bloodstream, inhibits various proteases (e.g., neutrophil elastase), and thereby protects the lung interstitium from elastase degradation. (B) In patients with AAT deficiency, mutant ATZ proteins polymerize in the ER of hepatocytes and cannot be released as functional proteins into the circulation; this results in an imbalance between elastases and elastase inhibition. This loss of AAT function promotes early-onset pulmonary emphysema. In the liver, polymerized ATZ proteins represent severe stress to hepatocytes (gain of function); this promotes liver injury and eventually leads to liver fibrosis, cirrhosis, or hepatocellular carcinoma. (C) Therapeutic intervention with the well-known anticonvulsant carbamazepine promotes proteolytic elimination of misfolded ATZ proteins by activating the autophagosomal degradation pathway and, to a lesser extent, the proteasomal degradation pathway in vitro and in a mouse model of AAT deficiency.
pathways for mutant ATZ proteins may, therefore, represent a realistic option in the near future. Nevertheless, several open questions remain. First, which of the potential drugs (carbamazepine, rapamycin, and possibly others) is most effective and best tolerated in patients with AAT deficiency? Second, is enhancing autophagy also an efficient option for advanced liver diseases (i.e., cirrhosis) in these patients? Third, how do the doses used in mice translate into humans? The carbamazepine doses necessary for beneficial effects in mice were approximately 10- to 20-fold higher (per body weight) than the therapeutic doses used in humans treated with carbamazepine for epilepsy. Fourth, will the activation of proteasomal degradation (observed in the carbamazepine-treated mice) affect normal hepatic protein synthesis as well? Nevertheless, the current studies provide a rationale for testing autophagy inhibition could also evolve as a therapeutic concept for liver diseases in which its activation is associated with cellular senescence or transdifferentiation.

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References

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Abstract
Lumiracoxib is a selective cyclooxygenase-2 inhibitor developed for the symptomatic treatment of osteoarthritis and acute pain. Concerns over hepatotoxicity have contributed to the withdrawal or nonapproval of lumiracoxib in most major drug markets worldwide. We performed a case-control genome-wide association study on 41 lumiracoxib-treated patients with liver injury (cases) and 176 matched lumiracoxib-treated patients without liver injury (controls). Several SNPs from the MHC class II region showed strong evidence of association (the top SNP was rs9270986 with P = 2.8 × 10⁻¹⁰). These findings were replicated in an independent set of 98 lumiracoxib-treated cases and 405 matched lumiracoxib-treated controls (top SNP rs3129900, P = 4.4 × 10⁻¹²). Fine mapping identified a strong association to a common HLA haplotype (HLA-DRB1*1501-HLA-DRB5*0101-HLA-DQA1*0102, most significant allele P = 6.8 × 10⁻⁵, allelic odds ratio = 5.0; 95% CI 3.6-7.0). These results offer the potential to improve the safety profile of lumiracoxib by identifying individuals at elevated risk for liver injury and excluding them from lumiracoxib treatment.

Comment
Despite its relatively infrequent occurrence, drug-induced liver injury (DILI) is the leading cause of
acute liver injury in the United States, an important cause of sporadic acute hepatitis in the community, a source of diagnostic and therapeutic challenges for treating clinicians and a common reason for premarketing and postmarketing drug withdrawals for pharmaceutical companies.

The selective cyclooxygenase-2 (COX-2) inhibitor, lumiracoxib, joins the long list of nonsteroidal anti-inflammatory drugs (NSAIDs) (bexaprofen, bromfenac, ibufenac) withdrawn due to their association with DILI.1 When first introduced, lumiracoxib appeared to fulfill many of the desired attributes of an NSAID: three-fold to four-fold lower gastrointestinal (ulcer-related) complications than naproxen and ibuprofen and a slightly better cardiovascular disease track record than rofecoxib, another recently withdrawn COX-2 inhibitor.2 Ironically, although gastroenterologists should have welcomed the introduction of such an agent, it turns out that lumiracoxib has the potential for rare but serious hepatotoxicity. Worldwide, at least 20 cases of severe DILI associated with lumiracoxib have been reported, including 14 with acute liver failure, two deaths, and three liver transplants.3 Most cases occurred several months after starting lumiracoxib, but early presentations were also noted. Many cases involved daily doses exceeding 100 mg, but severe DILI was also reported in those patients who were prescribed 100 mg/day.

The U.S. Food and Drug Administration (FDA) issued a “nonapprovable” letter for lumiracoxib in 2007. Although the passing of one more NSAID is likely to be soon forgotten, there are two lessons to be learned for prescribers. Yet again, postmarketing surveillance has identified serious instances of DILI that were not foreseen in clinical trials. In the large TARGET (Therapeutic Arthritis Research and Gastrointestinal Event Trial) study, 2.6% had aminotransferase (AT) elevations greater than three times the upper limit of normal (3 × ULN). There were six cases of probable or possible “clinical hepatitis,” but all resolved with cessation of the drug, and there were no reports of liver failure. Parallels can be drawn with troglitazone.4 However, whereas the relative rarity and unpredictability of many or now most causes of DILI has been recognized for more than 50 years, the genetic basis for such a host of susceptibility factors has been slow to document reliably since rare family clustering studies and indirect susceptibility tests were reported at least 25 years ago.5 The addition of lumiracoxib to the growing list of agents for which susceptibility to DILI has been linked to human leukocyte antigen (HLA) genotypes, as reviewed recently in HEPATOLOGY,6 provokes further consideration of the mechanistic significance and clinical utility of such associations.

The observations of Singer et al., who carried out a pharmacogenetic case-control analysis of participants enrolled into the two TARGET trials, are of particular interest.7 In the first phase of their study, 41 subjects with serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 5 × ULN (“cases”) and 176 age-matched, sex-matched, race-matched, and clinical trial–matched individuals (who took lumiracoxib but had normal ALT/AST; “controls”) were recruited for a genome-wide association study (GWAS). This was performed using the Affymetrix assay 6.0, which can detect more than 900,000 single-nucleotide polymorphisms (SNPs). Several SNPs from the major histocompatibility complex (MHC) class II region on chromosome 6 were significantly represented in cases with DILI, the leading contender being rs9270986 (P = 2.8 × 10−10). Thirteen SNPs, including seven from phase 1 of the trial, were reevaluated in the second (validation) phase of the study, involving 98 cases and 405 lumiracoxib-exposed controls, respectively. Cases were defined here by ALT/AST > 3 × ULN. The results of the replication phase confirmed the association of lumiracoxib-related DILI with the principal SNPs identified earlier, but did not find a similar relationship with cases of DILI drawn from small groups of controls receiving ibuprofen (n = 18) or naproxen (n = 9). Finally, fine mapping of the top SNPs showed strong association with a well-characterized MHC haplotype (HLA-DRB1*1501-HLA-DQB1*0602-HLA-DRB5*0101-HLA-DQA1*0102; most significant allele P = 6.8 × 10−25, allelic odds ratio = 5.0; 95% confidence interval [CI] = 3.6-7.0). Of these alleles, HLA-DQA1*0102 had the best negative predictive value (99%) and sensitivity (73.6%) in identifying cases at risk.

Before examining the implications of this study, it is worthwhile to look at the wider perspective of host/drug factors influencing susceptibility to DILI. Although the total dose of drug is critical in dose-dependent hepatotoxicity (e.g., acetaminophen), the relevance of this to idiosyncratic drug reactions is overshadowed by other host characteristics such as age, sex, comorbid illnesses, and coprescribed medications.8 A genetic predisposition to DILI is well recognized for drugs (phenytoin, sulfonamides) linked to hepatic injury as part of systemic hypersensitivity (“reactive metabolite syndrome”) and has been recognized for halothane.5 Other than these examples, the genetic contribution to DILI has only slowly been recognized, perhaps partly because of studies in the 1990s that
showed a lack of association between HLA markers and DILI. Although some HLA markers were overrepresented in some cases (e.g., HLA A-11 in 75% of cases of diclofenac hepatitis), no overall association between specific HLA alleles and DILI could be discerned. Another limitation was the use of insensitive serological methods to determine HLA status instead of high-resolution genotyping on large case and control populations that is currently favored. These studies were also underpowered to detect meaningful associations with individual drugs. This poses a considerable challenge because cases of DILI are infrequent (typically between 1 and 10 per 100,000 persons exposed) and collating a case series requires considerable collaborative efforts. Furthermore, careful case definition is necessary; for DILI, this itself poses a considerable challenge.

Studies have usually used one of the causality scoring systems, such as the CIOMS (Council for International Organizations of Medical Sciences), which although laudable in many respects, lack sensitivity and specificity for several phenotypes of DILI, as reviewed elsewhere. Thus, in the lumiracoxib study, only 41 cases were included, and these were defined by ALT/AST changes and not by "clinical hepatitis", which differs from another GWAS study that enrolled patients with fluvoxacin-associated DILI who had clinical features of liver disease. As a result of these logistic limitations, it is pertinent to consider whether the reported association between lumiracoxib-related AT elevations (DILI) and the HLA allele/extended haplotype is clinically meaningful. This cannot be conclusively determined from a study of this size, but supportive arguments have been put forward. Singer et al. noted the increasing sensitivity with increases in ALT rise; all patients with ALT > 20× ULN carried the specific HLA haplotype. Also, all three cases with substantial serum bilirubin increases that fulfilled "Hy's law" (ALT/AST > 3× ULN; serum bilirubin > 2 ULN), a reliable marker for high probability of significant hepatotoxicity, also carried the implicated HLA alleles. In other respects, the study by Singer and colleagues fulfills the necessary requisites for a GWAS: proper case definition (albeit by biochemical and not clinical presentation), matched controls in a ratio of cases:controls of 1:4, use of a replication cohort, and correction of P value for multiple comparisons.

At the end of all this, what are the implications of this study in terms of pathogenesis of DILI and whether these observations can be used to prevent DILI in the future?

The physiological role of HLA class I (A, B, and C) and class II (DP, DQ, and DR) molecules on the cell surface is to present endogenous (class I) or exogenous material such as drugs (class II) to T lymphocytes through engagement with the T cell receptor. Recognition of small molecular weight drug/drug metabolites by T cells will occur either if presented in combination with a protein ("hapten" hypothesis) and MHC class II molecule (MHC peptide-complex), or by direct engagement with the MHC molecule ("pharmaceutical interaction" concept). In either scenario, it is conceivable that alterations in MHC alleles will disrupt proper drug–T cell engagement. The species differences in MHC restriction would account for the failure to predict human hepatotoxicity despite apparent safety in animal models.

In the study by Singer et al., there were no functional analyses that could shed light on the precise mechanisms of lumiracoxib-related DILI. It is, however, interesting that lumiracoxib is bioactivated to a reactive quinone imine, and possibly noteworthy that the structure of lumiracoxib closely resembles diclofenac. The latter is also associated with hepatotoxicity, and has metabolic pathways that can generate reactive metabolites capable of forming adducts with hepatic proteins and evoking an immune response. On the other hand, lumiracoxib shows no structural similarity to abacavir, which is associated with a severe cutaneous hypersensitivity reaction linked to one of the same HLA haplotypes (HLA-B*5701) as lumiracoxib. Interestingly, association with HLA-B*5701 is also shared with fluvoxacin, a synthetic penicillin associated with severe cases of drug-induced cholestasis.

### Table 1. HLA Alleles Associated With DILI

<table>
<thead>
<tr>
<th>Drug</th>
<th>Therapeutic Group</th>
<th>HLA Allele</th>
<th>Odds Ratio for Developing DILI (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ticlopidine</td>
<td>Antiplatelet agent</td>
<td>HLA A*3303</td>
<td>36.5 (7.3-184)</td>
</tr>
<tr>
<td>Fluvoxacin*</td>
<td>Antibiotic</td>
<td>HLA B*5701</td>
<td>80.6 (22.8-284.9)</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>Antibiotic</td>
<td>HLA-DRB1*15</td>
<td>2.3 (1.0-5.26)</td>
</tr>
<tr>
<td>Lumiracoxib*</td>
<td>NSAID</td>
<td>HLA-DQA1*0102</td>
<td>6.3 (4.1-9.6)</td>
</tr>
<tr>
<td>Ximelagatran*</td>
<td>Oral direct thrombin inhibitor</td>
<td>HLA-DRB1*0701</td>
<td>4.4</td>
</tr>
<tr>
<td>Lopatinib*</td>
<td>Tyrosine kinase inhibitor used in advanced breast cancer</td>
<td>HLA-DQA1*201</td>
<td>9 (3.2-27.4)</td>
</tr>
</tbody>
</table>

*Genome-wide association study.
Unlike autoimmune hepatitis where specific HLA alleles can determine disease severity or treatment outcome, only limited genotype-phenotype correlations have been noted for instances of DILI. Interestingly, one of the same HLA haplotypes associated with lumiracoxib toxicity (HLA-DRB1*1501) is overrepresented among cases of liver injury resulting from amoxicillin-clavulanate. However, the latter causes early onset (<25 days) liver toxicity and has a completely different histologic pattern (mainly cholestatic injury), which differs from the usual late-onset hepatocellular reaction with lumiracoxib. Other recent associations of specific HLA alleles with DILI are listed in Table 1 and have been reviewed recently in Hepatology. It should be pointed out that not all HLA phenotypes are associated with increased susceptibility to DILI; HLA-DRB1*07 family of alleles conferred a reduced risk of DILI with amoxicillin-clavulanate as compared with population controls and treated nonaffected cases (odds ratio = 0.26 and 0.18, respectively). Overall, in most cases of DILI, the presence of a particular HLA allele is neither sufficient nor necessary for a particular adverse effect to occur. In addition to known and unknown host and environmental factors, the contributions of polymorphisms within drug-metabolizing systems, biliary transporters, and both innate and adaptive immune response pathways, as well as antioxidant, antiapoptosis, and other cell protective genes, need to be considered. It also remains possible that particular HLA alleles are in linkage disequilibrium with cardinal “susceptibility genes”, as turned out to be the explanation for the association between HLA A3 and C282Y, which led to the common form of genetic hemochromatosis.

Many consider the era of pharmacogenomic explanations for idiosyncratic adverse drug reactions to have begun with recognition of the association between hypersensitivity reactions to abacavir, a human immunodeficiency virus (HIV) protease inhibitor and HLA B*5701. Screening subjects for this HLA allele and withholding abacavir from those carrying it has almost completely abolished such reactions. However, unlike most cases of DILI, abacavir reactions are quite frequent (5%), and use of common agents like antimicrobials and NSAIDs is not usually subject to the same complex considerations as highly active antiretroviral therapy for HIV. A similar HLA-based screening strategy to exclude DILI is therefore unlikely to be logistically plausible or cost-effective unless screening costs become cheaper. In the case of lumiracoxib, excluding carriers of the HLA-DQA1*0102 allele would reduce the frequency of DILI to 1% but at the expense of excluding a considerable proportion (34%) of carriers, because less than 6% would actually develop hepatotoxicity. An alternative pharmacogenetic strategy is to restrict testing to those at increased risk of adverse drug reactions. For example, the FDA recommends screening Han Chinese patients for HLA-B*1502 before starting carbamazepine. Such screening is likely to be cost-effective because the allele in question is relatively common in that ethnic group (8%-12%) and, further, the odds ratio of developing a severe cutaneous reaction in persons carrying that allele is extremely high (>2500). This strategy would be useless in Caucasians who do not carry that specific HLA allele but also can develop similar reactions with carbamazepine. Likewise, a selective screening protocol cannot be applicable to lumiracoxib recipients because of the failure to identify specific characteristics that could be associated with a risk of DILI.

In the final analysis, routine pharmacogenetic testing would come down to costs, availability of alternative treatment options, and logistics (turn around times). Promising times lie ahead for the prospects of pharmacogenomic discovery to help unravel the multiple interactive mechanisms of DILI, but their impact on preventing DILI in the near future is still likely to be limited.

References
The Genetics of Primary Biliary Cirrhosis: The Revolution Moves On


Abstract

We genotyped individuals with primary biliary cirrhosis and unaffected controls for suggestive risk loci (genome-wide association \( P < 1 \times 10^{-8} \)) identified in a previous genome-wide association study. Combined analysis of the genome-wide association and replication datasets identified IRF5-TNPO3 (combined \( P = 8.66 \times 10^{-13} \)), 17q12-21 (combined \( P = 3.50 \times 10^{-13} \)) and MMEL1 (combined \( P = 3.15 \times 10^{-8} \)) as new primary biliary cirrhosis susceptibility loci. Fine-mapping studies showed that a single variant accounts for the IRF5-TNPO3 association. As these loci are implicated in other autoimmune conditions, these findings confirm genetic overlap among such diseases.


Abstract

A genome-wide association screen for primary biliary cirrhosis risk alleles was performed in an Italian cohort. The results from the Italian cohort replicated IL12A and IL12RB associations, and a combined meta-analysis using a Canadian dataset identified newly associated loci at SP1B (\( P = 7.9 \times 10^{-11} \), odds ratio (OR) = 1.46), IRF5-TNPO3 (\( P = 2.8 \times 10^{-10} \), OR = 1.63) and 17q12-21 (\( P = 1.7 \times 10^{-10} \), OR = 1.38).

Comment

The 2009 publication of the first genome-wide association study (GWAS) of primary biliary cirrhosis (PBC) represented a key point in the evolution of our understanding of the genetic basis and thus pathogenesis of this disease. This landmark study identified, in a reproducible fashion, genetic associations between PBC and human leukocyte antigen as well as polymorphisms in the genes encoding the interleukin-12 (IL-12) \( \alpha \)-chain and the IL-12 receptor \( \beta \)-chain. Two recent publications from Canadian, American, and Italian groups add an important further dimension to our knowledge base with respect to the genetic basis of PBC and build on the original study. Taken together, these two new studies replicate the original genetic associations with the IL-12 pathway, and importantly, through individual and combined analyses, they identify further associated loci. Critically, the newly identified loci are again associated with the biology of the interaction between antigen-presenting cells (APCs) and CD4\(^+\) T cells, which is thought to be critical to the development of the autoreactive immune responses underpinning PBC.

The advent of these new data make now a good time to reflect on what we now know and to identify potential future directions for research. Three important observations can be made about our new understanding of the genetic associations of PBC.
The strength and consistency of the findings in fully independent studies are themselves worthy of comment. This finding would confirm the view from population and twin-based studies that there is a significant genetic contribution to PBC. A further significant factor, however, in the clarity of the findings is the fact that PBC probably does constitute a single disease entity across different populations. Another factor is also likely to play a role in the consistency of the findings between the studies: the simplicity and accuracy of the diagnostic criteria for PBC. The combination of antimitochondrial antibodies on immunofluorescence (or anti-M2 antibodies on an enzyme-linked immunosorbent assay) and cholestatic liver function tests is 95% sensitive and specific for the diagnosis of PBC. This degree of diagnostic accuracy, which stands in contrast to many other disease states for which GWASs have given rise to weaker and more contradictory findings and for which diagnosis at the level of accuracy needed to avoid confounding genetic studies is more complicated, has the important benefit of effectively excluding the false-positive assignment of disease status, which introduces error and reduces power in GWASs. One of the conclusions that can be drawn from the PBC GWASs published to date is, therefore, that this disease is in fact an extremely valuable model with which to study genetic contributions to the pathogenesis of autoimmune disease.

The second observation that can be made is related to the nature of the associations found and replicated to date, all of which are for genes encoding proteins implicated in antigen presentation by APCs and the resultant induction of T cell immune responses. Major histocompatibility complex is clearly critical for the presentation of peptide epitopes, whereas the IL-12 pathway plays a key role in shaping the phenotype of the resulting T cell response and is essential for the development of proinflammatory T helper 1 (Th-1) type immune responses. The novel genetic associations with interferon regulatory factor 5 (IRF5)—transportin 3, SPIB, and the 17q12-21 chromosomal region that are reported in the two new studies (individually and in a meta-analysis) continue this theme. SPIB is a transcription factor that plays a role, among many others, in the pathway for the differentiation of plasmacytoid dendritic cells, which can also mediate and modulate the expression of CD40 (its interaction with the CD40 ligand has previously been identified as a key costimulatory/effecter pathway in PBC). IRF5 plays a key role in the innate immune response as part of the toll-like receptor signaling pathway and mediates apoptosis induced by tumor necrosis factor–related apoptosis-inducing ligand. Strikingly, IRF5 loci have previously been shown to be associated with autoimmune disease in the form of systemic lupus erythematosus, systemic sclerosis, and Sjögren’s syndrome; all these conditions are known to be associated with PBC. The 17q12-21 region contains a number of potentially biologically relevant genes and has itself previously been shown to be associated with other inflammatory and autoimmune diseases, including rheumatoid arthritis. What is striking is that all the identified associations are related to the immune response and, in particular, to the interactions relating to APC development, APC activation, epitope presentation, and the phenotype of the resulting T cell response. The inescapable conclusion is, therefore, that PBC is an immune disease, at least in genetic terms. It will be interesting to see whether the UK GWAS, which will be the largest to date and thus will have significantly augmented statistical power, identifies further genetic associations within this key pathway.

The third observation is related to a number of associations that can be hypothesized to be relevant to the pathogenesis of PBC on the basis of our ideas about its biology but that are not seen. To date, with the important caveat remaining about the power of GWASs necessary to identify all associations, no susceptibility loci related to the biology of the pyruvate dehydrogenase complex (the key disease autoantigen), the biology of biliary epithelial cells (the target cells for damage in PBC), or potential disease phenotype-controlling factors (the phenotype can have a big impact on the probability of a diagnosis being made) such as biliary transporter genes have been identified. It may be that a better powered GWAS or pathway analysis could identify such factors, but until this occurs, what we will see is an exclusively immunoregulatory portfolio of genetic associations.

What, therefore, do we know and still not know about PBC after the publication of these genetic studies? What is now absolutely clear (if it was not clear before) is that PBC is likely a disease of immune dysregulation. What predisposes a person to it is variability in the genes encoding the key proteins that regulate the normal immune response to an antigen. What we tantalizingly do not know yet and will not know until the results of functional studies emerge is whether PBC is associated with augmented Th-1 phenotype immunity or impairment. This is critical because it is conceptually possible to link augmented immunity and impaired immunity of the Th-1 phenotype with the pathogenesis of PBC through conventional autoreactivity and an impaired response to a pathogen model, respectively. This next stage of functional study is,
therefore, absolutely critical for the future direction of PBC immunology research.

The value of the GWAS approach has been questioned recently, with the argument put forward that it “flatters to deceive” and fails to deliver the anticipated paradigm shift in disease understanding. In this sense, therefore, PBC represents a triumph for GWASs because the PBC studies are likely to turn out to be landmarks in our understanding of the disease. It is also likely that the findings will translate into new approaches to therapy sooner than GWAS findings typically do, with modulation of the IL-12 pathway representing one obvious potential approach. The findings of these studies also, however, suggest that PBC may be not only an important disease to study in its own right but also an important paradigm for our understanding of immune regulation in humans as a result of its homogeneity and the diagnostic accuracy. It is often forgotten that PBC was a landmark disease in the study of autoimmunity and represented one of the first disease settings in which autoantibodies were described and in which the autoantigens associated with human disease were identified. We may now be at the point at which PBC returns to the forefront of the study of the mechanistic immunobiology of autoimmunity. These are interesting times.

References


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Potential conflict of interest: Nothing to report.