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Genetics and Genomics of PBC

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Abstract

The etiological and pathogenic factors contributing to PBC development, progression, response to treatment, and ultimately, outcome remain a mystery. This lack of knowledge can be attributed to the complexity of PBC, wherein a number of environmental triggers may be culpable, but require coexisting genetic susceptibility to exert their effect. Recognition of the genomic regions harboring these heritable risk factors has been hindered by the rarity and late onset of PBC, which has rendered the collection of adequate numbers of patients and family members for genetic analyses a difficult task. Recent advancements in the discipline of genomics holds promise to fundamentally change our understanding, prevention, and therapy of PBC. This chance arises from the development of new high-throughput approaches to genotyping, providing the means to rapidly uncover many of the genetic polymorphisms that are relevant to disease. In order to move ahead, large registries and biospecimen repositories of patients with PBC, their family members, and well-matched controls need to be established, maintained, and continually expanded. At the same time, sizeable, comprehensive haplotype mapping based association studies of functionally plausible candidate gene groups (e.g. immune function genes) as well as more far reaching genome-wide association studies will be necessary to form the basis upon which the genetic predisposition to PBC can be defined. This first set of experimental data will provide the means for future fine mapping studies, re-sequencing efforts, functional experimentation, and elucidation of gene-environment and gene-gene interaction; perhaps paving new paths in PBC research.

Keywords

Autoimmunity; complex disease; genomics; genetics; PBC

Introduction

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease that significantly diminishes the quality of life in those patients so affected. The driving force of the pathogenesis of PBC is now recognized as an autoimmune attack on the biliary epithelial cells of the small

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to medium intrahepatic bile ducts (1). However, the etiological factors contributing to disease development and biological features influencing disease progression, response to treatment, and ultimately, outcome remain an obscurity. In large part, this dearth of knowledge stems from the complexity of PBC, wherein any number of environmental triggers may be involved, but their relevance masked by the requirement for coexisting genetic susceptibility. Identification of the genomic regions harboring these heritable risk factors has been hampered by the relative rarity and late onset of PBC, which has made it difficult to collect sufficient numbers of patients and family members for robust genetic analyses.

Genetics and complex disease

As with the majority of autoimmune diseases, PBC is likely to be genetically complex. That is, the specific alleles contributing to disease will be neither sufficient nor necessary for PBC development, and instead act as risk factors modifying the likelihood of disease, through modulation of the pathogenic processes underlying its eventuation (2). The impact of such risk alleles on disease is likely to be tempered by interaction, possibly through an environmental substrate or by epistasis with other genetic variants at either linked or unlinked loci, adding to the perceived complexity.

The two most often utilized models to explain the genetic architecture of complex diseases are the common disease-common variant (3) and the common disease-rare variant (4) hypotheses. At odds between these theories is the expected level of complexity in the allelic spectra of most disease associated loci. Whereas the common-variant hypothesis predicts this spectra to be relatively simple, implicating common variants (i.e. minor allele frequency [MAF] >5%) as the primary instigators of disease (5), the rare-variant hypothesis makes the opposite claim, that the allelic spectra of disease loci will be highly diverse, and thus many rare variants would contribute to disease. At stake is our ability to identify the offending variants, as a high level of diversity at an individual locus (i.e. allelic heterogeneity) seriously hampers our association based mapping efforts, as new variants might arise on different haplotypes, effectively canceling each other out (6). Recent evidence supports that the common-variant hypothesis is likely applicable to many disease associated loci (6,7) offering great promise for significant genetic discoveries in the near term. However, it is apparent that locus heterogeneity (i.e. the number of loci contributing to a disease) is likely to be extensive for most complex diseases, and that the extent of allelic heterogeneity among these loci is likely to run the spectrum, such that both common and rare variants will have significant impact on most complex diseases, including PBC (7,8).

In order to alleviate some of the complexity arising from the locus and allelic heterogeneity implicit in complex disease, it is often useful to conceptualize the disease as a series of traits, in addition to presence of the overt disease state, when approaching the genetic analysis (9). For instance, serological presence of anti-mitochondrial antibodies (AMAs) is widespread among patients with PBC and thought possibly to contribute to disease pathogenesis (10), but they are not found in all cases, suggesting an underlying difference in the disease etiology of these individuals that to date has eluded explanation. Consideration of AMA status in the genetic analysis of PBC (i.e. AMA+ compared to AMA− PBC patients) represents a new approach, which may help to explain this conundrum. Antinuclear antibodies (ANAs) have also been recognized in patients with PBC, and while not as prevalent and specific to PBC as AMA, appear to correlate with disease severity (11), particularly when directed against the nuclear pore complex (12), and thus ANA profile seems an appealing trait for genetic study in PBC. Other more direct measures of severity, such as histological stage, Mayo PBC Risk Score, progression to orthotopic liver transplant (OLT), and presence of disease related complications, as well as response to treatment with ursodeoxycholic acid will also prove valuable in

dissecting the genetics of PBC. However, at present it remains unclear how best to approach these features, and to what extent ascertainment bias will affect the results.

The familial component of PBC

Similar to most autoimmune diseases, the genetic contribution to PBC risk and pathogenesis is thought to be quite important. This assertion is evidenced by familial clustering of PBC, high disease concordance in monozygotic twins, and increased prevalence of other autoimmune disorders in PBC patients and their family members (13).

Clustering of disease within families is often used as evidence of genetic influence on disease development as family members typically share more genetic material among themselves than with the general population, and thus are more likely to share genetic characteristics associated with increased risk of disease (14). It has long been appreciated that PBC aggregates in families, and the historical epidemiological studies have shown familial PBC prevalence (i.e. multiple cases of PBC within the same index family) to range between 1.0% and 6.4% (15,16).

Taken further, use of the sibling *relative risk ratio* (λ_s) is another means to estimate the level of the genetic impact on a disease, but in contrast to familial aggregation studies, provides a context by which comparison of family risk to that of the general population can be made. In the most comprehensive geographically based epidemiological study of PBC to date, the λ_s of PBC in the UK was determined to be 10.5, with 6.4% of patients reporting a family history of PBC, and 1% of their FDRs having the disease (17). More recently, in a study from the United States, 2.2% (24/1116) of the siblings of 379 PBC patients were reported to have PBC (18). Using the current best estimate for PBC prevalence in the US of 402/100,000 (19), the λ_s for PBC would be 54, much greater than that found in the UK study. However, the prevalence of familial PBC in this US study is high at 9.0% (18) compared to that found in a larger questionnaire based study of 1032 US PBC patients wherein 5.9% reported familial PBC (20), suggesting that a λ_s of 54 for PBC is somewhat inflated. Interestingly, this same study reported familial history of PBC to be the strongest identified risk factor for PBC development (5.9% PBC vs. 0.5% controls, OR 10.7, 95% CI 4.2–27.3, $p<0.001$) (20). While the λ_s values of PBC determined to date are far from definitive, they are of the same order of magnitude as other classic autoimmune diseases (13) and seem to implicate a strong role for genetically encoded risk on PBC.

Of course, caution needs to be taken when considering familial disease aggregation and λ_s values as evidence for the level of genetic involvement with disease. Biased sampling of the diseased population as well as inaccurate estimation of disease prevalence in the general population can significantly alter the calculated λ_s values, especially for rare diseases such as PBC. Perhaps more importantly, these studies are unable to address the role of shared environment and behavior among family members, phenomena that may play an important role in disease etiology and pathogenesis.

Presently, the best means for dissecting the contribution of shared-environment and genetic influences on complex disease is the comparison of disease concordance between monozygotic and dizygotic twins (21). As monozygotic twins share a common DNA sequence, disease concordance is highly suggestive of genetic influence and conversely, discordance is illustrative of environmental and/or epigenetic effects. Disease concordance between dizygotic twins is used as the control for the shared environment effect. In the only twins study of PBC to date, 63% (5/8) of monozygotic compared to 0% (0/8) of dizygotic twin pairs were found to be concordant for PBC (22), suggesting that the role of genetics in PBC is quite strong. However, caution should be taken in interpreting this finding as the study was quite small due to the rarity of PBC, and participation bias could not be adequately assessed.

The increased occurrence of other autoimmune disorders including systemic lupus erythematosus, autoimmune thyroid disease, Raynaud's syndrome, and Sjogren's syndrome (20) in PBC patients and their family members is well established and often invoked as further evidence for the genetic contribution to PBC. This is because autoimmune processes are thought to share overlapping mechanisms involved with immune control, and thus genetic propensity to the development of autoimmunity is partly borne in the general sense (23,24). It is within the context of this autoimmune-permissive genetic background that disease specific genetic susceptibility, in concert with disease specific environmental factors leads to the development of specific autoimmune diseases like PBC (23).

In addition to clustering of PBC and other autoimmune diseases in the families of patients with PBC, the presence of antimitochondrial antibodies (AMAs) has also been shown to aggregate in first degree relatives of afflicted individuals, regardless of familial history of PBC (18). Strikingly, in the largest such study to date, AMAs were detected in 13.3% of mothers, 15.6% of sisters, and 12.5% of daughters of 127 patients with PBC with no history of familial PBC, in contrast to 1.1% of the female controls (18). Also of interest, the frequency of circulating T-regulatory cells (Tregs) has been found to be significantly reduced among sisters and daughters of PBC patients compared to age-matched controls (25). Taken together, these findings illustrate the familial predisposition to pathogenic features underlying PBC, thus providing additional evidence for the likely influence of genetics on this disease.

Due to the rarity and late onset of PBC, the large registry-based twin studies, adoption studies, and multi-generational prevalence studies necessary to provide accurate quantitative estimates of the relative roles of genes and environment in the development of PBC are not feasible. However, the current familial evidence outlined above seems to suggest that the contribution of the genetic component to PBC is quite strong.

Genetic variation and PBC susceptibility

Traditional family-based linkage studies, the genetic workhorse of the recent past, have identified candidate loci for a number of autoimmune disorders, providing insight as well as the basis for ongoing work into the genetics of these diseases (26). However, the infrequency of PBC, coupled with its delayed onset, has thus far precluded the collection of sufficient sample sets for linkage analysis in PBC, and thus no such study has been reported. Instead, the selection of gene candidates in the various PBC association studies published to date has been widely based on knowledge gained from the study of other autoimmune disorders, and to a lesser extent on mechanisms gleaned from the current PBC research. Below we present an overview of the important genetic findings for PBC generated to date.

MHC – HLA

The association between susceptibility to autoimmune disease and alleles of the human leukocyte antigen (HLA) genes has been long appreciated (27). These genes are located in the highly polymorphic, gene-dense major histocompatibility complex (MHC) genomic region at chromosome 6p21 which, in addition to class-I and class-II HLA, harbors numerous genes influencing immune function such as complement factor and cytokine genes, as well as many others with no obvious immune connection (28). Genetic variants in antigen presenting HLA genes have been associated with over 100 diseases, including the vast majority of autoimmune disorders (29). While the specific mechanisms behind the associations with autoimmune disease remain unclear, it is thought that the particular disease associated variants might enhance the binding or presentation of specific self-peptides to the T-cell receptor, increasing the potential for development of an autoimmune response (30). Due to the long held realization of HLA involvement with autoimmunity, it is not surprising that the majority of past efforts at

deciphering the genetics of PBC have focused on this region. Indeed, over 20 studies have been published on the HLA association with PBC in the past 25 years (15,31–52).

Historically, the most commonly detected HLA association with PBC has been with the class II *DRB1*08* allele family; specifically *DRB1*0801* in European and North American Caucasians (32,41,49–51), and *DRB1*0803* in the Japanese (38). The *DRB1*0801* association was recently confirmed in a report from the UK, by far the largest HLA study in PBC to date, involving 492 patients from the UK and Italy as well as 331 regionally matched controls (49). In addition to the *DRB1*0801* allele, the haplotype consisting of *DRB1*0801-DQA1*0401-DQB1*402* has been associated with PBC in studies of patients from the US (51), UK, and Italy (49). However, strong linkage disequilibrium (LD) in the region, the fact that the prevalence of this haplotype is somewhat less than that of the *DRB1*0801* allele, and the finding that the Japanese risk haplotype is comprised of *DRB1*0803-DQA1*0103-DQB1*0601* (38) suggests that the *DRB1*08* allele family is most likely the primary determinant of PBC risk. While the *DRB1*08* family does appear to impose a significant risk of PBC, demonstrating odds ratios (ORs) in the range of 3 or greater in the larger Caucasian (32,41,49,50) and Japanese (38) studies, a recent study from China reported the *DRB1*0701* and *DRB1*03* alleles to be significantly increased in the PBC patients compared to controls, with no difference in the prevalence of the *DRB1*08* alleles (43). This finding seems to suggest that regional differences, perhaps variation in environment or lifestyle, or possibly some recent selective pressure on the underlying gene structure, have an influence on MHC encoded PBC risk. However, this study was small, involving only 65 patients and 431 healthy controls (43), and needs to be expanded before logical hypotheses regarding the pathogenic mechanisms behind these differences can be explored.

In addition to associations with risk of PBC development, a few class II HLA alleles have demonstrated protective associations against PBC. These include DQA1*0102 in US and Japanese studies (32,38) and DQB1*0602 in a US study (32), neither of which has been confirmed by larger studies. More recently, DRB*13 was found to be protective against PBC in both UK and Italian PBC patients and DRB1*11 was protective in Italian, but not UK patients (49,53). Interestingly, in their latest report, the UK group compared the differences between the most commonly implicated DRB1*08 risk allele and the DRB1*11/*13 protective alleles, from which they postulated that differences in the hydrophobicity or size of four DRB1 amino acids may alter the binding properties of the MHC molecule, influencing the development of PBC (49). Confirmation of this hypothesis would be of great interest, as it would strengthen the evidence for the genetic associations while demonstrating their functional relevance to disease.

A limited amount of effort has been placed on assessing the potential association of HLA class I alleles with PBC, with a handful of small studies finding no associations (34–36,38,45,46, 48). More recently, a few rare HLA-B alleles were found to be associated with PBC in an Italian study of 112 PBC patients and 558 controls (53). However, there have been no follow-up studies to confirm these findings. While the current evidence suggests little if any role for class I MHC alleles in PBC, due to the relative lack of coverage and number of patients assessed, the genes in this region remain prime candidates for further investigation.

The class III MHC genes also remain virtually unexamined in PBC, perhaps with the exception of tumor necrosis factor alpha (TNFα). As TNFα had been long appreciated as an important inflammatory mediator with potential involvement in PBC (57), and it had been demonstrated that a G/A polymorphism at position −308 in the promoter of the TNFα gene resulted in altered TNF expression (54,55); a number of small PBC association studies were performed (13,56–58). Interestingly, the first study reported that the less common −308*2 (A) allele was reduced in patients with PBC compared to controls, with also a significant finding for an increase in

the −308*1/*1 (GG) genotype in PBC (57), suggesting the allele shown to express higher levels of TNFα was somewhat protective against PBC. Subsequently, two studies reported to find no association between the allele and PBC, but both had relevant findings using different measures of severity (56,59). However, these two studies were at odds as the smaller of them found significantly higher Mayo PBC Risk Scores in patients heterozygous for the −308 allele (*1/*2) compared to the −308*1/*1 homozygotes, suggesting the −308*2 allele increased disease severity (56); whereas the larger study found a significant elevation of −308*1/*1 homozygosity in patients with more advanced disease by histological stage, suggesting that the −308*1 allele was at fault (59). A subsequent study showed no association with this allele, but was too small to draw any real conclusions (58). To date, the potential association between PBC and alleles affecting the expression of TNFα remains unresolved. However, in light of strong evidence for the role of −308*2 in autoimmunity (60), and on development of hepatic fibrosis (61), further study of this variant in PBC is imperative.

“Autoimmune” genes

Over the past few years, a number of genes have been implicated with multiple autoimmune disorders and thus have come to be known as “common autoimmune genes” (62). The products of these genes are thought to be essential to the maintenance of tolerance, and consequently even slight functional variation can tip the immunological scales to a more autoimmune permissive state. Among these, the most well established and thoroughly studied are PTPN22, CTLA4, and PDCD1 however several others have been proposed. Here we provide a brief background and review the current research on these common autoimmune genes in PBC.

The protein tyrosine phosphatase 22 (PTPN22) gene encodes lymphoid tyrosine phosphatase (LYP), a molecule which through association with C-terminal Src-kinase (CSK) has an inhibitory effect on T-cell activation following engagement of the T-cell receptor (63). A single nucleotide polymorphism (SNP) in this gene known as C1858T (rs2476601) results in an arginine to tryptophan substitution at codon 620 (R620W) which appears to significantly alter the function of both T- and B-lymphocytes (64) and has been associated with many autoimmune diseases including Hashimoto’s thyroiditis (HT) and systemic lupus erythematosus (SLE), with odds ratios in the neighborhood of 1.5 (62). To date, two studies have been published regarding this variant in PBC. The first included 154 Italian PBC patients and 166 ethnically matched controls, which found no association with PBC overall, but did report an association of the minor T allele with AMA negative PBC compared to controls (OR 3.6, 95% CI 1.4–9.2, p=0.01) (65). The second consisted of 160 AMA positive Caucasian PBC patients from Canada and 290 healthy controls, and also found no association of either allele or genotypes with PBC (66). Similar results were obtained in a study of 368 US PBC patients and 206 healthy controls, however the association with AMA negativity was not replicated (unpublished data, personal communication with Dr. Lazaridis), providing additional evidence that the C1858T variant of PDCD1 does not directly confer risk for PBC.

The cytotoxic T-lymphocyte antigen 4 (CTLA4) gene encodes an immunoreceptor that through competition with CD28 for CD80/86 on antigen presenting cells inhibits T-cell activation upon MHC presentation of antigen to the T-cell receptor (TCR), and has been shown to play an important role in the maintenance of tolerance and prevention of autoimmunity (67). Genetic variants of this gene, most often 49AG (rs231775) and/or CT60 (rs3087243), have been found to be associated with an assortment of autoimmune diseases including autoimmune thyroid disease, type I diabetes (T1D), and SLE (68).

Early studies of CTLA4 in PBC identified an association with the G allele of 49AG in both UK (69) and Chinese (58) populations, that was not confirmed in a small study from Brazil (31). However, a later study by the UK group which investigated additional SNPs across CTLA4 in an expanded patient population using a more suitable control group, failed to

replicate their initial findings of the 49AG association with PBC, calling into question this SNPs relevance to disease (70). Interestingly, this study did identify borderline associations of 2 of the CTLA4 SNPs with PBC related fatigue, as assessed by PBC-40 Fatigue Domain score (70), which if confirmed would offer relevant insight into an important life altering aspect of this disease. More recently, the 49AG and CT60 SNPs were assessed in 351 PBC patients and 205 healthy controls from the US, and neither SNP was significantly associated with PBC (2), in agreement with the latter UK study. However, the 49AG:CT60 haplotype was found to be significantly associated with progression to orthotopic liver transplantation (OLT) (global p=0.03) in this study (2). As well, homozygosity for the G allele of the 49AG SNP was strongly associated with AMA positivity among the PBC patients (19% AMA+ vs. 2% AMA−, OR 9.96 (1.34–73.97), p=0.02) (2). A separate study involving 154 Italian PBC patients, reported a significant decrease of homozygosity for the A allele of the CT60 SNP in AMA negative patients (which was not found to be different in the US study) (2), but this Italian study did not address the 49AG variant (65). In light of these recent findings, the continued investigation of CTLA4 genetic variation in PBC is clearly warranted.

The programmed cell-death 1 (PDCD1) gene encodes a receptor with T-cell inhibitory function similar to CTLA4, but without an apparent stimulatory competitor (71). Of interest, recent findings have suggested that PDCD1 and CTLA4 work synergistically through distinct mechanisms targeting the phosphoinositide 3-kinase/Akt signaling pathway in order to inhibit T-cell activation and promote self-tolerance (72,73). Studies have shown PDCD1 and its ligands PDL1 and PDL2 to be upregulated in the livers of PBC, autoimmune hepatitis (AIH), and chronic hepatitis C patients, but not in normal controls (74,75) demonstrating a potential, albeit not necessarily disease specific, role in PBC.

A SNP in the PDCD1 gene, known as PD1.3 (rs11568821) has been implicated in a number of autoimmune disorders (76–78) and is thought to disrupt a RUNX1 transcription factor binding site, potentially altering the regulation of PDCD1 (77). To date, the only published study of this variant in PBC included 351 PBC patients and 205 controls from the US (2) in which no PBC association was found. However, homozygosity for the putative general autoimmune risk A allele was found to be significantly increased in PBC patients who had previously required OLT compared to those patients who had not, suggesting this variant might be involved with severity or progression of disease. Although, caution should be taken in the interpretation of this result as the number of OLT patients and frequency of the genotype were quite low and thus prone to error. Moreover, this same study reported an increased risk of PBC associated with an interaction between the PD1.3 A allele and the putative autoimmune protective CTLA4 49AG:CT60 A:A haplotype (2). This finding suggests that the PD1.3 variant might impact disease in the context of CTLA4, consistent with the assertion of functional synergy between these genes.

N-RAMP—While SLC11A1 (formerly NRAMP1) is an interesting gene which seems to act at the intersect of infection, autoimmunity, and cancer and might prove to be relevant for PBC, we found only 1 reported study, which was from 2000, and included only 46 patients with PBC. In this review we have elected to focus on the larger studies.

Other PBC candidate genes

In addition to the “common autoimmune” genes and those of the MHC, a number of genes have been studied based on evidence or potential for their direct involvement with features of PBC. This rational candidate gene approach is well intended, offering a focus upon which to perform genetic interrogation in lieu of a viable candidate genomic region identified through traditional familial linkage studies. However, this tactic has yielded little in the form of insight into the genetic features underlying PBC, most often due to conflicting findings and the

inability to confirm positive associations. Indeed, many of the published studies on genetic variation in PBC have utilized small patient sets with relatively little power to reliably conclude whether the studied variant is associated with the disease or not, and many of these have been reviewed elsewhere (15,16). That said a couple of interesting larger genetic studies of candidate genes have been performed for PBC as of late, and recently reported mouse models have offered novel insights identifying new gene candidates for future assessment in human disease. These findings are highlighted below.

Selected genetic polymorphisms in genes with an influence on xenobiotic metabolism and transport were assessed in 169 Italian PBC patients and 225 healthy controls in order to address the hypothesis that halogenated xenobiotics might alter self-molecules and facilitate the breakdown of tolerance to mitochondrial antigens (79). Addressed in this study were a polymorphism of the multidrug resistance 1 gene (MDR1) that has been associated with decreased intestinal expression, three SNPs in the nuclear receptor gene PXR (pregnane × receptor) whose activity regulates expression of MDR1 in the liver and intestine, and various polymorphisms of the drug metabolizing cytochrome P450 genes CYP2D6 and CYP2E1 (79). None of the polymorphisms studied was found to be associated with the development of PBC, but the C2 allele of CYP2E1 was found to be significantly increased among patients with advanced stages of PBC compared to those with earlier stage PBC, as assessed by Ludwig stage, and history of cirrhotic complications (79). Indeed, 9 of the 10 patients with PBC with the C2 allele were in the advanced disease group, suggesting that patients carrying this allele may be more prone to disease progression (79), although the mechanism behind this phenomenon remains unclear. While the frequency of this allele was quite low (3% of CYP2E1 alleles) in the patient population, it could prove to be a useful prognostic indicator and might shed light on new features of disease pathogenesis. Further study including larger patient populations and a more comprehensive assessment of variation in the CYP2E1 gene will be of great interest.

A number of genetic variants potentially involved with T-cell proliferation were investigated in a study of 154 Italian PBC patients and 166 healthy controls, including single SNPs in the FOXP3, ICOS, IL2Rα (CD25), PTPN22, and CTLA4 genes (65). The only positive associations were found with AMA status and polymorphisms of the common autoimmune genes PTPN22 and CTLA4, which are described above. However, the three other genes tested, which were far from comprehensively addressed, remain interesting candidates for PBC involvement due to the direct role of their expressed products in immune function.

This assertion is particularly important for the IL2Rα gene, which encodes the alpha subunit of the interleukin-2 receptor, and is a marker for Tregs (CD25). In fact, a recent case report describes the complete lack of IL2Rα on the peripheral lymphocytes of a 5 year old boy presenting with liver dysfunction and serological expression of PBC, including the development of AMA specific for PDC-E2 (80). This case lends credence to the notion that Tregs, which are reduced in PBC patients, as well as in their sisters and daughters (25), exert a protective influence against the development of autoimmunity and particularly PBC. As a follow-up to this case report, IL2Rα deficient ($IL2R\alpha^{-/-}$) mice were generated and assessed for the development of clinical features found in PBC (81). Notably, these mice developed portal inflammation and bile duct damage, demonstrated increased levels of IgG and IgA, and produced PDC-E2 specific AMA; all hallmarks of human disease (81). While genetic polymorphisms of IL2Rα have yet to be assessed for association with PBC, these recent findings illustrate the potential for genetically encoded deficiencies in these genes to impact human disease.

In addition to the $IL2R\alpha^{-/-}$ mice, transgenic mice expressing a dominant negative form of TGF-β receptor II exclusively in T lymphocytes also have been shown to develop PBC specific

characteristics, including lymphocytic infiltration, biliary damage, and AMA specific for PDC-E2, BCOADC-E2, and OGDC-E2 (82). The observation that lack of TGF-β signaling in these mice leads to loss of tolerance to antigenic liver proteins suggests that genetic aberrations affecting the function of this pathway could contribute to human PBC. More clues to the possible location of genetic variants involved with PBC come from another mouse model known as NOD.c3c4 (83,84). These are congenic mice with insulin dependant diabetes (*Idd*) loci from diabetes-resistant strains introgressed onto the highly autoimmune prone non-obese diabetes (NOD) background (83). Interestingly, these mice are completely protected from diabetes, but instead develop a form of autoimmune cholangitis with similarities to human PBC, including the development of PDC-E2 reactive AMAs (84).

Subsequent congenic mapping of these mice identified the first autoimmune biliary disease locus in the mouse, *Abd1*, which is thought to contain alleles resulting in the switch between autoimmune targeting of the pancreatic islet and the biliary tract (84). However, coexistence of the autoimmune permissive NOD genetic background appears to be essential to development of the liver directed phenotype (83). This mouse has very interesting implications for human PBC in that the findings seem to suggest that alleles leading to the specific targeting of bile ducts, in combination with a genetic background rendering one prone to autoimmune processes, are likely to contribute to the etiology of PBC. Moreover, the *Abd1* locus identified in these mice could plausibly harbor genes involved with human disease. The murine *Abd1* locus is large, covering around 30 Mb of mouse chromosme 4, the homologous regions of which are distributed among 3 regions of human chromosomes 9 and 1, which are near perfectly syntenic (i.e. they share order of homologous genes with the mouse). Together, these areas cover about 40 Mb of the human genome and contain some 250 classified genes, the majority of which are homologs with similar functions in mice and humans. These include several genes encoding cytokines and their regulators, as well as genes involved with cell-cycle arrest, apoptosis, and control of bile acid synthesis. While this region is large, it is suitable for association mapping using emerging technologies, and is a viable candidate region for genetic study of PBC.

The sex chromosomes and PBC

The overwhelming preponderance of women in the PBC affected population clearly implicates sexually derived differences in the etiology of PBC, but what exactly these disparities are remains unclear. The differential effect of sex hormones on the immune system is the traditional concept for the female predominance of autoimmunity. Indeed, Th1 and Th2 responses seem to be clearly affected, with androgens favoring the activation of CD8 cells and development of a Th1 response, whereas estrogens promote the Th2 response through the activation of B-lymphocytes and subsequent induction of antibody production (85).

Microchimerism has also been suggested as an inducer of autoimmunity, and while there are many potential sources of microchimerism, pregnancy is far and away the most prevalent (86) and thus invoked as a mechanism contributing to the female autoimmune majority. This phenomenon of fetal-maternal chimerism has been studied in PBC but no strong evidence for a significant involvement with disease has been found (87,88–90). However, microchimerism could be a significant factor on the etiology of PBC for a subset of individuals, likely in concert with further genetically encoded autoimmune propensity, and therefore should not be so quickly discounted.

While the differential activity of sex chromosomes and increased exposure to microchimerism offer clues as to the reasons that autoimmunity predominates among females, these explanations give relatively little insight into why some females develop autoimmunity when most do not. To begin addressing this, Invernizzi et al investigated the frequency of X chromosome monosomy in women with PBC, because genes implicated with immune function

can be found on the X chromosome, and women with Turner's syndrome, characterized by the congenital loss of one X, often display autoimmune features and cholestatic manifestation (91). Interestingly, they found PBC patients to have an increased frequency of X chromosome monosomy in peripheral blood cells overall, as well as in all tested white blood cell subpopulations, suggesting an instabilty of the X chromosome related to PBC (91). Further work by this same group using quantitative flourescent PCR found the pattern of X chromosome loss to be preferential in PBC, compared to healthy and hepatitis C virus infected controls (92). In this same study, X chromosome inactivation (XCI) was also assessed, and no significant association between PBC and degree of XCI skewing was identified (92). Taken together, these findings suggest that particular X-linked alleles or haplotypes, likely in genes escaping XCI, might predispose to the development of autoimmunity as the result of haploinsufficiency due to the acquired monosomy, providing us with novel candidate regions for which to search and address in PBC.

The future of PBC genetics

Innovation arising from increased investment in genotyping technologies following the initial phases of the HapMap project positions us on the cusp of unprecedented potential for documentation of genetic variation underlying PBC. Rapidly decreasing costs have led to an explosion of genome-wide association (GWA) studies for all manner of diseases, and it is only a matter of time until the first GWA for PBC will appear. As well, large-scale custom genotyping and gene resequencing technologies are becoming more accessible to the average researcher, complementing GWA and allowing for the application of new targeted approaches toward the dissection of the genetic contributors to disease.

During this "genetic renaissance" of the approaching years we face many challenges. Foremost, the rarity and late onset of PBC makes recruitment of patients and their family members (especially their parents) into genetic studies a difficult task, and thus the initial large scale efforts will likely lack power to reliably detect risk factors of weaker effect (i.e. OR <1.5). Moreover, this rarity will make it difficult for individual programs to rapidly collect confirmatory groups of patients from similar populations, and therefore at minimum, cooperation, if not outright collaboration, between investigators will be essential. Careful selection of control populations to minimize statistical artifacts will be important, especially during the "rush to GWA", and it may prove useful to utilize multiple control groups, including family based controls, when considering the analysis of the limited number of patients ultimately available for study. Functional experimentation to determine the mechanisms and impact of associated genetic variants will also be important to confirming their relevance to disease, however the expected subtlety and complexity of the resulting effects will require new approaches, and initial efforts are likely to be poorly conceived.

Also contributing to our challenge is the presumed underlying latency of PBC, which has limited our ability to understand the natural history of its etiology and observe the pivotal events early in disease that could help to stratify the patients for genetic analysis. Such knowledge, as well as other novel and sophisticated methods of disease sub-classification would be beneficial to our short term efforts to identify the common genetic variants contributing to PBC, but will be paramount in future efforts to identify rare genetic contributors, and to evaluate the effects of environmental interaction and genetic epistasis on disease. To overcome this challenge, inclusion of at-risk populations, particularly the adult children of PBC patients, in ongoing PBC registries and biospecimen repositories will provide a base upon which to draw for future at-risk cohort studies of PBC development. Combined with genotyping, such studies would allow us to develop and test predictive algorithms, initially for PBC onset, but subsequently for response to treatment; as well as allowing us to better assess the influence of environmental exposure on disease.

Conclusions

The advances in the discipline of genomics over the past decade holds promise to radically change our understanding, prevention, and therapy of PBC. This opportunity stems from the continuous development of novel technologies related to high-throughput genotyping, providing the means to uncover the multiple genetic polymorphisms that are germane to PBC. In order to step forward, large prospective registries and linked biospecimen repositories of PBC patients, their family members, and well-matched controls have to be established, maintained, and continually expanded. Concurrently, sizeable comprehensive haplotype mapping based association studies of functionally plausible candidate gene groups (e.g. immune function genes) as well as more far reaching GWA studies will be necessary to begin defining the genetic predisposition to PBC. This first set of experimental data will provide the means for future fine mapping studies, re-sequencing efforts, functional experimentation, and elucidation of gene-environment and gene-gene interaction, which will pave new paths in PBC research.

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