

The Relation of HLA Genotype to Hepatitis C Viral Load and Markers of Liver Fibrosis in HIV-Infected and HIV-Uninfected Women

Mark H. Kuniholm,¹ Xiaojiang Gao,^{2,6} Xiaonan Xue,¹ Andrea Kovacs,⁷ Darlene Marti,^{2,6} Chloe L. Thio,³ Marion G. Peters,⁸ Ruth M. Greenblatt,⁹ James J. Goedert,⁵ Mardge H. Cohen,¹⁰ Howard Minkoff,¹¹ Stephen J. Gange,⁴ Kathryn Anastos,¹ Melissa Fazzari,¹ Mary A. Young,¹² Howard D. Strickler,^{1,a} and Mary Carrington^{2,6,a}

¹Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, New York; ²Cancer and Inflammation Program, Laboratory of Experimental Immunology, SAIC-Frederick, NCI-Frederick, ³Department of Medicine, Johns Hopkins School of Medicine, and ⁴Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, and ⁵Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, Maryland; ⁶Ragon Institute of MGH, MIT and Harvard, Charlestown, Massachusetts; ⁷Department of Pediatrics, University of Southern California, Los Angeles, and Departments of ⁸Medicine and ⁹Clinical Pharmacy, University of California, San Francisco; ¹⁰CORE Center, Cook County Bureau of Health Services, Chicago, Illinois; ¹¹Department of Obstetrics and Gynecology, Maimonides Medical Center, Brooklyn, New York; ¹²Department of Medicine, Georgetown University Medical Center, Washington, DC

Background. Human leukocyte antigen (HLA) class I and II genotype is associated with clearance of hepatitis C virus (HCV) infection, but little is known regarding its relation with HCV viral load or risk of liver disease in patients with persistent HCV infection.

Methods. High-resolution HLA class I and II genotyping was conducted in a prospective cohort of 519 human immunodeficiency virus (HIV)–seropositive and 100 HIV-seronegative women with persistent HCV infection. The end points were baseline HCV viral load and 2 noninvasive indexes of liver disease, fibrosis-4 (FIB-4), and the aspartate aminotransferase to platelet ratio index (APRI), measured at baseline and prospectively.

Results. DQB1*0301 was associated with low baseline HCV load ($\beta = -.4$; 95% confidence interval [CI], $-.6$ to $-.3$; $P < .00001$), as well as with low odds of FIB-4–defined (odds ratio [OR], $.5$; 95% CI, $.2$ – $.9$; $P = .02$) and APRI–defined liver fibrosis (OR, $.5$; 95% CI, $.3$ – 1.0 ; $P = .06$) at baseline and/or during follow-up. Most additional associations with HCV viral load also involved HLA class II alleles. Additional associations with FIB-4 and APRI primarily involved class I alleles, for example, the relation of B*1503 with APRI–defined fibrosis had an OR of 2.0 (95% CI, 1.0 – 3.7 ; $P = .04$).

Conclusions. HLA genotype may influence HCV viral load and risk of liver disease, including DQB1*0301, which was associated with HCV clearance in prior studies.

The natural history of hepatitis C virus (HCV) infection is highly variable. Approximately 20%–30% of individuals clear HCV spontaneously after acute infection, and only a subset of those with persistent HCV infection

develop progressive liver disease [1]. Considerable heterogeneity in the course of infection was observed, for example, even among a group of women infected with HCV through a single-source exposure [2]. These data suggest that genetic differences and other host factors probably influence the risk of HCV-related liver disease progression. However, the relevant genes remain largely unknown.

Recent epidemiologic studies have shown strong associations between human leukocyte antigen (HLA) genotype and spontaneous clearance of HCV (reviewed in [3, 4]). Briefly, HLA genes are highly polymorphic and encode molecules that present peptides to T cells on cell surfaces and thereby play a major role in the immune response to viruses. HLA class I molecules present

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^aH. D. S. and M. C. contributed equally to this work.

Correspondence: Mark H. Kuniholm, PhD, Department of Epidemiology and Population Health, Albert Einstein College of Medicine, 1300 Morris Park Ave, Belfer Bldg #1308, Bronx, NY 10461 (mark.kuniholm@einstein.yu.edu).

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peptides from intracellular pathogens (eg, viruses) to CD8⁺ T cells, whereas peptides derived from extracellular pathogens are generally complexed with class II molecules and are recognized by CD4⁺ T cells.

Despite associations of both HLA class I and class II genotype with HCV clearance [3, 5–7], an effect of HLA genotype on the development of liver disease in those with chronic HCV is not well established. Some, but not all, prior studies have shown an inverse association between DRB1*11 and the cross-sectional presence of advanced liver disease (reviewed in [8]). Most studies, however, did not assess HLA class I genotype—an important limitation given the role of CD8⁺ cytotoxic T lymphocytes in control of viral infections. Of the studies that did evaluate HLA class I [9–14], to our knowledge, none conducted high-resolution genotyping, which is needed to fully assess the associations between HLA and viral disease.

Determining the relation of HLA with the rate of liver disease progression has also been difficult, because in most instances the date that HCV infection occurred is unknown. Prior studies of HLA and HCV-related liver disease typically assessed disease at a single point in time (ie, in cross-sectional or case-control studies). An alternative, prospective design in an HCV-seroprevalent cohort is to control statistically for baseline disease status and then estimate the relationship between HLA genotype and the rate of disease progression from that time forward. However, no studies of this type have been reported.

Data regarding HCV viral load and HLA genotype is likewise limited [5, 15–18]. Although there are conflicting reports regarding the association of HCV viral load with liver disease progression [19–22], HCV viral load predicts responsiveness to antiviral therapy, and may correlate with transmissibility [23, 24]. Furthermore, many HCV viremic women and men worldwide are coinfecting with human immunodeficiency virus (HIV) [25], but there are few data regarding the impact of HLA on HCV disease progression in HIV coinfecting populations.

To address these issues we conducted high-resolution HLA class I and II genotyping in a large prospective cohort of US women with chronic HCV infection and a high prevalence of HIV coinfection. Specifically, we studied associations between HLA genotype and (1) HCV viral load at baseline, (2) liver disease at baseline, (3) the incident development of liver disease, and (4) the detection of liver disease at any visit, including at baseline or during follow-up. The presence of liver disease was assessed using 2 noninvasive indexes of liver disease, fibrosis-4 (FIB-4) and the aspartate aminotransferase to platelet ratio index (APRI). Although FIB-4 and APRI are insensitive to intermediate levels of liver disease, a number of studies have shown that elevated APRI and FIB-4 levels are useful biomarkers of advanced liver disease [26–30] and predict liver-related mortality [31].

SUBJECTS AND METHODS

Study Population

The Women's Interagency HIV Study (WIHS) is a prospective, multicenter cohort study of 2793 HIV-seropositive and 975 at-risk HIV-seronegative women enrolled through similar sources at 6 clinical sites. The initial enrollment was conducted between October 1994 and November 1995, and a second recruitment occurred during 2002. WIHS women are followed semiannually with physical examinations, specimen collection (including blood), and detailed questionnaires regarding health and behavior [32]. The WIHS protocol was approved by each local institutional review board, and all participants provided written informed consent. Follow-up data were available through February 2008.

The current investigation focused on women in the WIHS who were HCV-seropositive and positive for HCV RNA ($n = 860$) at the baseline study visit and was limited to those who provided consent for genetic testing ($n = 668$). Another 35 women were excluded because of specimen or laboratory issues (eg, insufficient sample volume, inadequate DNA amplification). We also excluded women who had received active antiretroviral therapy (ART) before study enrollment ($n = 11$) because they represented a small, potentially unusual group, and HIV-positive women with missing baseline CD4⁺ T-cell count data ($n = 3$).

Laboratory Testing

HCV Testing. HCV serostatus was determined in all subjects at enrollment using a commercial second- or third-generation enzyme immunoassay. HCV viremia was determined for HCV-seropositive women using either the COBAS Amplicor Monitor 2.0 or the COBAS Taqman assay (both from Roche Diagnostics), as described elsewhere [33]. HCV genotyping was conducted in a subset of HCV viremic women using the NC TRUGENE HCV 5 NC Genotyping Kit (Bayer HealthCare) [34].

HLA Genotyping. Genomic DNA was prepared from subjects' lymphoblastoid B cell lines or from peripheral blood lymphocytes. Briefly, HLA class I genes were amplified using locus-specific polymerase chain reaction (PCR) primers flanking exons 2 and 3, and the PCR products were hybridized with a panel of sequence-specific oligonucleotide (SSO) probes. The HLA alleles were assigned by the reaction patterns of the SSO probes, with any ambiguous SSO probing resolved by sequencing, as described elsewhere [35]. HLA class II typing used high-resolution SSO typing of exon 2, as described elsewhere [3]. The number of women with complete allele information varied by HLA locus: 613 for HLA-A, 602 for HLA-B, 587 for HLA-Cw, 316 for HLA-DQA1, 316 for HLA-DQB1, and 565 for HLA-DRB1.

Liver Disease Assays. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels and platelet counts were determined annually (1994–2000) or biannually (2001–2008) using standard laboratory protocols at the 6 clinical sites. We calculated the APRI index ($100 \times [\text{AST}/\text{AST ULN}]/\text{platelet}$

count [$10^9/L$]) [30] and the FIB-4 index ($[\text{age} \times \text{AST}]/[\text{platelet count } [10^9/L] \times \text{ALT}^{1/2}]$), as described elsewhere [28]. In keeping with other studies [28, 30], an APRI of $\leq .5$ was defined as the threshold indicating no significant liver fibrosis, whereas an APRI of > 1.5 was considered evidence of significant fibrosis. For FIB-4, these thresholds were < 1.45 and > 3.25 , respectively. For simplicity, we refer to APRI and FIB-4 values of $\leq .5$ and < 1.45 , respectively, as values in the “normal range” and APRI and FIB-4 values of > 1.5 and > 3.25 , respectively, as “APRI- or FIB-4–defined liver fibrosis.” An extensive literature has validated the use of APRI, including in populations with HCV-HIV coinfection (reviewed in [27]), whereas FIB-4 was developed to detect fibrosis in populations with HCV-HIV coinfection and has areas under the receiver operating characteristic curves of .77–.93 for the prediction of advanced fibrosis [26, 28, 29]. We used the kappa statistic [36] to calculate agreement between APRI and FIB-4.

Statistical Analysis

Our initial statistical analyses examined the relation of HLA genotype with baseline HCV viral load, as well as with APRI- and FIB-4–defined liver fibrosis at baseline. HCV viral load values were normalized using \log_{10} transformation and analyzed using multivariable linear regression. APRI- and FIB-4–defined fibrosis were analyzed by means of multivariable logistic regression, using women with normal values for these indexes as the comparison group.

All models in this study adjusted for self-reported race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other), age at baseline, HIV serostatus, and, among the HIV-seropositive women, also for $CD4^+$ T-cell count (>500 , 200–500, or <200 cells/ μL) and HIV RNA level ($\leq 100,000$ vs $>100,000$ copies/mL). In prospective analyses (described below), all models adjusted for $CD4^+$ T-cell count and HIV RNA level (modeled as time-dependent covariates), as well as baseline liver disease status (APRI, $\leq .5/.5$ – 1.0 ; FIB-4, $< 1.45/1.45$ – 2.35). However, statistical studies have demonstrated that genetic association models are generally unaffected by control for multiple covariates, and these investigators have recommended restricting the number of adjustment variables [37]. Therefore, behavioral factors associated with liver fibrosis such as smoking, alcohol consumption, and hepatotoxic antiretroviral therapies (eg, stavudine), which are very unlikely to be associated with the HLA genes under study, were not included in our models. To address concerns regarding multiple comparisons related to our assessment of 90 alleles and allele groups, we used a Bonferroni correction (ie, $P < .05/90 = .0006$) to define statistical significance. Other, less conservative methods for addressing multiple comparisons that are commonly employed cannot take covariates into account.

To evaluate the relation of HLA genotype with incident APRI- and FIB-4–defined liver fibrosis (APRI, > 1.5 ; FIB-4, > 3.25) we

limited the data set to women who had baseline values considerably below the threshold for liver disease (APRI, < 1.0 ; FIB-4, < 2.35 ; midpoint between normal and disease cutoffs for both indexes). It was possible to incorporate women with intermediate values in these prospective analyses along with women who had normal values, because we used Cox models that controlled statistically for baseline disease status (initial APRI and FIB-4 level). As mentioned in the introduction, by controlling for disease status at baseline, these analyses estimated the contribution of HLA genotype to the risk of disease progression from that time forward.

Furthermore, to reduce the chance that transient liver enzyme elevation might affect the findings of these prospective analyses, we required that to be defined as cases of incident APRI- or FIB-4–defined fibrosis, subjects had to have values above threshold for ≥ 2 sequential visits; those lost to follow-up or with missing data for > 1 visit were time censored. Subjects without APRI- or FIB-4–defined fibrosis were similarly time censored if they lacked the required data for > 1 visit.

Finally, as an additional analytical approach to maximize statistical power, we incorporated all cases (prevalent cases detected at baseline and incident cases detected during follow-up) as a single group, and compared them with women who were non-cases at all visits, using multivariable logistic regression models. We could combine prevalent and incident cases in this way, because HLA genotype is a time-independent host factor.

RESULTS

Demographic and Clinical Characteristics of the Study Population

Selected baseline characteristics of the 619 women with chronic HCV infection are shown in Table 1. The subjects had a median age of 40 years, the majority were black, non-Hispanic (63%), and most were HIV seropositive (84%). Nearly half of the HIV-seropositive women had $CD4^+$ T cell counts of 200–500 cells/ μL , and a quarter each had $CD4^+$ T cell counts of either >500 or <200 cells/ μL . Based on a random subset of 228 women who underwent HCV genotyping, most subjects had HCV genotype 1. Moreover, the women who were included in our analyses did not differ from those who were excluded (primarily because they did not provide consent for genetic testing) in terms of age, race, HIV serostatus, or $CD4^+$ T-cell count among the HIV-seropositive women (all $P > .10$).

Host immune status, categorized as HIV seronegative or HIV seropositive with $CD4^+$ T cell counts of >500 , 200–500, or <200 cells/ μL , was significantly associated at baseline (all $P_{\text{trend}} < .01$) with HCV viral load (median \log_{10} HCV viral load, 5.9, 6.1, 6.3, and 6.5 IU/mL, respectively), APRI (median, .4, .5, .6, and .6, respectively), and FIB-4 level (median, 1.1, 1.2, 1.5, and 1.8, respectively).

Table 1. Baseline Characteristics of Women With Chronic Hepatitis C Virus (HCV) Infection (*n* = 619)

Characteristic ^a	Data
Age, median (IQR), years	40 (36–43)
Race/ethnicity, no. (%)	
Black, non-Hispanic	389 (63)
White, non-Hispanic	98 (16)
Hispanic	121 (20)
Other	11 (2)
HIV status, no. (%)	
Positive	519 (84)
Negative	100 (16)
Recruitment cycle	
1994–1995	594 (96)
2002	25 (4)
HCV log ₁₀ IU/mL viral load, median (IQR)	6.3 (5.8–6.6)
HCV genotype, no. (%) ^b	
1	199 (87)
2	9 (4)
3	15 (7)
4	5 (2)
CD4 ⁺ T cell count in HIV-positive women, no. (%)	
<200 cells/μL	136 (26)
200–500 cells/μL	236 (45)
>500 cells/μL	147 (28)

NOTE. ^aHIV, human immunodeficiency virus; IQR, interquartile range.

^bPercentages among the 228 women with HCV genotype data.

HLA and Baseline HCV Viral Load

We examined all 90 HLA class I and II alleles and allele groups with a frequency of >5% in our study population. Table 2 shows the alleles and allele groups that had ≥1 statistically significant association with study end points. Results for all other alleles are shown in Supplementary Table 1

Six alleles and allele groups were significantly associated with baseline HCV viral load (Table 2). All but one of these HLA alleles was class II. DQB1*0301, for example, had a strong association with low HCV viral load ($P < .00001$), and this association was similar in subjects with good immune status (HIV negative or HIV positive with CD4⁺ T-cell count > 500 cells/μL; $\beta = -.44$; 95% confidence interval [CI], $-.74$ to $-.15$; see Table 2 for explanation of β) and those who were more immunosuppressed (HIV positive with CD4⁺ T-cell count < 500 cells/μL; $\beta = -.42$; 95% CI, $-.67$ to $-.17$; $P_{\text{interaction}} = .97$). Similarly, B*5101 (the single HLA class I allele; $P = .02$), DQA1*0500 ($P = .005$), DRB1*0804 ($P = .02$), and the rare DRB1 group ($P = .02$) each had significant inverse associations with baseline HCV viral load that did not differ by immune status. Only one allele association showed interaction by immune status: DRB1*0701 ($P_{\text{interaction}} = .03$) was associated with higher HCV load in women with good immune status ($P < .01$) but not among women who were more immunosuppressed ($P = .44$). Only the association with DQB1*0301, however,

retained significance after multiple-comparison adjustment ($P_{\text{corrected}} = .0004$).

HLA, APRI, and FIB-4

At the baseline visit, 75 women (12%) had APRI values above the threshold reported to indicate significant fibrosis (APRI, > 1.5), whereas 264 (43%) had values below this threshold but above normal and 278 (45%) had APRI values in the normal range (APRI, ≤ .5). For FIB-4, these percentages were 11%, 36%, and 53%, respectively. Furthermore, FIB-4 values were highly correlated with APRI ($r = .90$), and there was very good agreement between APRI and FIB-4 regarding the presence of significant liver fibrosis ($\kappa = .72$).

We examined HLA associations with FIB-4 and APRI in 3 ways: (1) comparing women who had prevalent FIB-4- or APRI-defined fibrosis at baseline with those who had normal values, using multivariable logistic regression; (2) studying the incidence rates of new cases of FIB-4- or APRI-defined fibrosis during follow-up, using multivariate Cox models; and (3) comparing women who had FIB-4- or APRI-defined fibrosis at any visit (including baseline or during follow-up) with those who were non-cases at all visits, using multivariable logistic regression. This third approach maximized statistical power by incorporating all cases, taking advantage of the fact that HLA genotype is a time-independent factor.

The best evidence of a biologic relationship between HLA and liver disease would be consistent associations in all models. However, as shown in Table 2, none of the alleles we studied had statistically significant associations with both FIB-4- and APRI-defined fibrosis in each of these 3 analyses (total, 6 models).

Further examination did show that the allele most strongly associated with HCV viral load, DQB1*0301, had a significant inverse association with FIB-4-defined liver fibrosis detected at any visit (odds ratio [OR], = .5; 95% CI, .2–.9; $P = .02$) and a similar relationship of borderline significance with APRI-defined fibrosis detected at any visit (OR, = .5; 95% CI, .3–1.0; $P = .06$). Furthermore, though not statistically significant, DQB1*0301 had very similar associations in each of the additional models; for example, in our prospective analysis of incident FIB-4-defined fibrosis, DQB1*0301 had an HR of .5 (95% CI, .3–1.2; $P = .13$).

Twenty additional alleles and allele groups also had ≥1 significant association with FIB-4- and/or APRI-defined liver fibrosis (Table 2). Sixteen of these involved HLA class I alleles and allele groups. B*1503 was notable because it had associations with liver fibrosis in 5 of the 6 analyses. Specifically, B*1503 had a significant positive association with both APRI-defined fibrosis (OR, 2.0; 95% CI, 1.0–3.7; $P = .04$) and FIB-4-defined fibrosis (OR, 1.9; 95% CI, 1.0–3.6; $P = .05$) at any visit, as well as borderline associations with incident APRI-defined fibrosis and both APRI- and FIB-4-defined fibrosis at baseline. Similarly, A*3303 and the A homozygous group also showed consistent results across analyses; each with significant or borderline associations with

Table 2. HLA Alleles and Allele Groups Significantly Associated With Hepatitis C Virus (HCV) Viral Load and Liver Disease as Detected by Aspartate Aminotransferase to Platelet Ratio Index (APRI) and Fibrosis-4 (FIB-4)

Allele ^a	No. of women ^b	Baseline HCV viral load (n= 619), β (95% CI) ^c	Baseline APRI >1.5 (n= 353; 75 events) OR (95% CI) ^d	Baseline FIB-4 >3.25 (n= 397; 68 events), OR (95% CI) ^d	Incident APRI >1.5 (n= 367; 64 events), HR (95% CI) ^e	Incident FIB-4 >3.25 (n= 383; 87 events), HR (95% CI) ^e	APRI >1.5 at any visit (n= 617; 139 events), OR (95% CI) ^f	FIB-4 >3.25 at any visit (n= 617; 155 events), OR (95% CI) ^f
A. Alleles significantly associated with both HCV viral load and liver disease detected by APRI and FIB-4								
DQB1*0301	94	-.4 (-.6 to -.3)^g	.6 (.3-1.4)	.5 (.2-1.3)	.6 (.2-1.4)	.5 (.3-1.2)	.5 (.3-1.0)^h	.5 (.2-.9)ⁱ
B. Alleles significantly associated with HCV viral load								
B*5101	49	-.3 (-.6 to 0) ⁱ	1.5 (.6-3.5)	1.5 (.5-4.2)	2.1 (.9-4.8) ^h	.8 (.3-1.8)	1.9 (1.0-3.6)	1.1 (.6-2.2)
DQA1*0500	110	-.3 (-.5 to -.1) ^j	.7 (.3-1.6)	.5 (.2-1.3)	.8 (.4-1.7)	.8 (.4-1.4)	.6 (.3-1.2)	.7 (.4-1.3)
DRB1*0701	107	.3 (.1-.4) ^{jk}	1.4 (.7-2.7)	1.7 (.8-3.6)	1.3 (.7-2.4)	1.1 (.6-1.8)	1.4 (.9-2.3)	1.5 (.9-2.4)
DRB1*0804	35	-.4 (-.7 to -.1) ⁱ	.3 (.1-1.6)	.2 (0-1.6)	.7 (.2-2.4)	1.3 (.5-3.0)	.6 (.2-1.6)	.7 (.3-1.6)
Rare DRB1	129	-.2 (-.4 to 0) ⁱ	.9 (.5-1.7)	.8 (.4-1.7)	1.0 (.5-1.9)	.9 (.6-1.6)	1.1 (.7-1.8)	.9 (.5-1.4)
C. Alleles significantly associated with liver disease detected by APRI and FIB-4								
A*2301	87	0 (-.3 to .3)	2.1 (1.0-4.3) ⁱ	1.3 (.6-2.9)	1.4 (.8-2.7)	1.7 (1.0-3.1) ^h	1.9 (1.2-3.3) ⁱ	1.6 (.9-2.7) ^h
A*3001	54	.1 (-.2 to .3)	1.5 (.6-3.5)	.9 (.3-2.7)	1.5 (.7-3.4)	2.2 (1.2-4.2) ⁱ	1.8 (.9-3.4) ^h	1.7 (.9-3.3) ^h
A*3002	75	0 (-.2 to .3)	.2 (0-.8) ⁱ	.5 (.2-1.5)	.8 (.3-1.8)	.8 (.4-1.5)	.4 (.2-.8) ⁱ	.6 (.3-1.1)
A*3303	49	.1 (-.2 to .4)	.5 (.1-1.6)	.5 (.1-2.1)	.3 (.1-1.2)^h	.5 (.2-1.5)	.4 (.1-.9)ⁱ	.4 (.2-.9)ⁱ
A*6802	51	.1 (-.1 to .4)	4.1 (1.6-10.3) ^{jk}	2.7 (.9-8.4) ^h	1.4 (.6-3.6)	1.0 (.4-2.3)	1.8 (.9-3.6) ^h	1.3 (.7-2.5)
A*7401	55	.1 (-.1 to .4)	.6 (.2-1.9)	.9 (.3-2.5)	2.2 (1.2-4.1) ⁱ	1.3 (.7-2.5)	1.8 (1.0-3.5) ^h	2.0 (1.1-3.6) ⁱ
B*1402	33	0 (-.3 to .3)	.5 (.1-1.9)	.4 (.1-2.6)	.4 (.1-3.0)	.3 (0-1.8)	.4 (.1-1.3)	.2 (.1-.9) ⁱ
B*1503	53	.1 (-.2 to .3)	2.2 (.9-5.7)^h	2.4 (.9-6.2)^h	1.9 (.9-4.1)^h	1.4 (.7-3.0)	2.0 (1.0-3.7)ⁱ	1.9 (1.0-3.6)ⁱ
B*3501	72	0 (-.2 to .2)	1.5 (.6-3.5)	2.6 (1.1-6.2) ⁱ	.9 (.4-2.0)	1.0 (.5-1.9)	1.0 (.6-1.9)	1.7 (1.0-2.9) ^h
B*4403	58	.2 (0-.5) ⁱ	1.7 (.7-4.2)	1.8 (.7-4.5)	.8 (.3-2.1)	1.4 (.8-2.7)	1.2 (.6-2.3)	2.1 (1.1-3.8) ⁱ
Cw*0401	189	.1 (-.1 to .2)	2.0 (1.1-3.6) ⁱ	3.1 (1.6-5.9) ^g	1.3 (.8-2.3)	1.2 (.7-1.9)	1.4 (.9-2.1)	1.7 (1.1-2.5) ⁱ
Cw*0802	54	-.1 (-.3 to .2)	.3 (.1-1.0) ⁱ	.3 (.1-1.2) ^h	.8 (.3-2.3)	.8 (.3-1.9)	.5 (.2-1.1) ^h	.4 (.2-1.0) ⁱ
DRB1*0301	99	-.1 (-.3 to .1)	1.2 (.6-2.4)	1.2 (.5-2.8)	1.8 (1.0-3.2) ⁱ	1.4 (.9-2.4)	1.6 (1.0-2.6) ^h	1.5 (.9-2.5)
A homozygous	41	-.1 (-.4 to .2)	.4 (.1-1.7)	.6 (.1-2.6)	.4 (.1-1.6)	.1 (0-.8)ⁱ	.4 (.1-1.0)^h	.3 (.1-.8)ⁱ
Rare B	152	.1 (-.1 to .2)	.7 (.4-1.3)	.4 (.2-1.0) ⁱ	.6 (.3-1.2)	.8 (.5-1.4)	.8 (.5-1.2)	.8 (.5-1.2)
C1 homozygous	165	-.1 (-.2 to .1)	.2 (.1-.5) ^g	.4 (.2-.9) ⁱ	1.0 (.6-1.8)	.9 (.5-1.4)	.5 (.3-.9) ⁱ	.6 (.4-1.0) ⁱ
C2 homozygous	108	.2 (0-.3) ^h	1.9 (1.0-3.7) ⁱ	2.3 (1.1-4.7) ⁱ	1.0 (.5-2.0)	1.2 (.7-2.0)	1.5 (.9-2.4)	1.5 (.9-2.4)
Class II homozygous	121	-.1 (-.3 to .1)	1.2 (.6-2.5)	1.0 (.4-2.4)	1.8 (1.0-3.3) ⁱ	1.3 (.8-2.2)	1.3 (.8-2.1)	.9 (.6-1.5)
Rare DQA1	84	0 (-.2 to .2)	.8 (.3-1.7)	1.2 (.5-3.3)	2.4 (1.1-5.1) ⁱ	1.6 (.8-3.3)	1.4 (.8-2.4)	1.0 (.6-1.9)
Common DQB1	98	-.2 (-.4 to .0) ^h	.8 (.3-1.7)	1.3 (.5-3.5)	.7 (.3-1.6)	.4 (.2-1.0) ⁱ	.8 (.4-1.4)	.7 (.4-1.3)

NOTE. Bold alleles and allele groups were noted to be of special interest, as discussed in the text. CI, confidence interval; HR, hazard ratio; OR, odds ratio.

^a In addition to individual alleles, the analyses included HLA-B and -C alleles that act as ligands for killer immunoglobulinlike receptors, namely, Bw4 and Bw4I80 groups [12] and groups C1 and C2 [42, 43]; allele zygosity [13, 44]; and allele rarity [45-47]. Allele rarity was examined by comparing alleles with moderately common genotypes (second and third quartiles of allele frequency) with those with rare genotypes (first quartile) or common genotypes (fourth quartile of allele frequency) [45, 46].

^b Number of women homozygous or heterozygous for a given allele or allele group in the total study population. Analysis-specific allele frequencies are shown in Supplementary Table 1.

^c Linear regression models of \log_{10} normalized HCV RNA levels adjusted for race/ethnicity, age, baseline CD4⁺ T cell count, and baseline human immunodeficiency virus (HIV) RNA level (β represents the absolute difference in \log_{10} HCV viral load associated with the presence of a given allele or allele group; eg, women with the DQB1*0301 have, on average, .4 \log_{10} lower HCV viral loads than women without DQB1*0301).

^d Logistic regression models of baseline liver disease adjusted for race/ethnicity, age, baseline CD4⁺ T cell count, and baseline HIV RNA level, using women with normal values for these indexes as the comparison group, as discussed in the text.

^e Cox models of incident of liver disease adjusted for race/ethnicity, age, baseline APRI or FIB-4, time-dependent CD4⁺ T cell count, and time-dependent HIV RNA level. Data set was limited to women who had baseline values considerably below the threshold for liver disease, as discussed in the text.

^f Logistic regression models of liver disease at any visit adjusted for race/ethnicity, age, baseline CD4⁺ T cell count, and baseline HIV RNA level.

^g $P < .001$ unadjusted for multiple comparisons.

^h $.05 \leq P < .10$, unadjusted for multiple comparisons.

ⁱ $.01 \leq P < .05$ unadjusted for multiple comparisons.

^j $.001 \leq P < .01$ unadjusted for multiple comparisons.

^k Significant interaction by immune status (HIV-negative women and HIV-positive women with CD4⁺ T cell counts >500 cells/ μ L vs HIV-positive women with CD4⁺ T cell counts <500 cells/ μ L).

decreased risk of fibrosis in 3 of 6 models. Most other alleles lacked this level of consistency, including the only allele or allele group to retain a significant association after multiple comparison adjustment, the C1 homozygous group; C1 homozygosity was inversely associated with APRI-defined fibrosis at baseline (OR, .2; 95% CI, .1–.5; $P_{\text{corrected}} = .04$). One allele, A*6802, had significant interaction by host immune status (see Table 2).

DISCUSSION

Although a strong association of HLA genotype and HCV viral clearance has been reported by our group and others, prior studies relating HLA with HCV viral load and with liver disease have had inconsistent findings. Few of these prior studies, however, conducted HLA class I genotyping. Most studies of HCV-related liver disease were also cross-sectional. In the current investigation, therefore, we conducted high-resolution HLA class I and II genotyping in a large prospective cohort of HCV viremic women at elevated risk of HCV disease progression due to a high prevalence of HIV coinfection.

Our data analysis identified 6 alleles and allele groups that were significantly associated with HCV viral load. Interestingly, 5 of these were HLA class II. The strongest relationship was the association of DQB1*0301 with low HCV viral load ($\beta = -.4$; $P < .00001$). This finding is noteworthy, because DQB1*0301 is also one of the alleles that has been most consistently associated with clearance of HCV viremia (reviewed in [3, 4, 8]). At least 5 prior studies reported data regarding HCV viral load and HLA [5, 15–18]. However, there was no agreement among these studies, or with the findings of the current study. The main advantage to the current investigation was statistical power, this study being 3-fold larger than prior studies.

Our data additionally suggested a possible association of DQB1*0301 with reduced risk of FIB-4 and APRI diagnosed fibrosis. This included a statistically significant 40%–50% reduction in risk of FIB-4–defined liver fibrosis detected at any visit and an association of similar strength but of borderline statistical significance with APRI-defined liver fibrosis at any visit. Though the effect estimates in 4 other models did not approach statistical significance, they showed the same 40%–50% decreased risk of fibrosis. Thus, the consistency of the DQB1*0301 findings across these several analyses—inverse associations like the one observed in its relation with HCV viral load—made these results notable. Further, 2 small independent studies reported similar results regarding DQB1*0301 and liver disease [14, 38]. Whereas our study did not demonstrate the inverse association between DRB1*11 and liver fibrosis reported elsewhere (reviewed in [8]), it is interesting to note that DQB1*0301 and DRB1*11 are in linkage disequilibrium [39]. In the current study, excluding women with the DQB1*0301-DRB1*1101 haplotype did not meaningfully change the results (data not shown), suggesting that our findings related specifically to DQB1*0301.

Several additional HLA alleles were also associated with FIB-4 and APRI-defined fibrosis in ≥ 1 of our analyses, but none were significant in all 6 models. B*1503 was notable because it had associations with increased risk of fibrosis that reached statistical significance or borderline significance in 5 of the 6 analyses. Although no prior studies have observed an association of B*1503 with HCV disease progression, we note that this allele has been associated elsewhere with poor prognosis after HIV-2 infection [40]. In contrast, A*3303 and homozygosity at the A locus showed consistent inverse associations across analyses. Interestingly, most of the alleles associated with FIB-4 and APRI-defined fibrosis were HLA class I, whereas most of those related to HCV viral load were class II alleles. If correct, the data could be interpreted to mean that CD4⁺ T cell responses are important for controlling HCV viral load, whereas CD8⁺ T cell responses play a larger role in determining the rate of disease progression.

This study had several limitations. First, most of our significant findings did not retain significance after adjustment for multiple comparisons and the results await confirmation. The major finding regarding DQB1*0301 and HCV viral load, however, remained highly significant after Bonferroni correction. Furthermore, DQB1*0301, B*1503, A*3303, and the A homozygous group had consistent associations across multiple if interrelated fibrosis end points that reduced the possibility that they occurred by chance. A second concern was the unavailability of histologic data, the reference standard for diagnosis of liver fibrosis. Nonetheless, there are advantages to prospective observational cohorts involving noninvasive follow-up, in that large numbers of participants can be studied and repeated testing with long follow-up is possible—as in the current study. A third concern was that our study population was racially heterogeneous, and we cannot exclude the possibility of confounding by population substructure. We did, however, control for self-reported race/ethnicity, and in the US, these self-reported categories have been shown to correlate well with genetically determined ancestry [41]. Fourth, we did not conduct haplotype analyses (except as noted for DQB1*0301-DRB1*1101); haplotype frequencies vary considerably by race/ethnicity, and in a multiracial population such as ours it would require larger numbers of subjects within each racial group to study haplotype associations adequately. Finally, because of the extensive linkage disequilibrium in the HLA region, it is possible that the alleles identified in our analyses are markers for other, causal, genetic variants that affect HCV pathogenesis.

Because most of the women in our study population were coinfecting with HCV and HIV, we took several steps to minimize the possibility that the associations we observed might be due to the relation of HLA alleles with HIV, and not to their relationship with HCV. Specifically, each of our analyses adjusted for HIV serostatus, CD4⁺ T-cell count, and HIV RNA level. We further examined each of our significant associations for interaction by HIV serostatus and CD4⁺ T-cell count, but few significant interactions were observed.

In summary, we found that DQB1*0301 was associated with low HCV viral load and also possibly with a low risk of liver fibrosis, as measured using 2 noninvasive indexes of liver fibrosis, FIB-4 and APRI. Similar results regarding DQB1*0301 and liver disease progression had been noted in other studies, and DQB1*0301 is in linkage disequilibrium with DRB1*11, which some prior studies associated with disease progression. Prior data have additionally suggested DQB1*0301 may play a role in HCV clearance. Although most other associations with HCV load in our study involved HLA class II alleles, additional associations with fibrosis primarily involved class I alleles. Overall, although the findings of this study await confirmation, they add to the growing evidence that HLA genotype, and DQB1*0301 in particular, influence both the early and late stages of HCV-related liver disease.

Supplementary Data

Supplementary data are available at <http://jid.oxfordjournals.org> online.

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