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Interacting Alleles of the Coinhibitory Immunoreceptor Genes Cytotoxic T-Lymphocyte Antigen 4 and Programmed Cell-Death 1 Influence Risk and Features of Primary Biliary Cirrhosis

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Abstract

Autoimmune diseases such as primary biliary cirrhosis (PBC) result from failure in the immune mechanisms that establish and maintain self-tolerance. Evidence suggests that these processes are shared among the spectrum of autoimmune syndromes and are likely genetically determined. Cytotoxic T-lymphocyte antigen 4 (CTLA4) and programmed cell-death 1 (PDCD1) are two genes encoding coinhibitory immunoreceptors that harbor polymorphisms with demonstrated associations to multiple autoimmune disorders. We aimed to assess functional single nucleotide polymorphisms (SNPs) in these two genes for association with PBC. SNPs in CTLA4 and PDCD1 were genotyped in 351 PBC patients and 205 controls. Allele and genotype frequencies were evaluated for association with PBC and/or antimitochondrial antibody (AMA) positivity with logistic regression. Haplotypes were inferred with an expectation-maximization algorithm, and allelic interaction was analyzed by logistic regression modeling. Individual SNPs demonstrated no association to PBC. However, the GG genotype of CTLA4 49AG was significantly associated with AMA positivity among the PBC patients. Also, individual SNPs and a haplotype of CTLA4 as well as a rare genotype of the PDCD1 SNP PD1.3 were associated with orthotopic liver transplantation. As well, we identified the influence of an interaction between the putatively autoimmune-protective CTLA4 49AG:CT60 AA haplotype and autoimmune-risk PDCD1 PD1.3 A allele on development of PBC.

Conclusion—Our findings illustrate the complex nature of the genetically induced risk of PBC and emphasize the importance of considering definable subphenotypes of disease, such as AMA positivity, or definitive measures of disease severity/progression, like orthotopic liver transplantation, when genetic analyses are being performed. Comprehensive screening of genes involved with immune function will lead to a greater understanding of the genetic component of autoimmunity in PBC while furthering our understanding of the pathogenic properties of this enigmatic disease.

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Primary biliary cirrhosis (PBC) is an autoimmune disease of the liver with complex etiology and a strong genetic component.[1] The hallmark of liver-directed autoimmunity in PBC is the development of antimitochondrial antibodies (AMAs) reactive with the inner lipoyl domain of the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2).[2] These autoantibodies are often detectable in the serum; their presence can precede PBC development by years, and their existence likely increases one's risk of future progression to overt disease.[3,4] Interestingly, autoimmune-mediated destruction in PBC is primarily limited to the biliary epithelial cells lining the small intrahepatic bile ducts, even though PDC-E2 is ubiquitously expressed.[5] This targeted attack is thought to develop subsequent to an initial inflammatory insult during which tolerance to PDC-E2 self-antigens is lost.[5]

Autoimmune diseases are presumed to be the cumulative result of minor variations in the immune mechanisms that establish and maintain self-tolerance.[6] Evidence suggests that some of these processes are shared among auto-immune syndromes, and their impact on mediating autoimmune risk is likely to be genetically determined.[7–9] Thus, it is quite plausible that PBC, a disease overlapping with other autoimmunity and thought to have a strong genetic component,[10] may develop in part because of these shared genetic risks.

The inhibition of T-cell activation is one means by which the immune system regulates self-tolerance. Cytotoxic T-lymphocyte antigen 4 (CTLA4) and programmed cell-death 1 (PDCD1) are two genes involved with this process and have been implicated with generalized risk to the development of autoimmunity.[11,12] Both encode coinhibitory immunoreceptors and harbor putatively functional polymorphisms associated with several to multiple autoimmune disorders.[11,12] Recent findings suggest that these receptors work synergistically through distinct mechanisms targeting the phosphoinositide 3-kinase/protein kinase B signaling pathway in order to inhibit T-cell activation and promote self-tolerance in the periphery.[13,14]

Two of the single nucleotide polymorphisms (SNPs) in the CTLA4 gene, 49AG (rs231775) and CT60 (rs3087243), have been implicated in a number of autoimmune diseases, including PBC.[15–19] The 49AG SNP lies in the coding region of the CTLA4 signal peptide and is characterized by a threonine (A allele) to alanine (G allele) substitution, which has been shown to impact the cell surface expression of CTLA4.[20,21] The G allele is thus proposed to contribute to autoimmune risk by reducing the amount of cell-surface CTLA4, resulting in increased T-cell proliferation in response to immune activation. The CT60 SNP of CTLA4 is located in the 3′ untranslated region of the gene, and the slightly less common A allele is suggested to be protective for autoimmunity.[18] Conversely, the G allele is thought to impart autoimmune risk by interfering with splicing processes, resulting in reduced production of a soluble form of CTLA4[18] that has been shown to inhibit T-cell activation *in vitro*.[22]

In an Italian study of 154 PBC patients and 166 controls, homozygosity for the autoimmune-protective A allele of the CTLA4 CT60 polymorphism was shown to be decreased in PBC patients compared to controls (17.5% versus 26.7%, $P = 0.0411$); however an increase in homozygosity of the autoimmune-risk G allele was not observed.[23] Moreover, in a study of 200 PBC patients and 200 controls from the United Kingdom investigating the CTLA4 49AG polymorphism, the autoimmune-risk G allele (45.0% PBC versus 30.5% controls, $P < 0.0002$) and GG genotype (18.5% PBC versus 10.5% controls, $P = 0.00006$) were both reported to be associated with PBC.[15] However, this finding was not replicated in a larger study by the same group[24] or by a smaller study from Brazil,[25] calling into question the role of CTLA4 genetic variation in PBC.

The PD1.3 polymorphism (rs11568821) of the PDCD1 gene has also been implicated in a number of autoimmune disorders[26–28] but has not yet been thoroughly investigated in PBC. PD1.3 is an A/G polymorphism located in the fourth intron of the PDCD1 gene, in which the A allele disrupts a RUNX1 transcription factor binding site, potentially altering the regulation of PDCD1[27] and increasing the risk of developing autoimmunity. However, the specific mechanism by which this dysregulation leads to a change in PDCD1 function affecting self-tolerance has yet to be elucidated.

Considering the previous findings of CTLA4 SNPs in PBC, the lack of investigation into PDCD1, and the putative synergistic effects that these genes have in negative T-cell regulation and mediation of immunologic self-tolerance, we aimed to evaluate these polymorphisms in our group of PBC patients and controls.

Patients and Methods

Study Participants

All participants in this study, 351 well-documented PBC patients and 205 clinic-based controls, had been previously recruited into our Mayo Clinic PBC Genetic Epidemiology Registry and Biospecimen Repository, which was initiated with the aim of elucidating the genetic and environmental contributors to PBC pathogenesis.[29] Diagnosis of PBC was confirmed by chart review evidencing a persistent biochemical profile of cholestasis (greater than 6 months) in the absence of other known liver disease, compatible liver histopathology, and/or detectable AMA in serum. Controls were recruited from the Mayo Clinic Division of General Internal Medicine during annual visits for routine checkups and were matched by age (±2.5 years), sex, and state of residence to individual PBC patients. Control exclusion criteria included evidence of prior or current liver disease. Demographic features of the patient and control groups are presented in Table 1.

Informed consent was obtained from all participants in the study. The registry and current study conform to the ethical guidelines of the 1975 Declaration of Helsinki and have been approved by the Mayo Clinic Institutional Review Board.

Sample Handling and DNA Preparation

The collection of blood specimens from participants in the Mayo Clinic PBC Genetic Epidemiology Registry was facilitated by bar-coded, mail-in kits that were prepared by the Mayo Central Laboratory for Clinical Trials. The blood specimens were either collected on-site at one of the out-patient phlebotomy stations at the Mayo Clinic in Rochester, MN, or collected off-site at the convenience of the study participants and shipped to us via overnight courier following blood draw. Blood processing, assessment, quality control, and distribution were performed under the supervision of the Mayo Central Laboratory for Clinical Trials.

Isolation of DNA from the blood samples was performed by the Mayo Clinic General Clinical Research Center using the PureGene kit (Gentra Systems, Minnesota) as samples were received. Subsequent handling of the DNA specimens, including dilution to working concentrations and quality control, was performed in our own laboratory.

AMA Testing

Serum was prospectively tested for the presence of AMA at the time of study enrollment. The AMA test was performed by the Mayo Clinic Diagnostic Immunology Laboratory using a commercially available enzyme-linked immunosorbent assay (Diastat AMA, Euro-Diagnostica, Malmo, Sweden) specific for the PDC-E2 (M2) antigen. The resulting

absorbance units were classified as negative (≤0.1 units) or positive (≥0.2 units) for AMA detection, as described previously.[29]

SNP Typing

The CTLA4 polymorphisms 49AG (rs231775) and CT60 (rs3087243) were typed by commercially available TaqMan allelic discrimination assays (Applied Biosciences, California) with an Applied Biosciences 7500 fast real-time polymerase chain reaction system. The PDCD1 polymorphism PD1.3 (rs11568821) was typed by polymerase chain reaction–restriction fragment length polymorphism. Briefly, a 322– base pair fragment of PDCD1 containing the PD1.3 (A/G) variant was amplified from genomic DNA (primer 1, 5′CAGGCAGCAACCTCAATCCCTAAA3′; primer 2, 5′CTGAAATGTCCCTGGCATTCTTGC) using recombinant taq polymerase (Invitrogen, California). The resulting product was digested with the restriction enzyme PstI (New England Biolabs, Massachusetts), which specifically cuts products containing the A allele, and visualized on ethidium bromide–stained agarose gels to determine genotype.

Statistical Analysis

Differences in characteristics of compared groups were assessed by an unpaired Student *t* test (for means) or Fisher's exact test (for proportions). All analyses were two-sided; actual *P* values are reported. Disease association with individual SNPs was performed with logistic regression to determine statistical significance along with odds ratios (ORs) and 95% confidence intervals (CIs); multiple inheritance models (that is, dominant, recessive, and additive) were considered. Haplotypes were inferred with the expectation-maximization (EM) algorithm[30] and studied for association with PBC, AMA status, and prior orthotopic liver transplantation (OLT) with a score test [31] and a max-stat test, assuming multiple inheritance models. The interaction between the two-SNP CTLA haplotype and the PDCD1 SNP was modeled with logistic regression, in which the posterior probabilities of the possible haplotypes for each subject were included as the covariates in the model. Age and sex were included as adjustment factors in all the models. All analysis was completed with Splus, version 8.0. Nominal *P* values are reported.

Results

CTLA4 49AG, CTLA4 CT60, and PDCD1 PD1.3 Are Not Individually Associated with PBC; However, Genotype Associations with AMA Status and Prior OLT Were Identified

Each of the three SNPs genotyped was found to be in Hardy-Weinberg equilibrium. The counts and frequencies of CTLA4 49AG, CTLA4 CT60, and PDCD1 PD1.3 genotypes were determined and are shown in Table 2A. No significant allele or genotype association between the overall PBC group and the controls was detected (Table 2B). However, we found the frequency of the CTLA4 49AG GG genotype to be significantly lower in AMA-negative PBC patients than in AMA-positive patients (18.8% AMA+ PBC versus 2.3% AMA− PBC, $P = 0.02$; Table 2B). Demographic and clinical features of the AMA-positive and AMA-negative groups were compared and found to be similar (Supplementary Table 1). Considering this genotypic disparity in PBC patients due to AMA status, we compared CTLA4 49AG between the group of 303 AMA-positive PBC patients and the 205 controls and found that the GG genotype was not significantly associated with AMA-positive PBC (18.3% AMA+ PBC versus 13.2% controls, OR 1.5, 95% CI 0.91–2.49, $P = 0.11$), which was consistent with the findings from the larger second United Kingdom study.[24]

To assess the potential affect of these SNPs on disease progression, we compared genotype frequencies between those PBC patients who had received OLT and those who had not (Table 2B). OLT was chosen as the progression metric as it is a definitive clinical outcome

illustrative of significantly severe and advancing disease. Other measures of progression such as serial biopsies and Mayo PBC risk scores were considered, but sufficiently extensive observations of any such parameter were available for only a small percentage of individuals. Characteristics of the OLT and non-OLT groups are presented in Table 3.

Interestingly, significant associations of all 3 tested SNPs were detected in this analysis. The CTLA4 49AG GG genotype was significantly increased in the OLT group compared to the non-OLT group (30.0% versus 15.5%, $P = 0.04$; Table 2B) and more so when compared to the controls (30.0% OLT PBC versus 13.2% controls, OR 2.99, 95% CI 1.23–7.28, $P = 0.02$). Conversely, the frequency of the CTLA4 CT60 A allele was significantly decreased in the OLT group compared to both the non-OLT group (25.9% versus 44.3%, $P = 0.008$) and controls (25.9% versus 42.9% controls, $P = 0.015$), as well as this was largely the result of differences in the homozygous genotypes (that is, AA and GG; Table 2A) and was reflected by positive findings in both the dominant and recessive models (Table 2B). Moreover, the rare AA genotype of PDCD1 PD1.3 was significantly increased in the OLT group compared to the PBC patients without prior OLT (6.7% versus 0.6%, $P = 0.01$) and was elevated but not statistically different when compared to the controls (6.7% OLT PBC versus 2.0% controls, OR 3.58, 95% CI 0.62–20.76, $P = 0.16$).

The CTLA 49AG:CT60 Haplotype Is Not Associated with PBC Risk or AMA Status but Is Significantly Associated with Prior OLT Among the PBC Patients

Frequencies of the CTLA4 49AG:CT60 haplotype were inferred by the EM algorithm and analyzed for association with PBC, AMA status, and prior OLT with score and max-stat tests under an additive model. No significant association between CTLA4 haplotype and PBC was detected (Table 4). Of interest, the haplotype composed of the G allele of 49AG and the A allele of CT60 was not identified in the population, illustrative of strong linkage between the SNPs. Although the CTLA4 haplotype was not globally significant for AMA status, not surprisingly, the frequency of the GG haplotype was found to be significantly higher in AMA+ PBC patients compared to AMA− PBC patients (40.9% versus 29.5%, $P = 0.04$), reflective of the diminished presence of the 49AG G allele in the AMA− patients.

Notably, there was a significant association of the CTLA4 49AG:CT60 haplotype with prior OLT (global P value = 0.030; Table 4). Specifically, the frequency of the GG haplotype was increased in the OLT patients (51.7% OLT versus 38.7% non-OLT, $P = 0.04$), whereas the AA haplotype was decreased (25.9% OLT versus 44.3% non-OLT, $P = 0.009$). Again, this was in concordance with the genotypic disparities observed between these two groups.

Interaction Between the CTLA4 49AG:CT60 Haplotype and the PDCD1 PD1.3 Allele Contributes to Risk of Developing PBC but Not to AMA Status or Progression to OLT

The proposed synergistic effect of CTLA4 and PDCD1 on the negative regulation of T-cell activation and control of peripheral tolerance led us to test the hypothesis that interaction between the putatively functional variants of these genes might influence PBC risk in lieu of any detectable association of the individual SNPs.

Although 49AG was historically the most plausible CTLA4 SNP for involvement with PBC risk, the strong linkage between the 49AG and CT60 SNPs led us to conclude that testing for interaction between the CTLA4 49AG:CT60 haplotype and PDCD1 PD1.3 allele would be more appropriate. To assess this interaction, we first inferred the relative frequencies of the possible 49AG: CT60 haplotype–PD1.3 allele combinations in the groups using the EM algorithm, and then we analyzed for association using a score and max-stat test under an additive model; the results are presented in Table 5.

The distribution of the haplotype-allele combination frequencies was not globally significant between any of the three groups. However, there was a significant difference in frequency of the CTLA4 AA haplotype and PD1.3 A allele combination between the PBC patients and controls (6.1% PBC versus 2.2% controls, $P = 0.05$), which was also marginally different between AMA+ and AMA− PBC ($P = 0.06$). As well, a difference in the CTLA4 GG haplotype and PD1.3 G allele combination was identified between the AMA+ and AMA− PBC patients ($P = 0.02$); this observation was driven by the reduced prevalence of the CTLA 4 GG haplotype in the AMA− patients. Differences in the haplotype-allele combination frequencies were also noted in the OLT patients compared to the non-OLT group (that is, AA-G and GG-A), reflective of the noted CTLA4 haplotype disparities.

To better assess the potential interaction between the CTLA4 haplotype and PD1.3 allele, we used logistic regression and analyzed using both dominant and additive effect models. Specifically, the interaction models consisted of 3 variables: (1) the presence of one or more copies of the CTLA4 haplotype, (2) the presence of one or more copies of the PD1.3 allele, and (3) the interaction (that is, the combination of the CTLA4 haplotype and PD1.3 allele). The only significant interaction detected was between the PBC patients and controls; the results are presented in Table 6. Interestingly, the interaction between the CTLA4 AA haplotype and the PDCD1 A allele was found to exhibit a significant influence on PBC risk (17.1% PBC versus 11.7% controls, $P = 0.039$ for the dominant model, $P = 0.008$ for the additive model; Table 6). This finding did not appear to be due to the CTLA4 AA haplotype as the overall frequency (42.7% versus 42.9%) of this haplotype was nearly identical between PBC patients and controls. As well, the role of the PD1.3 A allele was not driving the results either, as the frequency (12.5% versus 11.7%) of this allele was also similar between the PBC and control groups.

We conclude from this analysis that the proposed general-autoimmune-risk A allele of PDCD1 PD1.3 has an impact on PBC development in the context of the putative autoimmune-protective CTLA4 49AG:CT60 AA haplotype. Certainly, a more in-depth analysis of other variants in the region will be required to clarify this association.

Discussion

We found no association of CTLA4 SNPs or haplotypes with overall risk of PBC, in agreement with recent findings from the United Kingdom.[24] However, we have demonstrated for the first time the increased risk of PBC associated with an interaction between alleles of CTLA4 and another coinhibitory immunoreceptor gene, PDCD1. We also identified the association of CTLA4 49AG with AMA positivity among PBC patients. Specifically, the frequency of the 49AG GG genotype was greatly reduced in the AMA-negative PBC patients compared to both the AMA-positive patients and the controls. As well, we made the novel observation that CTLA4 SNPs and haplotypes are associated with progression to OLT among PBC patients, with evidence for both increased-risk and protective effects. Homozygosity for the A allele of PDCD1 PD1.3 was also found to be significantly associated with OLT (OR 14.75, 95% CI 1.89–114.9, $P = 0.01$) but was present only in a small number of individuals (6.7% OLT versus 0.6% non-OLT). These findings illustrate the importance of considering disease subphenotypes like AMA status and severity/progression metrics such as OLT in the analysis of genetic data for complex diseases such as PBC.

The genetic variants that we tested in CTLA4 and PDCD1 have demonstrated associations with other autoimmune disorders and consequences that could plausibly affect immune function and contribute to the development of the autoimmune phenotype seen in PBC. Indeed, the significantly reduced frequency of the GG genotype of CTLA4 49AG in AMA-

negative PBC patients identified in our study (19% AMA+ PBC, 13% normal controls, 2% AMA− PBC) suggests a role for this variant in influencing the development and expression of clinically detectable levels of serum AMA within the genetic context of PBC.

More evidence for the role of CTLA4 in PBC comes from our analysis of patients who had previously received OLT. Notably, a significant increase in the autoimmune-risk G allele and GG genotype of 49AG and a decrease in the autoimmune-protective A allele and AA genotype of CT60 were observed in the PBC patients who had received OLT compared to those who had not. These findings were significant at the haplotype level as well, with an increase of the 49AG:CT60 GG haplotype and decrease of the AA haplotype in OLT patients. Interestingly, these associated haplotypes contain either both putative protective alleles (that is, AA) or risk alleles (that is, GG), whereas the AG (protective-risk) haplotype was not significantly different between the groups but was elevated in OLT (22.4% OLT versus 17.1% non-OLT), and the GA (risk-protective) haplotype did not exist in the population. In addition to CTLA4, PDCD1 also appears to play a role in progression to OLT as homozygosity for the autoimmune-risk A allele was found to be considerably elevated in the OLT group; however, this genotype was found in only a limited number of individuals.

As OLT is a definitive clinical endpoint that implies a severe and progressing course of disease, its use negates the possibility of misclassifying the progressive group, substantially increasing its utility in genetic analysis. That said, it is certainly likely that members of the non-OLT group will eventually require OLT, so as an effort to discount this bias, we compared the OLT group with a subset of the non-OLT group who were greater than 65 years old at the time of enrollment in our study, an age near the upper limit for liver transplantation eligibility in the US, and found the results to be quite similar to those of the complete non-OLT group (Supplementary Table 2).

Our novel finding suggesting that the PD1.3 A allele of PDCD1 may contribute to risk of PBC in the context of the CTLA4 49AG:CT60 AA haplotype is interesting because this haplotype is composed of 2 SNPs thought to be protective against autoimmunity in a general sense. Indeed, the interaction between this protective CTLA4 haplotype and autoimmune-risk PD1.3 allele appears to significantly influence the development of PBC when both dominant and additive genetic models are considered. The mechanisms favoring the development of PBC and perhaps autoimmunity in general that are invoked by these interacting alleles are unclear and necessitate further study. Extension of the CTLA4 haplotype may uncover additional variants in linkage disequilibrium with the observed 49AG:CT60 haplotype that may further strengthen and potentially explain our findings. More globally, our finding implies that some portion of the risk to PBC is born within complex genetic patterns that cannot be appreciated at the level of the individual variant, the effects of which will likely be difficult to detect and even harder to explain.

At a minimum, a more comprehensive investigation of the variation in and around CTLA4 and PDCD1 and throughout the genes comprising the human immunome, as well as consideration of observable subphenotypes such as AMA status and severity/progression metrics such as OLT, will be required if we are to gain greater insight into the genetic basis of this perplexing disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AMA	antimitochondrial antibody
CI	confidence interval
CTLA4	cytotoxic T-lymphocyte antigen 4
EM	expectation-maximization
OLT	orthotopic liver transplantation
OR	odds ratio
PBC	primary biliary cirrhosis
PDCD1	programmed cell-death 1
PDC-E2	pyruvate dehydrogenase complex E2 subunit
SNP	single nucleotide polymorphism
UDCA	ursodeoxycholic acid

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Table 1

Demographics of PBC Patients and Controls

	PBC Patients (n = 351)	Controls (n = 205)
Sex		
Female	92.6%	90.7%
Male	7.4%	9.3%
Race		
Caucasian	100%	100%
Mean age (years) *	60.5 (35–85)	64.4 (35–88)
Mean age at diagnosis (years) *	50.9 (29–77)	–
Disease duration (years) *	9.6 (0–31)	–
Biopsy at diagnosis (n = 230)		
Stage I–II	68.3%	–
Stage III–IV	31.7%	–
Liver transplanted	8.3%	–
UDCA therapy	86.2%	–

UDCA indicates ursodeoxycholic acid.

* Values are expressed as mean (range).

Table 2

SNP Data and Analysis

A. Counts and Frequencies of Individual SNP Genotypes*

	All PBC (n = 351)	Control (n = 205)	AMA+ PBC (n = 303)	AMA− PBC (n = 44)	OLT PBC (n = 29)	Non-OLT PBC (n = 322)
CTLA4 49AG						
AA	131 (0.37)	79 (0.39)	112 (0.37)	19 (0.43)	8 (0.28)	123 (0.38)
AG	161 (0.46)	99 (0.48)	134 (0.44)	24 (0.55)	12 (0.41)	149 (0.46)
GG	59 (0.17)	27 (0.13)	57 (0.19)	1 (0.02)	9 (0.31)	50 (0.16)
CTLA4 CT60						
GG	117 (0.33)	70 (0.34)	105 (0.35)	10 (0.23)	15 (0.52)	102 (0.32)
AG	168 (0.48)	94 (0.46)	142 (0.47)	24 (0.55)	13 (0.45)	155 (0.48)
AA	66 (0.19)	41 (0.20)	56 (0.18)	10 (0.23)	1 (0.03)	65 (0.20)
PDCD1 PD1.3						
GG	267 (0.76)	161 (0.79)	236 (0.78)	29 (0.66)	22 (0.76)	245 (0.76)
AG	80 (0.23)	40 (0.20)	64 (0.21)	15 (0.34)	5 (0.17)	75 (0.23)
AA	4 (0.01)	4 (0.02)	3 (0.01)	0	2 (0.07)	2 (0.01)

B. Statistical Analysis of SNP Genotypes Based on Logistic Regression and Adjusted for Age and Sex

	All PBC Versus Controls		AMA+ Versus AMA− PBC		OLT Versus Non-OLT PBC	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
CTLA4 49AG						
Log-additive	1.11 (0.86–1.42)	0.44	**1.67 (1.03–2.71)**	**0.04**	**1.69 (1.00–2.88)**	**0.05**
Dominant	1.05 (0.73–1.51)	0.79	1.35 (0.71–2.58)	0.36	1.71 (0.73–4.00)	0.22
Recessive (GG)	1.33 (0.81–2.20)	0.26	**9.96 (1.34–73.97)**	**0.02**	**2.46 (1.05–5.76)**	**0.04**
CTLA4 CT60						
Log-additive	1.00 (0.78–1.27)	0.97	0.71 (0.45–1.12)	0.14	**0.44 (0.24–0.81)**	**0.008**
Dominant	1.05 (0.72–1.52)	0.80	0.55 (0.26–1.17)	0.12	**0.43 (0.20–0.93)**	**0.03**
Recessive (AA)	0.92 (0.59–1.43)	0.71	0.73 (0.34–1.58)	0.43	**0.13 (0.02–0.99)**	**0.05**
PDCD1 PD1.3						
Log-additive	1.05 (0.72–1.55)	0.78	0.62 (0.33–1.17)	0.14	1.35 (0.62–2.93)	0.46
Dominant	1.12 (0.74–1.71)	0.59	0.54 (0.27–1.08)	0.08	1.01 (0.41–2.47)	0.99
Recessive (AA)	0.55 (0.13–2.36)	0.43	–	–	**14.74 (1.89–114.9)**	**0.01**

* Values are expressed as count (frequency).

Bold text indicates statistically significant findings (i.e., $P \leq 0.05$).

Table 3

Characteristics of OLT and Non-OLT PBC Patients

	OLT (n = 29)	Non-OLT (n = 322)	*P* Value
Male/female ratio	4:25	22:300	0.253
Age (years) *	55.9 (41–68) †	60.2 (35–85)	**0.034**
Age at diagnosis (years) *	47.4 (33–60)	51.2 (29–77)	**0.039**
Disease duration (years) *	8.5 (2–21) †	9.0 (0–31)	0.689
AMA-positive	89.3%	87.1%	1.000

* Values are expressed as mean (range).

† At time of liver transplantation.

Bold text indicates statistically significant findings (i.e., $P \leq 0.05$).

Table 4

Frequencies and Analysis of the CTLA4 49AG:CT60 Haplotype: Additive Model

	Frequency			Frequency			Frequency		
CTLA4 49AG: CT60	**PBC (n = 351)**	**Control (n = 205)**	***P* Value**	**AMA+ PBC (n = 303)**	**AMA− PBC (n = 44)**	***P* Value**	**OLT (n = 29)**	**Non-OLT (n = 322)**	***P* Value**
A:A	0.4274	0.4292	0.97	0.4191	0.5000	0.16	0.2586	0.4426	**0.009**
A:G	0.1752	0.1976	0.36	0.1716	0.2046	0.38	0.2241	0.1708	0.34
G:G	0.3974	0.3732	0.44	0.4093	0.2954	**0.04**	0.5172	0.3867	**0.04**
Global *P* value		0.589			0.125			**0.030**	

Bold text indicates statistically significant findings (i.e., $P \leq 0.05$).

Table 5

Frequencies and Analysis of the CTLA4 Haplotype–PD1.3 SNP Interaction: Additive Model

	Frequency			Frequency			Frequency		
CTLA4-PDCD1	**PBC (n = 351)**	**Control (n = 205)**	***P* Value**	**AMA+ PBC (n = 303)**	**AMA− PBC (n = 44)**	***P* Value**	**OLT (n = 29)**	**Non-OLT (n = 322)**	***P* Value**
A:A-G	0.366	0.408	0.47	0.362	0.427	0.42	0.237	0.378	**0.03**
A:A-A	0.061	0.022	**0.05**	0.057	0.073	0.06	0.031	0.066	0.28
A:G-G	0.153	0.170	0.45	0.152	0.177	0.43	0.214	0.150	0.29
A:G-A	0.022	0.028	0.49	0.020	0.028	0.59	0	0.023	0.44
G:G-G	0.355	0.306	0.26	0.370	0.225	**0.02**	0.424	0.351	0.17
G:G-A	0.043	0.068	0.43	0.039	0.070	0.48	0.093	0.033	0.06
Global *P* value	0.132			0.274			0.104		

Bold text indicates statistically significant findings (i.e., $P \leq 0.05$).

Table 6

Statistical Analysis of the CTLA4 AA Haplotype–PD1.3 SNP Interaction Based on Logistic Regression After Adjustment for Age and Sex

	Frequency	
CTLA4-PDCD1	**PBC (n = 351)**	**Control (n = 205)**
A:A carrier–A carrier	0.17	0.12
A:A carrier–not A carrier	0.49	0.54
Not A:A carrier–A carrier	0.07	0.10
Not A:A carrier–not A carrier	0.27	0.24
Interaction *P* value (dominant model)	**0.039**	
Interaction *P* value (additive model)	**0.008**	

Bold text indicates statistically significant findings (i.e., $P \leq 0.05$).