

Genetics in PBC: What Do the “Risk Genes” Teach Us?

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Abstract Primary biliary cirrhosis is characterised by a progressive and destructive lymphocytic cholangitis, targeting small intra-hepatic bile ducts. In association with the histologic liver injury, patients characteristically express highly specific auto-antibodies that recognise a conserved epitope of the pyruvate dehydrogenase complex found on the inner membrane of the mitochondria. Family studies demonstrate a clear increased incidence and prevalence of associated autoimmune diseases; and historically, a clear HLA association with disease has been evident. With the use of a high-throughput whole-genome array technology, significant insights into the non-HLA loci associated with risk for disease development have been made. These studies, which have primarily incorporated genome-wide association screens and targeted analysis of immune genes, have highlighted the integral roles for immune cell development and function in disease risk. This has revealed the IL-12/JAK-STAT signalling pathway as a key etiologic factor. In conjunction with a better understanding of environmental triggers, such work lays the foundation for better disease insights mechanistically and, hopefully, therapeutically. Obstacles to uncovering all the associated genetic risk and the correlation between genotype and phenotype remain to be circumvented, as do better appreciation of the processes that underpin not only disease initiation but also presentation and outcome.

Keywords Genetics · Primary biliary cirrhosis · Autoimmune liver disease

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Introduction

Autoimmune liver diseases are rare chronic immune-mediated liver injuries, with predominant hepatic and/or biliary inflammation as defining features. Three major diseases are recognised that reflect the classical distinctions of patient presentation in a clinic [1–3]. These diseases are autoimmune in nature but lack definable causes at present, with pathophysiologic models highlighting interacting genetic and environmental risks. Of the three main patterns of immune-mediated liver injury, primary biliary cirrhosis (PBC) is the most classic, as well as the most frequent, affecting 1 in 1,000 women over the age of 40. It is characterised by a progressive lymphocytic granulomatous cholangitis in tight association with anti-mitochondrial antibodies, in nearly all patients.

Whilst the pathogenesis of autoimmune liver diseases remains unclear, cumulative data from epidemiological and laboratory studies consistently highlight the combined importance of environmental and genetic influences on risk [4]. How these factors combine to engender such risk requires further investigation, but the role of genetics is revealed by the familial clustering of autoimmune disease, and in the case of PBC, the not infrequent occurrence of other autoimmune diseases such as celiac disease, scleroderma, Sjögren’s syndrome and rheumatoid arthritis in a patient or a patient’s first degree relatives. There is thus evidence among the autoimmune liver diseases, and autoimmune diseases in general, of common etiologic mechanisms related to immune pathways and tissue response to injury [5].

Autoimmune risk factors include a shared host genetic predisposition and a breadth of potential environmental challenges (in PBC, for example, xenobiotics, molecular mimicry, infective triggers) that may lead to the loss of tolerance in an individual host with a “permissive” genetic predisposition. It is this coalescence of variants that influence immune reactivity and exposure to environmental triggers that is widely believed to evoke and then perpetuate immune dysfunction and downstream tissue injury and damage. Thus, an individual association need not be disease-specific nor necessarily the pivotal

factor in disease aetiology. Each is more likely to represent a piece of the risk and should be viewed in terms of the collective picture.

This brief review, with reference to, and help from, original publications as well as reviews and editorials already in press from the authors and others, highlights some aspects of genetic susceptibility now acknowledged as important in underpinning disease risk in patients with PBC. In particular, we seek to put in perspective how such new information can illuminate improved treatment strategies for patients.

Genetic Risk Discovery—Methodologic Concepts

In making the most of the welcome, but nevertheless voluminous, data on genetic risk in PBC, it is highly pertinent to consider the technology and approaches used to study the genetic influences for disease risk. These, in particular, relate to efforts to map the non-HLA genetic risk of disease. It is imperative to always consider the population studied, including the controls, used to determine genetic distinction between affected and unaffected individuals. It is also key to consider what phenotype of disease has been studied and how robust a phenotype may be. Hence, in comparing non-alcoholic fatty liver disease [6] with PBC, one might expect that the number of published gene loci associated with disease would be the same, because apparently rare, but reported, monozygotic twin concordance for relevant phenotypes is about the same (0.6). However, it is inherently easier to study a homogenous group of patients with AMA reactivity and cholestatic liver chemistry, than patients with fatty liver, whose phenotype may be “defined” for example by liver enzymes, liver fat, liver histology or insulin resistance [7, 8]. Furthermore, there remains a bias in the GWAS studies published to date towards the analysis of Caucasian populations—the data from the single robust study of a non-HLA risk for PBC from a Japanese population stands out for divergence to other GWAS results [9], despite analysis of a very reproducible, heritage free phenotype. The translation of any association (by which one refers to a significant difference in allele frequency at a certain locus between subjects and controls) to the clinical context is also limited to the phenotype studied. Hence, for a disease like PBC, we recognise a multistep pathogenic model that links immune regulation to biliary epithelial cell injury and the cholestatic response to duct injury. The genetic data presented in the current literature addresses merely the first part of the story that is the loss of tolerance to mitochondrial antigens associated with biliary injury. The genetic contribution to the response to injury, treatment and “symptomatic” phenotype of disease remains to be evaluated, and if there are genetic risks underpinning these clinical variations, they may diverge from those engendering risk. This distinction is

important, in minimising the misuse or misinterpretation of genetic data, particularly in directing therapeutic decisions.

Technologic and methodologic advances in genetics have also been at the forefront of study design of late, with the emphasis on investigating candidate genes increasingly diminished. In screening for non-*HLA* gene associations with disease, multiple gene array technology and/or sequencing efforts are standard. The gene arrays may provide a “genome-wide coverage” with different levels of certainty based on how many variants are tested and the frequency of those variants in the population evaluated or may be very focused towards genes with a particular interest such as the ImmunoChip array which was deliberately designed with bias towards genes of relevance to immune pathways. As technology costs fall, more focus will turn towards exome or whole genome sequencing, but such efforts are complicated by the bioinformatics challenges posed by the large datasets.

Despite the technologic advances in genetic sequencing, the reverse identification of candidates can still be revealing and has the ability to identify hitherto unexpected pathways relevant to disease pathophysiology. Thus, for example, it is possible to induce random mutations into mice, screen offspring for the particular phenotypes of interest and, in that way, identify pathways of relevance to disease—a pertinent example in liver disease is the identification of an association between variants in the *Gabrb1* gene and alcohol consumption [10]. Further approaches to understanding individual gene associations have also taken into consideration the concept that DNA sequences are in 3D “communication” across the genome. A good example is provided by a recent study (and accompanying commentary) investigating the basis for the association of the *FTO* gene with obesity [11, 12]. Studies initially naturally focused on exploring a direct role for *FTO* in disease, the variants associated with obesity risk being non-coding and presumed related to gene expression. However, attempts to link sequence to risk biology have failed, and this transpires to be because it has become clear that the association signal in fact arises from the gene encoding the *IRX3* transcription factor. By understanding the 3D structure of the chromosome segment containing both genes, it has been shown that the obesity-associated regions in *FTO* are physically in contact with the promoter of *IRX3*. Further studies revealed that *IRX3* is strongly linked with obesity that subjects with one of the obesity-associated mutations having a higher expression of *IRX3*, but not with *FTO*, in brain tissue and that mice lacking the *IRX3* gene are 25–30 % lighter than controls. This example from another disease setting is important for reinforcing the concept that GWAS data provide association with disease risk in the form of a marker on the genome; this is not necessarily the same as proving which gene is affected. This situation arises not only because of linkage disequilibrium across chromosomal regions but also because of epistatic/physical interactions existing between distant genes. Our

immunochip data in PBC [13] indeed suggested an epistatic interaction between the *IL12RB2* risk locus at 1p31 and the *IRF5* risk locus at 7q32, implying a complementary effect of these loci in predisposing to disease.

Another insightful approach to clarifying disease aetiology is exemplified by efforts to correlate host genetic variation, with specific whole-cell transcriptomic expression patterns following definable and reproducible stimuli. Such work is key to the understanding of how new gene associations are relevant to biology. As an example, in a recent study aimed at identifying the genetic basis of variability in the host response to pathogens [14] (stimulation with *Escherichia coli* lipopolysaccharide, influenza virus or the cytokine interferon- β), a cohort of 534 individuals were subjected to both the genotyping of common DNA variants and analysis of transcriptional profiles in immune dendritic cells isolated from the peripheral blood, thus allowing transcriptional profiles to be aligned with the individuals' genetic variations. Investigators have also modified this approach by mapping inter-individual variation in gene expression as a quantitative trait, defining expression quantitative trait loci (eQTLs). To investigate the effect of innate immune stimuli on eQTLs, for example, one group exposed primary CD14⁺ human monocytes from volunteers to the inflammatory proxies' interferon- γ or lipopolysaccharide and then performed eQTL mapping on a genome-wide basis [15]. With such an approach, the investigators were able to show how inter-individual variation in immune responses is accompanied by patterns of gene regulation dependent on the underlying genotype. In human monocytes, many regulatory variants displayed functionality only after relevant immune stimuli, supporting the concept that biologic risk is latently present, but only functionally important after certain extrinsic challenges.

HLA Associations with PBC

Whilst much of the recent excitement related to genetics of autoimmune disease has revolved around the discovery of novel and statistically robust (i.e., observations corrected for the denominator of variants tested) non-*HLA* associations, it is the HLA region that is the strongest, yet still not understood, association with an autoimmune liver disease risk [16]. Variants in genes for the proteins encoded by the human leukocyte antigen (HLA) system are associated with most autoimmune diseases, as is consistent with pivotal roles for HLA proteins in the specificity of immune responses. The relevant genes encode cell-surface antigen-presenting proteins corresponding to MHC class I (A, B and C) that presents intracellular-derived peptides. HLAs corresponding to MHC class II present antigens originating extra-cellularly from T-lymphocytes. The class II proteins combine as heterodimeric ($\alpha\beta$) protein receptors that are found on the surface of antigen-presenting

cells, and which stimulate T-helper cells, in turn stimulating antibody-producing B-cells.

For PBC specifically, the human MHC has long been implicated in the etiopathogenesis of disease [17]. Genome-wide association studies (GWAS) of PBC cohorts reproducibly show that the strongest genetic associations with the disease derive from single-nucleotide polymorphisms (SNPs) within the HLA region [9, 18–20]. In these studies, the peak association signal is between *HLA-DQA1* and *HLA-DQB1*. Multiple studies of PBC further refine the association with particular classical HLA alleles in PBC. Most of the HLA association can be attributed to specific associations with *DRB1*08*, *DRB1*11*, *DRB1*14* and *DPB1*03:01*, and the majority of the associated amino acids are both nearly unique to the specific HLA classical alleles and also correspond to critical residues in the antigen-binding pocket. Interestingly, preliminary data further suggested differences in HLA association when patients were stratified by the presence or absence of specific anti-nuclear antibody reactivity (sp100). In Japanese subjects, the significant associations do differ with an evidence of increased PBC susceptibility associated with the *DRB1*08:03-DQB1*06:01* and *DRB1*04:05-DQB1*04:01* haplotypes. Significant protective associations are with the *DRB1*13:02-DQB1*06:04* and *DRB1*11:01-DQB1*03:01* haplotypes. Understanding such distinctions may be beneficial to disease modelling, as is the case with the non-*HLA* distinctions that arose in the Japanese GWAS of PBC.

Non-*HLA* Associations with PBC

Candidate gene studies have generally been small, highly focused and prone to over the estimation of significance, making much of these data unreliable. Exceptions exist wherein a gene has been associated with other autoimmune diseases, the best example being perhaps *CTLA-4/ICOS* which to date has not emerged as a risk locus in PBC by GWAS (interestingly, *CD28* has in primary sclerosing cholangitis), but sufficient candidate gene studies do support an association, albeit complex, in keeping with an unusual linkage disequilibrium in the locus of interest [21]. Robust non-*HLA* associations with PBC risk have however arisen from new genome-wide studies, in large populations, and have underscored adaptive regulatory immune pathways as important in PBC risk. Several large-scale genetic studies (Table 1) have used subjects with PBC and controls from North America [13, 18, 22, 23], Italy [19], the United Kingdom [20, 24] and Japan [9]. In keeping with other autoimmune diseases, it seems likely that there will be few, if any, PBC-specific genetic associations. Many of the PBC-related variants have been identified in other GWAS of immune-related diseases, with a different mosaic of disease-specific

Table 1 Current non-HLA associations recognised for primary biliary cirrhosis

Chromosome (Position Mb)	Probable gene association	Other disease associations from GWAS
1 (2.5)	<i>MMEL1, TNFRSF14</i>	CeID, MS, RA, UC, PSC
1 (67.7)	<i>IL12RB2</i>	SS
1 (197.5)	<i>DENND1B</i>	Asthma, CroD
2 (191.8)	<i>STAT1, STAT4</i>	CeID, RA, SS, SLE
2 (228.7)	<i>CCL20</i>	HT
3 (17.0)	<i>PLCL2</i>	
3 (119.2)	<i>CD80</i>	CeID
3 (159.7)	<i>IL12A, SCHIP1</i>	CeID, MS
4 (103.4)	<i>NFKB1</i>	Schizophrenia
5 (35.9)	<i>IL7R</i>	UC, MS, T1D
5 (158.7)	<i>IL12B</i>	AnkS, CroD, MS, Ps, PID, UC
6 (137.5)	<i>IL22RA2</i>	MS
7 (36.9)	<i>ELMO1</i>	CeID
7 (128.6)	<i>IRF5, TNPO3</i>	SS, SLE, RA, UC
9 (117.5)	<i>TNFSF15</i>	CroD, UC
11 (64.1)	<i>RPS6KA4</i>	
11 (111.2)	<i>POU2AF1</i>	
11 (118.8)	<i>CXCR5, DDX6</i>	MS
12 (6.4)	<i>TNFRSF1A</i>	MS, PID
12 (111.8)	<i>SH2B3, ATXN2</i>	CeID, CholM, CAD, RA, T1D
13 (43.1)	<i>TNFSF11 (RANKL)</i>	CroD
14 (68.2)	<i>RAD51L1</i>	
14 (103.6)	<i>TNFAIP2</i>	
16 (11.3)	<i>CLEC16A, SOCS1</i>	CeID, IgA deficiency, MS, SLE, T1D, UC
16 (85.9)	<i>IRF8 - FOXF1</i>	SLE, UC
17 (38.1)	<i>IKZF3, ZBP2, GSDMB, ORMDL3</i>	Asthma, CroD, RA, T1D, UC
17 (44.0)	<i>MAPT</i>	Parkinson's disease
19 (10.5)	<i>TYK2</i>	CroD, PID, Ps, T1D
19 (50.9)	<i>SPIB</i>	
21 (45.6)	<i>ICOSLG</i>	CeID, UC
22 (39.8)	<i>MAP3K7IP1, SYNGR1</i>	CroD

Table is based on reference [5]; for gene name abbreviations, see <http://www.ncbi.nlm.nih.gov/gene>

AnkS ankylosing spondylitis, *Alk Phos* alkaline phosphatase, *BRIC* benign recurrent intra-hepatic cholestasis, *Ca* cancer, *CAD* coronary artery disease, *CeID* celiac disease, *CholM* cholesterol metabolism, *CLL* chronic lymphocytic leukaemia, *CroD* Crohn's disease, *DILI* drug-induced liver injury, *GD* Grave's disease, *GCH* giant cell hepatitis, *HCC* hepatocellular carcinoma, *HT* hypertension, *IBD* inflammatory bowel disease, *ICP* intra-hepatic cholestasis of pregnancy, *LPAC* low phospholipid-associated cholelithiasis, *MS* multiple sclerosis, *PFIC* progressive familial intra-hepatic cholestasis, *PID* primary immunodeficiency syndromes, *Ps* psoriasis, *PSC* primary sclerosing cholangitis *RA* rheumatoid arthritis, *SLE* systemic lupus erythematosus, *SS* systemic sclerosis, *T1D* type 1 diabetes, *T2D* type 2 diabetes, *TrigM* triglyceride metabolism, *UC* ulcerative colitis, *Viti* vitiligo

risk contributing to the pathogenesis of PBC: nevertheless the data to date confirm overtly and clearly that PBC is a

classic autoimmune disease, for which the genetic risk of development is associated with multiple, overlapping, immunoregulatory pathways. Overall, a reasonable inference is for important contributions from a number of immune pathways to the development of PBC, particularly highlighting a strong implied role for the interleukin (IL)-12 cytokine and downstream JAK-STAT signalling pathway. These data need to be interpreted in the context of a large pre-existing body of evidence supporting immune models of disease from murine and human studies, and thus the present list of associated genes sustains a model heavily focused towards the effects of balancing immunoregulatory pathways, in particular Th cell lineages including Th1 and Th17 cells [2, 25]. Furthermore, key biologic studies are still needed to confirm the etiologic relevance of these associations. Such studies will include therapeutic intervention studies in patients, which may prove the only way to fully understand the significance of particular pathways to disease. The challenges to understanding also need to recognise that over time the relative impact of adaptive immunity to PBC progression may change: hence, whilst all stages of disease can be evident on a liver explant from a PBC patient, the persistent immune damage and recurrence of disease post-transplant may imply an eventual dominance of innate immune responses over time. The association of PBC with genes involved in the IL-12 signalling pathway is consistent with the major immunoregulatory roles of 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 is central to generating Th1 immune responses directed towards the clearance of intracellular pathogens, and interferon gamma production inhibits IL-23-driven induction of IL-17-producing helper T lymphocytes. A role for IL-35 is also worthy of investigation, given that the subunits of the IL-35 cytokine and its receptor, respectively, include IL-12 p35 and IL-12R $\beta 2$. It is also notable that the regulation of *IL12RB2* expression in T regulatory cells appears to be important in determining Th cell lineage effects, 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. Many of the loci associated with PBC, such as *IRF5*, *SOCS 1* and *NF- κ B*, further suggest that Toll-like receptor signalling upstream to the IL-12 production may be relevant to disease. For example, *IRF5* interacts with nuclear factor κ B, which consequently causes an expression of a number of potentially relevant Th1 cytokines, including IL-12.

Future Opportunities

Genetic discoveries do not negate the importance of environmental factors in determining disease, but highlight a number

of mechanistic pathways that are presumed to be differentially relevant to disease in different patients. In this way, it is therefore now possible to focus biologic experiments on these pathways in the hope that the results will inform the design of more rational and effective therapies for patients. However, such studies need to be conducted in the context of refined genetic mapping, because as demonstrated for obesity and the *FTO* gene, gene involvement in disease cannot be presumed from GWAS data alone.

Future work will therefore focus not only on the biologic implications of these discoveries but also on defining a genetic risk related to the selected sub-phenotypes of disease, such as the characteristics of presentation, treatment response or symptoms. In this way, the mosaic of disease biology will be pieced together, and investigators will gain insights into why patients develop disease and whether intervention targeted to risk pathways can change the clinical course for individual patients. Already large-scale studies in related diseases such as rheumatoid arthritis and inflammatory bowel disease are working towards this goal. Application of novel bioinformatics approaches is key to success, particularly in seeking new therapies for disease, which indeed may arise from the “re-purposing” of existing therapies. In rheumatoid arthritis, a major study performed recently involved a genome-wide association study meta-analysis in a total of nearly 30,000 RA cases, evaluating <10 million single-nucleotide polymorphisms [26]. Moreover, than the discovery of a further 42 novel risk loci, the group used bioinformatics methods based on functional annotation, *cis*-acting expression quantitative trait loci and pathway analyses, alongside methods based on genetic overlap with human primary immunodeficiency, haematological cancer somatic mutations and knockout mouse phenotypes, to identify biological candidate genes and show that these genes are already the targets of approved therapies for RA and further suggest that drugs approved for other indications might be re-purposed for treatment.

Overall, for PBC, and related autoimmune liver disease, the true power of genetic studies will only be fully harnessed when cohorts are as large as possible and when genomic, meta-genomic and environmental data can be mapped to clinical phenotype. In this way, a stratified approach to therapy is hoped to arise, that is focused to carefully selected patients. Because of the complexity of disease, however, inevitably, this is likely to be a slow process, because fundamentally, it requires a shift in the paradigms of clinician understanding—whilst it is already accepted in colorectal cancer to think of molecular phenotypes [27], this is some way off for PBC, a much rarer clinical disease. The true application therefore of genetic studies can only come in parallel to a shift in clinical care concepts and delivery.

Conflicts of Interest None related to the content of this review.

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