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Specific HLA Class I and II Alleles Associated with Hepatitis C Virus Viremia

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Abstract

Studies of human leukocyte antigen (HLA) alleles and their relation with hepatitis C virus (HCV) viremia have had conflicting results. However, these studies have varied in size and methods, and few large studies assessed HLA class I alleles. Only one study conducted high resolution class I genotyping. The current investigation therefore involved high-resolution HLA class I and II genotyping of a large multi-racial cohort of US women with high prevalence of HCV and HIV.

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Our primary analyses evaluated associations between twelve HLA alleles identified through a critical review of the literature and HCV viremia in 758 HCV-seropositive women. Other alleles with >5% prevalence were also assessed; previously unreported associations were corrected for multiple comparisons. DRB1*0101 (prevalence ratio [PR] = 1.7; 95% confidence interval [CI] = 1.1–2.6), B*5701 (PR=2.0; 95% CI = 1.0–3.1), B*5703 (PR = 1.7; 95% CI = 1.0–2.5), and Cw*0102 (PR = 1.9; 95% CI = 1.0–3.0) were associated with the absence of HCV RNA (i.e., HCV clearance), while DRB1*0301 (PR = 0.4; 95% CI = 0.2–0.7) was associated with HCV RNA positivity. DQB1*0301 was also associated with the absence of HCV RNA but only among HIV-seronegative women (PR = 3.4; 95% CI = 1.2–11.8). Each of these associations was among those predicted. We additionally studied the relation of HLA alleles with HCV infection (serostatus) in women at high risk of HCV from injection drug use (IDU; N=838), but no significant relationships were observed.

Conclusion—HLA genotype influences host capacity to clear HCV viremia. The specific HLA associations observed in the current study are unlikely to be due to chance since they were *a priori* hypothesized.

Keywords

human leukocyte antigen; HIV; injection drug user; multiple comparisons; killer immunoglobulin-like receptor

INTRODUCTION

Over 4 million women and men in the United States and 150 million people worldwide are estimated to be hepatitis C virus (HCV) seropositive.(1;2) Most of these individuals are chronically infected with the virus and are at high risk of cirrhosis, hepatocellular carcinoma, and liver-related death. The natural history of HCV infection however is highly variable. Some individuals do not become HCV infected despite high levels of exposure.(3) Other individuals may clear HCV RNA following acute infection, and while some individuals with long-term HCV viremia remain clinically asymptomatic, others have progressive disease.(4) Indeed, marked variability in natural history is seen even among groups of individuals with single source exposure to HCV, as occurred in a population of Irish women exposed to HCV-contaminated anti-D immune globulin.(5) Together these observations suggest that host factors, particularly host immune response, play a key role in the regulation of HCV pathogenesis.

Human leukocyte antigen (HLA) genes are critical to the regulation of both cellular and innate immunity and are among the most polymorphic in the human genome. HLA genes are clustered together on the short arm of chromosome 6 and encode HLA molecules that form stable complexes when bound to foreign peptides. These complexes are presented on cell surfaces where they are recognized and bound by T-cells initiating a cascade of immune responses capable of clearing foreign material. The diversity of HLA variants, or alleles, is a critical factor in the ability of HLA to bind a wide variety of antigens, and for the immune system to respond to a wide variety of pathogens.

Several strong associations between HLA alleles and infectious agents have been reported, and recent genome-wide association studies of HIV disease progression have provided further evidence of the importance of HLA polymorphism in host control of viral infections. Over forty studies have examined the relationship between HLA alleles and HCV natural history (reviewed in (6;7)) but many of these studies have been small and/or had important limitations (e.g. absence of a control group). In addition, despite the major role of class I HLA-restricted T-cell responses in the control of HCV pathogenesis,(8;9) few studies have

examined associations between HCV natural history and HLA class I alleles.(10–15) Among the studies that did examine HLA class I alleles, several interesting findings have been reported, but the results were inconsistent across studies. Only one of these studies utilized high resolution genotyping of class I alleles.(12) Lastly, there are also few data regarding associations between HLA alleles and risk of initial HCV infection (HCV seropositivity) in highly exposed populations.(16–18)

To address these issues we conducted high-resolution HLA class I and II genotyping in a large multi-racial population of US women with a high prevalence of HCV and HIV infection. Furthermore, we focused primarily on associations between HCV disease phenotypes and a narrow set of *a priori*-defined HLA alleles identified as part of a critical review of the literature. By specifying in advance those associations with the highest prior probability, we intended to reduce concerns regarding multiple comparisons a major difficulty for the interpretation of HLA data due to the large number of distinct HLA alleles and to make this study largely an assessment of a small number of discrete hypothesized relationships.

METHODS

Review of the HCV-HLA Literature

We searched the PubMed database using the search string “hepatitis C” and “HLA” (or “human leukocyte antigen”). We limited our search to epidemiologic studies focused on the associations of HLA alleles with (i) HCV viremia (i.e. presence/absence of HCV RNA) among HCV-seropositive individuals, or (ii) HCV infection (i.e. serostatus) in high-risk populations, and to studies published in English. We additionally examined the references cited in each paper, including several review papers.(6;7;19;20)

We then critically evaluated the identified studies for the appropriateness of their research design (e.g. existence of a suitable comparison group, adequacy of sample size) and statistical methods. Based on this evaluation we constructed a list of HLA class II alleles (4 digit resolution) and allele groups (2 digit resolution) associated with HCV viremia or HCV infection that had been reported in at least two studies that in our subjective view had been appropriately conducted. Alleles and allele groups meeting these criteria were considered to have a high prior probability of association with one or both of the HCV phenotypes of interest.

Because there was much less data reported regarding associations between HCV and HLA class I alleles (compared to class II alleles) we set a slightly less stringent threshold for designating class I alleles as having a high prior probability of association with HCV. Specifically, an HLA class I HCV association could either have (as above for class II) been reported in at least two prior studies, or it could have had a particularly strong (i.e., an odds ratio greater than or equal to 3.0) relationship with HCV in one prior study. This odds ratio threshold was selected since threefold and greater risks are considered to be strong and less likely to be due to confounding.(21)

Our review identified six HLA class II alleles and three HLA class I allele groups associated with HCV viremia in two or more studies. An additional two HLA class I allele groups were strongly associated (OR>3.0) with HCV viremia in a single study (shown in Table 1). In contrast to HCV viremia, however, we found only three studies that examined the relation of HCV serostatus with HLA alleles in high-risk populations(16–18) and there were no consistent or strong findings among these three studies.

Study Population

The Women's Interagency HIV Study (WIHS) is a prospective, multicenter cohort study of HIV-seropositive (N=2,793) and HIV-seronegative (N=975) women enrolled through similar sources at six clinical sites (Bronx, NY; Brooklyn, NY; Chicago, IL; Los Angeles, CA; San Francisco, CA; and Washington, DC). The initial enrollment was conducted between October 1994 and November 1995, and a subsequent second recruitment cycle occurred in 2002. The recruitment methods and data collection procedures for WIHS have been described previously.(22) Briefly, subjects in this ongoing study are evaluated every 6 months with standardized interviews, physical examination, and a blood draw. The WIHS protocol was approved by each local institutional review board, and all participants signed informed consent.

In the current investigation we focused on WIHS women who, at the enrollment visit, had either self-reported a history of injection drug use (IDU) and were therefore considered at high risk of HCV infection and/or were HCV seropositive. Among HCV seropostive women (N=1,204) we limited our analysis to women with known HCV RNA status (N=1,070), self-reported White non-Hispanic, Black non-Hispanic, or Hispanic race/ethnicity (N=1,046), and had complete HLA data at one or more HLA loci (N=758). Among the IDU (N=1,161), we limited our analyses to women with known HCV serostatus (N=1,129), self-reported White non-Hispanic, Black non-Hispanic, or Hispanic race/ethnicity (N=1,098), and had complete HLA data at one or more HLA loci (N=838).

Laboratory Methods

HCV Testing—HCV serostatus was determined in all WIHS 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, which has a linear range of 600 5.0×10^5 IU/ml, as previously described,(23) or the COBAS Taqman assay, which has a linear range of 10 2.0×10^8 IU/ml (both from Roche Diagnostics, Branchburg, New Jersey, USA). Previous testing demonstrated a high correlation of HCV RNA levels in the two assays, and a 10% resample of patients with undetectable HCV RNA in the Amplicor assay were also undetectable in the TaqMan assay (data not shown). HCV genotyping was conducted on a subset of HCV viremic women using the NC TRUGENE HCV 5 NC Genotyping Kit (Bayer HealthCare LLC, Tarrytown, NY), as previously described.(24)

HLA Genotyping—Genomic DNA was prepared from subjects' lymphoblastoid B cell lines or from peripheral blood lymphocytes. Protocols for HLA genotyping have been standardized through the International Histocompatibility Working Group (http://www.ihwg.org). Briefly, HLA class I genes (HLA-A, −B, and −C) were amplified using locus-specific PCR primers flanking exons 2 and 3, the polymorphic segments of the class I genes. The 1 kb PCR products were blotted on nylon membranes and hybridized with a panel of sequence-specific oligonucleotide (SSO) probes. The HLA alleles were assigned by the reaction patterns of the SSO probes, according to known HLA sequences. Any ambiguous SSO probing was resolved by sequencing analysis, as previously described.(25) HLA class II typing was conducted using high resolution SSO typing for HLA-DQA, −DQB, and −DRB1 loci, using the polymorphic exon 2. DRB genotyping involved a two-step procedure. First, the broad serological DR types were determined using a pair of DRB generic primers and a panel of SSO probes. Allele-level DRB typing was then achieved by using group-specific primers to amplify the DRB alleles determined in the generic typing followed by SSO hybridization. For DQA and DQB, locus-specific PCR were performed followed by SSO hybridization.

Statistical Methods

Descriptive statistics for demographic and clinical variables were calculated for the HCV-seropositive women and the IDU. We examined differences in these characteristics between HCV RNA-positive versus HCV RNA-negative women and between HCV-seropositive versus HCV-seronegative women using the T-test (for continuous data), Mann-Whitney U test (for continuous data with small subgroups), chi-square test (for categorical data), and Fisher's exact test (for categorical data with small subgroups).

The principal analyses focused on the associations between HLA alleles and HCV viremia among HCV-seropositive women and between HLA alleles and HCV infection (serostatus) among women who reported IDU. In our *a priori*-planned analyses of HLA alleles with a high prior probability of association with HCV viremia, we included those alleles present in at least 3% of the women studied (i.e., 23 or more of the 758 HCV seropositive women heterozygous or homozygous for a given allele). In our exploratory analyses, which examined alleles without a high prior probability of association (i.e., alleles not included in Table 1) we focused on alleles that had at least 5% prevalence, since exploratory analyses are more prone to spurious findings (particularly when data are limited). Our analyses employed log-binomial regression models to calculate both unadjusted and race/ethnicity-adjusted prevalence ratios. However, when assessing alleles without a high prior probability of association (i.e. alleles not included in Table 1) we corrected the P-values for multiple comparisons via permutation resampling using PROC MULTTEST; a method that empirically incorporates correlations within and between loci.(26)

Because some prior studies have described variation in HLA associations by race,(27;28) we assessed potential heterogeneity in effect estimates (i.e. interaction) by race/ethnicity. In contrast, we did not expect to observe heterogeneity by HIV-serostatus or CD4+ T-cell count among HIV-seropositive women. A variety of sources suggest that HCV infection generally occurs prior to HIV infection in new IDU,(29–31) and therefore that the majority HCV RNA clearance/persistence occurs without relation to HIV. It is possible, however, that HIV preceded HCV infection in some women. For completeness, therefore, we assessed heterogeneity by HIV serostatus/CD4+ T-cell count (HIV-seronegative, HIV-seropositive with CD4+ T-cell count ≥ 500 cells/mm^3, and HIV-seropositive with CD4+ T-cell count < 500 cells/mm^3). Lastly, we examined whether groups of HLA alleles that act as ligand for killer immunoglobulin-like receptors (KIR) were associated with HCV infection and HCV viremia. KIR play a major role in the activation of natural killer (NK) cells and the innate immune response and specific combinations of KIR and HLA ligands have been associated with clearance of HCV RNA previously.(32;33) These ligand groups were Bw4 reflecting 141 HLA-B alleles, Cw group 1 reflecting 48 HLA-Cw alleles, and Cw group 2 reflecting 43 HLA-Cw alleles.(32) All statistical analyses were performed using SAS 9.1 (SAS Institute, Inc., Cary, NC).

RESULTS

Demographic and Clinical Characteristics of the Study Population

Selected characteristics of the 758 HCV-seropositive women with and without detectable HCV RNA are shown in Table 2a. Most HCV-seropositive women reported IDU, and this did not vary according to HCV RNA positivity. The HCV RNA-positive women, though, were more likely than those who were HCV RNA-negative to be Black, non-Hispanic. HIV-seroprevalence did not differ between HCV RNA-positive/-negative women, but the CD4+ T-cell counts were significantly lower among those HIV-seropositives who had detectable HCV RNA. HCV genotype was determined for 226 of the women with detectable HCV viremia. The genotype distribution among these women was: 1a in 125 (55%) of the 226

women; 1b in 65 (29%); type 1 but with undetermined subtype in 8 (4%); 2a in 3 (1%); 2b in 6 (3%); 3a in 14 (6%); 3d in 1 (<1%); and 4a in 4 (2%) women.

Table 2b shows selected characteristics of the 838 IDUs with/without HCV infection (there was substantial, but not complete, overlap between the injection drug users evaluated to study association with HCV infection, and the HCV-seropositive women evaluated to study association with HCV viremia). HCV-seropositive IDU were older, more likely to be Black, non-Hispanic, and more likely to be HIV-seropositive than HCV-seronegative IDU. CD4+ T-cell counts were similar however between HIV infected HCV-seropositive and HCV-seronegative IDU.

HLA Alleles Associated with HCV Viremia in HCV-Seropositive Women

Our review of the HCV-HLA epidemiologic literature identified six class II alleles (4 digit resolution) and five class I allele groups (2 digit resolution) with a high prior probability of association with detectable HCV RNA (Table 1). Each of the six class II alleles had >3% prevalence among the HCV-seropositive subjects. The five class I allele groups were largely reflective of a single (4 digit) allele with >3% prevalence, except for the B*57 group which had two alleles with >3% prevalence (Table 3a). Therefore, a total of twelve individual alleles with a high prior probability of association with HCV viremia were included in our primary analyses of HCV clearance.

Of these twelve HLA alleles, six were found to have the predicted associations with detectable HCV RNA in both unadjusted and adjusted (for race/ethnicity) analyses. Specifically, DRB1*0101 (prevalence ratio [PR] = 1.7; 95% confidence interval [CI] = 1.1–2.6), B*5701 (PR = 2.0; 95% CI = 1.0–3.1), B*5703 (PR = 1.7; 95% CI = 1.0–2.5), and Cw*0102 (PR = 1.9; 95% CI = 1.0–3.0) were each associated with absence of HCV RNA (i.e., HCV clearance) in adjusted analysis, as was the B*57 allele group (PR =1.7; 95% CI = 1.1–2.4) as a whole. DRB1*0301 (OR = 0.4; 95% CI=0.2–0.7) in contrast was associated with the presence of HCV RNA. For a sixth allele with high prior probability of association, DQB1*0301, we observed significant statistical interaction by HIV serostatus/CD4+ T-cell count ($P_{interaction}$ = 0.02). Only among HIV- seronegative women (PR = 3.4; 95% CI = 1.2–11.8), and not among HIV-seropositive women with CD4+ T-cell count ≥ 500 cells/mm^3 (PR = 0.6; 95% CI = 0.2–1.4) or HIV-seropositive women with CD4+ T-cell count < 500 cells/mm^3 (PR = 1.7; 95% CI = 0.8–3.3) was there a significant association with between DQB1*0301 and HCV viremia. In contrast, there were no significant associations between HCV viremia and the other six alleles with high prior probability of association, namely, DRB1*0401, DRB1*1101, DRB1*1501, B*1801, B*2705, and Cw*0401.

Exploratory analyses of the 58 additional HLA class I and II alleles (which lacked a high prior probability of association) identified two additional alleles that were significantly associated with HCV viremia: DRB1*0701 and DRB1*1302 (see Supplementary Table 1). However, as seen in Table 3b, these allele associations became statistically non-significant after adjustment for multiple comparisons. We also studied associations with three broad groups of HLA class I alleles that can act as ligand for KIR, namely, Bw4, Cw group 1, and Cw group 2. Specifically, as in prior reports, we tested whether homozygosity for a given KIR ligand group (e.g., a woman with two Cw group 1 alleles) was associated with HCV viremia (32;33), but in our dataset no significant relationships were observed with KIR ligand groups.

HLA Alleles Associated with HCV Infection in Injection Drug Users

Our review of the HCV-HLA literature identified only three studies of HLA alleles and HCV serostatus in high-risk populations(16–18) and there were no consistent findings or

strong associations. We therefore treated all of the analysis of HCV serostatus and HLA alleles as exploratory, and all significant P-values were adjusted for multiple comparisons. Of the 64 HLA class I and II alleles with > 5% prevalence in the 838 IDUs studied, B*5703 (P = 0.03), Cw*0304 (P = 0.04), and Cw*0701 (P = 0.01), were significantly associated with HCV infection (see Supplementary Table 2), but these associations did not retain significance after adjustment for multiple comparisons (P = 0.99, 1.0, and 0.64 respectively). There were no significant interactions by race/ethnicity or HIV serostatus/CD4+ T-cell count.

DISCUSSION

We studied the relation of high-resolution HLA class I and II genotype with HCV viremia, and with HCV serostatus in injection drug users (IDU). While there have been a number of prior studies of HLA alleles and HCV viremia, many of the findings to date have been conflicting. Part of this variability may relate to aspects of study design, including differences in sample size, and whether an appropriate control group was used. Furthermore, few studies examined HLA class I alleles, with only one having conducted high resolution class I genotyping.(12) There has also been very little data regarding HLA and the prevalence of HCV infection (i.e. seropositivity) in high-risk populations.(16–18) To build upon the existing data, therefore, we conducted high-resolution HLA class I and II genotyping in a large multi-racial cohort of US women with high prevalence of HCV and HIV infection.

Several significant associations between HLA class I and II alleles and HCV viremia were observed. These included six of the twelve HLA alleles that we identified as having a high prior probability of association with HCV viremia based on a critical review of the literature. Because each of these associations represented discrete *a priori* hypotheses it is unlikely that they occurred by chance.

Both B*5701 and B*5703, for example, were significantly associated with absence of HCV RNA. Two earlier studies of HLA class I had reported similar associations with the B*57 allele group; the first was conducted in a large multi-racial, majority male population in the US,(12) while the second was conducted in a small, majority male population in West Africa.(13) A third study conducted in Ireland also found an association between the B*57 allele group and HCV viremia, though the results did not attain statistical significance.(14) In the current study the relation of the B*57 allele group with HCV viremia mainly reflected the combined effects of B*5701 and B*5703, but we caution that our dataset included too few women with other B*57 alleles (e.g. B*5702, B*5704) to study in detail.

It is interesting to note that B*5701 and B*5703 also appear to have a diverse range of other effects on host immune response. B*5701 for example has been associated with slow HIV disease progression,(34;35) as well as with abacavir hypersensitivity,(36) and with the autoimmune conditions psoriasis and psoriatic arthritis.(37) B*5703 is also associated with slow HIV disease progression(38) and with the autoimmune condition spondylarthropathy.(39) These associations could reflect the antigen binding characteristics of these alleles. However, an alternative or additional possibility is that the broad impact of B*5701 and B*5703 is explained by their ability to act as ligand for killer immunoglobulin-like receptors (KIR); receptors which help modulate the activation of NK cells and the innate immune system. Indeed, it has been reported that the B*57 group may be a particularly strong KIR ligand.(40) Although we did not observe a significant association between HCV viremia and the broader groups of alleles that act as ligand for KIR, KIR genotyping of our population will be needed to study this issue more comprehensively.

Similarly, DRB1*0101, DQB1*0301, Cw*0102, and DRB1*0301 were associated with HCV viremia in this and the other epidemiologic studies identified in Table 1, and may also be associated with various autoimmune conditions. DRB1*0101 and DQB1*0301 are reported to be risk factors for the autoimmune condition rheumatoid arthritis,(41;42) while Cw*0102 is associated with both psoriasis(43) and autoimmune hepatitis.(44) Cw*0102, like HLA*B57, can act as ligand for KIR.(45) Lastly, DRB1*0301 is inversely associated with rheumatoid arthritis,(46) consistent with its inverse association with HCV clearance, though we note it also has positive associations with autoimmune hepatitis(47) and systemic lupus erythematosus.(48)

The fact that both B*57 and Cw*01 can act as ligand for KIR could help explain their broad range of immunologic associations, since NK cells are not antigen-specific. However, we are unaware of any characteristics of the HLA class II alleles that might readily explain their mutual associations with both HCV viremia and autoimmune disease.

Six expected associations between HLA alleles and HCV viremia were not observed. Specifically, there were no significant relationships of DRB1*0401, DRB1*1101, DRB1*1501, B*1801, B*2705, or Cw*0401 with the presence/absence of HCV RNA despite their high prior probability of association based on earlier reports. The failure to replicate these predicted associations could have several explanations. First, in our study the vast majority of HCV infections were genotype 1, whereas in Europe genotypes 3 and 4 are also common;(49) i.e., differences in genotype-specific protein expression may affect HLA-restricted immune responses.(50) Differences in host characteristics could also explain the conflicting findings, such as differences in the prevalence of certain alleles. For example, in our population both DRB1*0401 and B*2705 were rare, and thus we had limited statistical power to examine associations with these alleles. There could also be differences in patterns of linkage disequilibrium between loci in different populations.

In contrast to HCV viremia, we found no significant associations of HLA alleles with HCV infection in high-risk women. We note that compared with the substantial literature regarding HCV viremia and HLA there has been little published data regarding HCV serostatus and HLA. To our knowledge, only three prior studies have been reported,(16–18) and they had conflicting results. With so little prior data for comparison we are unable to fully judge our null findings for HCV serostatus. It is interesting that prior to adjustment for multiple comparisons, B*5703, Cw*0304, and Cw*0701 were significantly associated with HCV infection among the women who reported IDU, but these associations were not reported in the three earlier studies. Therefore, while it might seem unlikely for there to be no associations at all between HLA alleles and the risk of HCV infection, it remains that there have been no reproducible associations observed to date.

This study had several limitations that must be considered in the interpretation of these data. One of the most important limitations was the lack of individualized data regarding the actual level of exposure to HCV which is needed to comprehensively assess the associations between HCV serostatus and HLA alleles. The fact that the women all reported IDU only identifies them as being in a high-risk group. Data regarding frequency of IDU, frequency of needle sharing, and whether IDU took place in a high-risk context (e.g. a shooting gallery) were unavailable in this study. On the other hand, our analysis of HCV RNA clearance was unlikely to be affected by issues of HCV exposure since all of the women analyzed were HCV-seropositive. The major limitation of our analysis of HCV viremia was sample size. Although this study was large compared with many prior reports, we still lacked the statistical power to study associations between HCV phenotypes and uncommon alleles. Given that our study population was largely Black and Hispanic, this applied especially to alleles that are mainly common among White, non-Hispanic populations. Lastly, as in all

genetic association studies, the associations we observed could relate to the alleles we assessed, or to linkage disequilibrium with unexamined genetic markers.

In summary, the current study provides new evidence that a small number of specific HLA alleles may be important mediators of host capacity to clear HCV infection. These results are unlikely to be due to chance since each of the associations had been *a priori* predicted based on a critical review of the literature. We additionally recognized that each of the alleles related to HCV viremia in this study has also been associated with one or more autoimmune conditions. Additional epidemiologic and laboratory studies to characterize the properties of these alleles is warranted, with the hope that knowledge of the factors involved in an effective immune response can be exploited in the development of novel HCV prevention and treatment strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations

HLA	human leukocyte antigen
HCV	hepatitis C virus
PR	prevalence ratio
CI	confidence interval
IDU	injection drug use
WIHS	Women's Interagency HIV Study
SSO	sequence-specific oligonucleotide
KIR	killer immunoglobulin-like receptor
NK	natural killer

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Table 1

HLA Alleles and Allele Groups with a High Prior Probability of Association with HCV Viremia *

a. Associations with HCV Viremia in HCV-Seropositive Individuals		
Allele	**HCV RNA Status**	**References**
DQB1*0301	Clearance	(28;51–56)
DRB1*0101	Clearance	(14;28;57;58)
DRB1*0301	Persistence	(14;28)
DRB1*0401	Clearance	(14;52)
DRB1*1101	Clearance	(51;54–56)
DRB1*1501	Clearance	(14;59)
B*18	Persistence	(14)
B*27	Clearance	(14)
B*57	Clearance	(12;13)
Cw*01	Clearance	(12;14)
Cw*04	Persistence	(12;15)

* HLA alleles associated with HCV viremia in two or more prior studies, or strongly associated with HCV viremia in a single study (see Methods).

Table 2

Demographic and Clinical Characteristics of the Study Population

a. HCV-seropositive women (evaluated to study the association with HCV viremia)		
Characteristic	**HCV RNA negative (N=136)**	**HCV RNA positive (N=622)**
Age, years (mean ± SD)	39.3 ± 6.9	39.8 ± 6.2
Race/ethnicity *		
White, non-Hispanic	23 (17%)	100 (16%)
Black, non-Hispanic	66 (49%)	398 (64%)
Hispanic	47 (35%)	124 (20%)
Injection drug use †	123 (90%)	577 (93%)
HIV positive	112 (82%)	524 (84%)
CD4 + count, cells/mm^3 in HIV-seropositive women		
Median (range) *	401 (4 1933)	333 (0 1499)
>500	39 (35%)	146 (28%)
200–500	46 (41%)	229 (44%)
< 200	27 (24%)	149 (28%)
b. Injection drug users (evaluated to study the association with HCV infection)		
Characteristic	HCV-seronegative (N=70)	HCV-seropositive (N=768)
Age, years (mean ± SD) *	33.0 ± 6.3	40.1 ± 5.9
Race/ethnicity *		
White, non-Hispanic	22 (31%)	132 (17%)
Black, non-Hispanic	30 (43%)	464 (60%)
Hispanic	18 (26%)	172 (22%)
HIV positive *	37 (53%)	646 (84%)
CD4 + count, cells/ mm^3 in HIV-seropositive women		
Median (range)	324 (4 831)	341 (0 1933)
>500	9 (32%)	177 (27%)
200–500	16 (43%)	287 (44%)
< 200	12 (24%)	182 (28%)

*Significantly different (P<0.05) between the two groups, based on the T-test (for continuous data), Mann-Whitney U test (for continuous data with small subgroups), chi-square test (for categorical data), and Fisher's exact test (for categorical data with small subgroups).

†As shown, there was substantial, but not complete, overlap between the injection drug users evaluated to study association with HCV infection, and the HCV-seropositive women evaluated to study association with HCV viremia (e.g., 577 HCV RNA positive and 123 HCV RNA negative women were injection drug users).

Table 3

HLA Alleles and Their Associations with HCV Viremia among HCV-Seropositive Women

Allele*	HCV RNA negative (N=136)	HCV RNA positive (N=622)	PR	95% CI	P-value	PR†	95% CI†	P-value†	P-value‡
a. Alleles identified from the literature as *a priori* associated with HCV viremia									
DQB1*0301	25 (39%)	95 (30%)	1.4	0.9, 2.2	0.15	1.4	0.9, 2.2	0.13	NA
DRB1*0101	17 (15%)	41 (7%)	1.8	1.1, 2.7	0.01	1.7	1.1, 2.6	0.01	NA
DRB1*0301	8 (7%)	99 (18%)	0.4	0.2, 0.7	0.01	0.4	0.2, 0.7	0.01	NA
DRB1*0401	6 (5%)	29 (5%)	1.0	0.4, 1.9	0.98	0.9	0.4, 1.8	0.84	NA
DRB1*1101	18 (15%)	75 (13%)	1.1	0.7, 1.7	0.58	1.3	0.8, 1.9	0.34	NA
DRB1*1501	9 (8%)	68 (12%)	0.7	0.3, 1.2	0.18	0.6	0.3, 1.0	0.10	NA
B*1801	12 (9%)	32 (5%)	1.6	0.9, 2.5	0.07	1.5	0.9, 2.4	0.09	NA
B*2705	7 (5%)	18 (3%)	1.6	0.8, 2.8	0.14	1.5	0.7, 2.6	0.22	NA
B*5701	9 (7%)	18 (3%)	2.0	1.0, 3.2	0.02	2.0	1.0, 3.1	0.02	NA
B*5703	14 (11%)	34 (6%)	1.7	1.0, 2.7	0.02	1.7	1.0, 2.5	0.03	NA
Cw*0102	8 (6%)	15 (3%)	2.0	1.0, 3.3	0.02	1.9	1.0, 3.0	0.03	NA
Cw*0401	35 (27%)	192 (33%)	0.8	0.6, 1.1	0.24	0.8	0.6, 1.2	0.28	NA
b. Exploratory alleles significantly associated with HCV viremia									
DRB1*0701	36 (31%)	107 (19%)	1.7	1.2, 2.3	0.004	1.6	1.1, 2.2	0.01	0.29
DRB1*1302	6 (5%)	87 (16%)	0.3	0.1, 0.7	0.01	0.4	0.1, 0.7	0.01	0.07

PR, prevalence ratio; 95% CI, 95% confidence interval; NA, not applicable.

*The number of women with complete allele information varied by HLA locus (749 for HLA-A, 734 for HLA-B, 719 for HLA-Cw, 380 for HLA-DQA1, 381 for HLA-DQB1, and 675 for HLA-DRB1). Percentages reflect the number of women who were homozygous or heterozygous for the indicated allele amongst those with complete allele information at that locus.

†Estimates are adjusted for race/ethnicity (White non-Hispanic, Black non-Hispanic, Hispanic).

‡Associations between HLA alleles and HCV infection without a high prior probability were corrected for multiple comparisons by permutation resampling.