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The Genetic Basis of Primary Biliary Cirrhosis: Premises, Not Promises

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Primary biliary cirrhosis (PBC) is considered a model for autoimmune disease based upon its hallmark anti-mitochondrial serologic response, the clinical homogeneity among patients, and the focused target destruction of biliary epithelial cells. The past decade has witnessed several key advances in understanding the effector mechanisms of PBC based upon rigorous dissection of the epitopes involved in the anti-mitochondrial response and the qualitative and quantitative characteristics of autoreactive T cells ¹. These data suggest that the primary event in PBC is the loss of tolerance to PDC-E2, the immunodominant mitochondrial autoantigen. They also suggest that the destruction of biliary epithelium is based in part upon its unique apoptotic properties in which the mitochondrial autoantigens remain immunologically intact ¹. Furthermore, several animal models with autoimmune cholangitis have now been described ^{2–7}. Despite these advances in effector mechanisms and animal models, the genetic basis of PBC remains elusive ⁸.

The majority of studies on the etiopathogenesis of PBC have focused upon candidate gene based association studies. In the current issue of *Gastroenterology*, Juran et al. ⁹ provide novel data that are relevant. In particular, they report a novel susceptibility SNP (among the 8 evaluated) within the cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene in PBC. The major strength of this study is the typing of this large collection of DNA from a single center and the use of current technology for SNP selection. We should note, however, that the role of CTLA-4 gene abnormalities in PBC is not entirely novel and has not always been associated with disease. Furthermore, the results herein, similar to other candidate gene descriptions, do not include functional analysis.

The current thesis on the etiopathogenesis of PBC implies that susceptibility is secondary to genetic predisposition elements that are permissive for host-environmental interactions which lead to loss of tolerance to PDC-E2^{8, 10, 11}. Recent data strengthen the relevance of the multifactorial genetic basis in PBC, including the incidence of disease among first-degree relatives ¹², a high concordance rate among monozygotic twins ¹³, and the observation that women with PBC have preferential loss of one X chromosome in peripheral white blood cells ^{14, 15}. In addition, in contrast to earlier work, PBC is not only associated with the HLA DRB1*08 allele but also with the protective HLA DRB1*11 and DRB1*13 alleles ^{16, 17}.

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Finally, we note the appearance of a PBC-like disease in a child born with IL2 receptor α deficiency¹⁸.

Autoimmune diseases result from a failure to control autoreactive immune cells, and a number of negative immune regulatory pathways have been characterized¹⁹. The cell surface CTLA-4 is a critical inhibitor of T-cell activation and a pivotal component of the regulatory systems that serve to maintain peripheral tolerance²⁰. In particular, it is likely that CTLA-4 has a facilitating role in the suppressive function of Tregs, although does not appear to be absolutely required for the development or function of these unique cells. Interestingly, CTLA-4 has also been successfully utilized for the therapeutic manipulation of immune responsiveness and has led to the development of recombinant soluble inhibitors of T cell/antigen presenting cell costimulation²¹. These agents have demonstrated efficacy in rheumatoid arthritis²².

The literature on autoimmunity contains large numbers of publications that have attempted to identify genes responsible for autoimmunity by evaluating small numbers of single nucleotide polymorphisms (SNPs) in one or few specific candidate genes by means of case control study designs. However, such approaches have led to very few insights into the genetic basis of these complex diseases. By contrast, we are now witnessing substantial advances because of the use of large-scale, high-density genome-wide association studies (GWA)²³. The latter approach has disclosed more than 50 disease-susceptibility loci and has provided insights into the allelic architecture of multifactorial traits. An updated list of published GWA studies can be found at the National Cancer Institute (NCI)-National Human Genome Research Institute (NHGRI)'s catalog of published GWA studies (<http://www.genome.gov/26525384>). Interestingly, this GWA publications' list include only those attempting to assay at least 100,000 SNPs.

Based upon the development of autoimmunity in mice deficient in CTLA-4, a number of genetic association studies suggest that this gene is a locus of susceptibility to loss of tolerance, although specific functional defects in humans have yet to be identified^{20, 24}. The human CTLA-4 gene is located on the long arm of chromosome 2 (2q33), with a high degree of sequence homology with the mouse gene²⁵. The CTLA-4 gene consists of four exons, with exon 1 encoding a leader peptide, exon 2 the ligand-binding domain, exon 3 the transmembrane domain, and exon 4 encoding the cytoplasmic tail. A number of SNPs within the CTLA-4 locus are associated with a variety of autoimmune diseases, including type 1 diabetes, Graves' disease, systemic lupus erythematosus, Addison's disease, rheumatoid arthritis, and celiac disease. Overall, the magnitude of disease susceptibility associated with these identified SNPs is generally small, but they suggest the need for fine gene mapping and functionality to correlate these data and the mechanisms of action that result from these allelic variants of the CTLA-4 gene.

Other studies have evaluated the CTLA-4 gene in PBC (Table 1). While two earlier studies from the U.K.²⁶ and China²⁷ found an association with the coding SNP 49AG (encoding threonine or alanine at amino acid level) and PBC, more recent data from Brazil²⁸, Italy²⁹, Germany³⁰, the U.K.³¹, and the U.S.³² failed to confirm this, including analysis of additional SNPs. Indeed, a follow-up study by the U.K. group³¹, failed to replicate their original positive finding²⁶. By contrast, the follow-up study by the U.S. group (published in the current issue of *Gastroenterology*⁹) found a novel, albeit weak, SNP ($P=0.003$) association in contrast with their original negative finding³². In particular, they enlarged their study population from 351 to 402 patients with PBC and from 205 to 279 controls, and considered six additional CTLA-4 SNPs^{9, 32}. Although they used an appropriate method to identify the SNPs to be evaluated³³, they did not provide the information necessary to localize these SNPs in the CTLA-4 gene, nor did they perform any imputing analysis (explain what this is), thereby potentially missing the opportunity to strengthen their finding and to find neighboring SNPs in linkage disequilibrium³⁴. In such cases, the Mantel-Haenszel test could have been applied to analyze

the “sequential” sampling design, thus allowing a better understanding to the contribution of each single “sub-sample” other than the entire sample, and also looking at the potential heterogeneity of the two sets of subjects. Such an approach would allow a focus on whether negative findings were due to a lack of power. Finally, although the permutation test is currently the gold standard in GWA studies, in the Juran study with limited SNPs, there are other methods to apply, i.e. Nyholt’s, which modifies the traditional Bonferroni’s while considering the linkage disequilibrium relationship across SNPs and, being less conservative than Bonferroni’s, provided the SNPs are not independent from each other³⁵. Indeed, permutation tests have unexpectedly provided a protective haplotype association driven by the susceptibility SNP rs231725.

In conclusion, we submit that solution to the genetic basis of PBC ? too strong (may) not occur with use of candidate genes. Rather, a large whole-genome approach is required to identify the genetic elements that lead to loss of tolerance in PBC, as currently underway in many other multifactorial and complex diseases²³. We believe that the study of SNPs in PBC should focus primarily on coding variants (both in exons or in promoter regions) of genes with a clear known involvement in the mechanisms of disease³⁶. A multi-team and multi-centric effort will be required to enroll sufficient subjects and controls for such analysis.

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Abbreviations

PBC	primary biliary cirrhosis
CTLA-4	cytotoxic T-lymphocyte antigen-4
SNPs	single nucleotide polymorphisms
GWA	genome-wide association studies

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Table 1

CTLA-4 genetic SNPs evaluated in PBC

SNP (rs ID)	Country (year)	PBC Patients (n)	Controls (n)	P Value	Reference
49/AG (rs231775)	U.K. (2000)	200	200	0.000063	26
	Brazil (2003)	50	67	NS	28
	China (2004)	77	160	0.0046	27
	Germany (2007)	180	202	NS	30
	U.K. (2007)	247	293	NS	31
	Italy (2007)	80	99	NS	31
	U.S. (2008 A)	351	205	NS	32
	<u>U.S. (2008 B)</u>	<u>402</u>	<u>279</u>	<u>NS</u>	9
-318 (rs5742909)	China (2004)	77	160	NS	27
	<u>U.S. (2008 B)</u>	<u>402</u>	<u>279</u>	<u>NS</u>	9
	Italy (2005)	154	166	NS	29
	U.K. (2007)	247	293	NS	31
CT60 (rs3087243)	Italy (2007)	80	90	NS	31
	U.S. (2008 A)	351	205	NS	32
	<u>U.S. (2008 B)</u>	<u>402</u>	<u>279</u>	<u>NS</u>	9
	U.K. (2007)	247	293	NS	31
T-1722C	Italy (2007)	80	90	NS	31
	U.K. (2007)	247	293	NS	31
	Italy (2007)	80	90	NS	31
	U.K. (2007)	247	293	NS	31
A-1661G	Italy (2007)	80	90	NS	31
	U.K. (2007)	247	293	NS	31
	Italy (2007)	80	90	NS	31
	U.K. (2007)	247	293	NS	31
C-658T	Italy (2007)	80	90	NS	31
	U.K. (2007)	247	293	NS	31
	Italy (2007)	80	90	NS	31
	U.K. (2007)	247	293	NS	31
C-319T	Italy (2007)	80	90	NS	31
	U.S. (2008 B)	<u>402</u>	<u>279</u>	<u>NS</u>	9
	<u>U.S. (2008 B)</u>	<u>402</u>	<u>279</u>	<u>NS</u>	9
	<u>U.S. (2008 B)</u>	<u>402</u>	<u>279</u>	<u>NS</u>	9
-1146 (rs16840252) +923 (rs231777) (rs11571319)	<u>U.S. (2008 B)</u>	<u>402</u>	<u>279</u>	<u>0.003</u>	9
	<u>U.S. (2008 B)</u>	<u>402</u>	<u>279</u>	<u>NS</u>	9
	<u>U.S. (2008 B)</u>	<u>402</u>	<u>279</u>	<u>NS</u>	9
	<u>U.S. (2008 B)</u>	<u>402</u>	<u>279</u>	<u>NS</u>	9

U.S. (2008 A): Juran BD, et al. Hepatology 2008;47:563-570.

U.S. (2008 B): Juran BD, et al. Gastroenterology 2008;135:000-000.