IL28B Polymorphism Does Not Determine Outcomes of Hepatitis B Virus or HIV Infection

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An *IL28B* haplotype strongly determines the outcome of natural and interferon-α treated hepatitis C virus (HCV) infection. To assess whether the polymorphism marking the haplotype (rs12979860) also affects other interferon-α responsive chronic viral illnesses, namely hepatitis B virus (HBV) and human immunodeficiency virus (HIV) type 1 infections, we genotyped 226 individuals with HBV persistence, 384 with HBV recovery, and 2548 with or at high risk for HIV infection. The C/C genotype of rs12979860 was not associated with HBV recovery (odds ratio, 0.99), resistance to HIV infection (odds ratio, 0.97), or HIV disease progression ($P > .05$). This *IL28B* single-nucleotide polymorphism affects the immune response to HCV but not to HBV or HIV.

IL28B (interferon [IFN] λ3) produces an antiviral state by triggering a cascade through the JAK-STAT pathway that up-regulates the IFN-stimulated genes (ISGs). The effects of IL28B are similar to those of IFN-α and -β; however, IL28B binds to a distinct receptor that may up-regulate a different set of ISGs [1]. This cytokine has been identified as a key modulator of the immune response to hepatitis C virus (HCV), because a single-nucleotide polymorphism (SNP) (rs12979860) upstream of the *IL28B* gene was associated with spontaneous and treatment-induced HCV clearance [2, 3]. The mechanism of how the SNP affects IL28B function has not been elucidated. One clue to the mechanism may come from determining whether the SNP affects the outcome of other chronic viral infections wherein IFN-α and ISGs are important in the host response, such as hepatitis B virus (HBV) and human immunodeficiency virus (HIV) type 1 infection.

After an HBV infection, 90%–95% of adults mount an effective immune response that leads to recovery from infection and development of hepatitis B surface antibodies (anti-HBs). For the remaining 5%–10%, chronic hepatitis B is established, which predisposes those infected to cirrhosis and liver cancer. As with HCV, IFN-α and ISGs are thought to be important in the immune response to HBV, and pegylated IFN-α is used to treat chronic hepatitis B infection. Thus, IL28B may be important in recovery from an HBV infection.

Despite repeated exposure to HIV, a small percentage of persons resist infection, allowing study of the host genetic basis of such protection. Among those who become infected with HIV, the rate of progression to AIDS varies markedly. As with HBV and HCV, IFN-α is active against HIV, probably through stimulation of ISGs [4]. Furthermore, IL28A (IFN-λ2) and IL29 (IFN-λ1), which bind the same receptor as IL28B, inhibit HIV-1 infection of macrophages [5]. Thus, IL28B may have a role in the pathogenesis of HIV infection. Given the varied outcomes after both HBV and HIV infections and the importance of IFN-α and ISGs in these infections, we tested whether the rs12979860 SNP is associated with HBV recovery, resistance to HIV infection, or HIV disease progression.

Methods. The subjects in the HBV cohort were participants in one of the following ongoing parent cohorts: (1) Multicenter AIDS Cohort Study, a study of 5622 men who have sex with men in the United States; (2) AIDS Link to Intravenous Experience, a study of 2921 injection drug users in Baltimore, Maryland; (3) Hemophilia Growth and Development Study, a study of 333 children and adolescents with hemophilia; and (4) Multicenter Hemophilia Cohort Study, a prospectively followed cohort of patients with coagulation disorder from 16 hemophilia treatment centers (for references with cohort descriptions, see [6]).

A nested case-control design was used, in which individuals with a persistent HBV infection were matched to 2 persons from the same cohort with HBV recovery, who were otherwise

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Table 1. rs12979860 and Likelihood of Developing Chronic Hepatitis B Virus (HBV) Infection

HBV genotype	HBV recovery (n = 384)	Persistent HBV infection (n = 226)	Comparison	OR (95% CI)[a]	P
C[b]	489 (63.7)	292 (64.6)	C vs T	1.04 (0.82–1.33)	.75
T/T	52 (13.5)	33 (14.6)	C/C vs T/T	0.90 (0.44–1.85)	.77
C/T	175 (45.6)	94 (41.6)	C/C vs C/T + T/T	0.99 (0.67–1.46)	.95
C/C	157 (40.9)	99 (43.8)	T/T vs C/C + C/T	1.20 (0.69–2.08)	.52

NOTE. Data are no. (%) of participants, unless otherwise indicated.

[a] Odds ratio (OR) for genotypes in Comparison column. ORs >1 were associated with increased likelihood for chronic HBV infection. Conditional ORs are adjusted for hepatitis C virus infection status, *CCR5Δ32*, and cohort. CI, confidence interval.

[b] Data are no. (%) of alleles.

similar with regard to nongenetic factors, as described elsewhere [6]. Subjects were considered persistently HBV infected if they had hepatitis B surface antigen (HBsAg) at 2 visits separated by ⩾6 months. Individuals with HBV recovery were positive for hepatitis B core antibody (anti-HBc) and anti-HBs without HBsAg at 2 time points separated by ⩾6 months.

The HIV cohort came from participants in the same parent cohorts as the HBV study, with the addition of the DC gay cohort, which includes men who have sex with men from Washington, DC, and the San Francisco City Clinic Cohort, which includes men who have sex with men from the San Francisco sexually transmitted disease clinic (for references with cohort descriptions, see [7]). Subjects who were persistently HIV negative despite ongoing high-risk behavior are considered high-risk seronegative (HRSN), as described elsewhere [7]. Subjects who were HIV positive at entry into the parent cohort are seroprevalent subjects, and those who became HIV positive while in the parent study are HIV seroconverters. Informed consent was obtained from all participants. This study was approved by the institutional review boards at participating institutions.

All serum specimens were stored at −70° C before testing. HIV antibody was determined by enzyme immunoassay with Western blot confirmation. HBsAg, anti-HBs, and anti-HBc were measured using commercially available kits according to the manufacturer's specifications (Auszyme, Ausab, and Corzyme, respectively; Abbott Laboratories). HCV antibody and HCV RNA were assayed using commercially available kits according to the manufacturer's specifications, as described elsewhere [2]. HCV infection was defined as positive results for HCV antibody and HCV RNA. Samples were genotyped for the C or T allele at rs12979860 using a predeveloped TaqMan allelic discrimination assay (Applied Biosystems), as described elsewhere [3].

The SNP was in Hardy-Weinberg equilibrium in the HBV and HIV cohorts, as determined by a χ^2 test using 1 degree of freedom. For the HBV study, the Cochran-Mantel-Haenszel test was used for the allelic analysis. For dominant and recessive models and for comparing C/C and T/T, conditional logistic regression analysis was used to adjust for cohort, HCV status, and the presence of a 32–base pair deletion in *CCR5* (*CCR5Δ32*), which is associated with HBV recovery [6]. All analyses were stratified by HIV status and ethnicity.

To determine whether the SNP was associated with resistance to HIV infection, we compared the allele frequency between the HRSN and the HIV-infected subjects (HIV seroconverter and seroprevalent subjects combined), using the Cochran-Mantel-Haenszel test. We tested dominant and recessive models, in addition to comparing C/C with T/T, using logistic regression analysis to adjust for cohort, ethnicity, and HCV status. To determine whether the SNP was associated with HIV disease progression in the HIV seroconverters, Cox proportional hazards models were used to test the following AIDS-related outcomes: CD4 T cell count <200 cells/μL, progression to AIDS according to the 1987 and 1993 Centers for Disease Control definitions [8, 9], and death from an AIDS-related cause.

Results. In the HBV cohort, there were 226 individuals with HBV persistence and 384 with HBV recovery; thus, only 1 matched control was available for 68 subjects. No significant differences were detected between these HBV groups in terms of chronic HCV prevalence (21.5% vs 16.9%). The matched nongenetic factors were also similar (median age, 31 years; 75% white and 20% black; 98% male; 69% HIV positive).

The C allele frequency was significantly higher in white subjects (69.1%) than in black subjects (44.4%) ($P < .001$) and was similar to population frequencies [2]. However, there was no significant difference in C allele frequency between subjects with HBV persistence and those with HBV recovery (64.6% and 63.7%, respectively; $P = .76$) (Table 1). Stratification by ethnicity ($P = .51$ for both blacks and whites) or HIV status ($P = .94$ for HIV-positive, $P = .74$ for HIV-negative) also did not reveal an association with HBV outcome.

Because the homozygous C/C genotype was most strongly protective in HCV, we next compared the frequency of C/C with that of C/T or T/T (dominant model) and the frequency of T/T with that of C/C or C/T (recessive model) in subjects

Table 2. rs12979860 and Likelihood of Acquiring Human Immunodeficiency Virus (HIV) Infection

HIV genotype	HIV positive (n = 1221)	HRSN (n = 291)	Comparison	OR (95% CI)	P
C[a]	1478 (60.5)	346 (59.5)	C vs T	1.05 (0.87–1.26)	.63
T/T	189 (15.5)	50 (17.2)	C/C vs T/T	0.98 (0.59–1.60)	.92
C/T	586 (48.0)	136 (46.7)	C/C vs C/T + T/T	0.97 (0.72–1.31)	.84
C/C	446 (36.5)	105 (36.1)	T/T vs C/C + C/T	1.04 (0.69–1.57)	.86

NOTE. Data are no. (%) of participants, unless otherwise indicated. HIV-positive group includes HIV seroconverters and seroprevalent subjects. Odds ratio (OR) for genotypes in Comparison column. ORs >1 were associated with increased likelihood of acquiring HIV infection. Except for C vs T, ORs are adjusted for race, cohort, and hepatitis C virus infection status. CI, confidence interval. HRSN, high-risk seronegative.

[a] Data are no. (%) of alleles.

with HBV recovery or persistence (Table 1). In the adjusted multivariate analysis, neither model showed an association with HBV recovery, nor did a comparison between subjects with C/C and those with T/T genotype. Stratification of these analyses by race or HIV status (data not shown) did not demonstrate a difference.

The HIV portion of the study included 1512 individuals of whom 291 were HRSN, 1036 were HIV seroconverters, and 185 were HIV seroprevalent. The cohort was 68% white, 26% black, and 6% other ethnicities. The HCV infection status was known for 1323 (87.5%) of the cohort; of those, 46% were HCV infected.

As in the HBV study, the C allele was more frequent in whites than in blacks ($P < .001$), and the frequencies were similar to population frequencies [2]. The C allele frequency did not differ between the HIV-infected and HRSN subjects (Table 2). Neither the C/C genotype nor the adjusted genotype models showed an association with resistance to HIV infection.

The influence of rs12979860 on the rate of progression to AIDS was determined in 1036 HIV seroconverters, as described

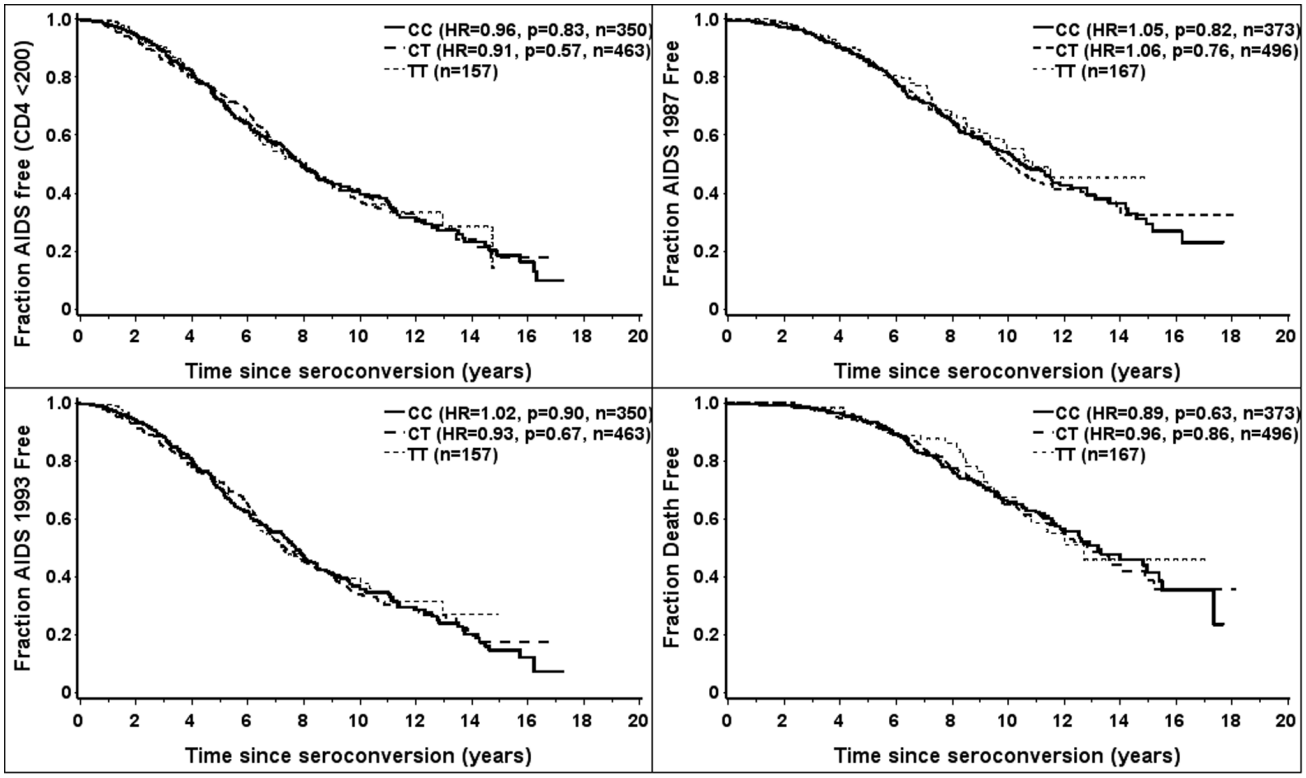

Figure 1. Kaplan-Meier survival curves combining all ethnic groups demonstrate no difference in the time to various outcomes of human immunodeficiency virus (HIV) infection based on rs12979860 genotypes C/C, C/T, T/T in 1036 HIV seroconverters. HR, hazard ratio.

in Methods. The adjusted genotype models did not demonstrate an association of HIV disease progression to the outcomes when examined across ethnic groups (Figure 1) or stratified by ethnic group (data not shown).

Discussion. This study clearly demonstrates that the SNP 4 kilobases upstream of *IL28B* (rs12979860) was not associated with outcomes of 2 common chronic viral infections, namely, HBV recovery and HIV infection and disease progression. Prior studies have shown that this SNP is associated with spontaneous and treatment-induced clearance of HCV and is one of the strongest known genetic associations with any chronic viral infection to date. Thus, even though both IFN-α and IL28B signal through the JAK-STAT pathway, this *IL28B* SNP apparently has a distinct effect on the immune response to HCV.

It is somewhat surprising that there was no association, given that IL28B stimulates ISGs, which play an important role in the immune response to HIV and HBV infections. In HBV transgenic mice, ISGs are a major mechanism of noncytolytic inhibition of HBV replication [10]. Furthermore, in an immortalized murine hepatocyte cell line that replicates HBV infection, exogenous murine IFN-λ2 (IL28A) inhibited HBV replication by >90%, inhibition that was thought to be mediated by up-regulation of ISGs [11]. Studies of nonhuman primate models of HIV infection also demonstrate that ISGs are up-regulated in blood and lymph nodes during acute infection [12].

It is not known how rs1297860 affects the function of IL28B, but presumably it alters the immune function to HCV but not to HBV or HIV. IL28B has interesting characteristics that may explain this finding. In addition to inducing ISG expression, IL28B may activate alternate antiviral pathways, such as the adaptive immune response, which may be more important in HCV. This is supported by a study in which IL28B, when used as a vaccine adjuvant, significantly decreased splenic regulatory T cells, increased splenic and peripheral blood $CD8^+$ T cells, and led to increased antigen-specific perforin induction and degranulation [13]. IL28B may also lead to a different antiviral state than IFN-α, because these 2 molecules up-regulate different ISGs and do so with different kinetics [1]. It was also shown that phosphorylation of STAT1 and STAT2 by IFN-α is slower but more sustained than phosphorylation by IFN-λ, suggesting differences in signal transduction between these 2 IFNs [1].

It is also possible that the IL28B pathway may have a more dominant response to HCV than to HBV or HIV; therefore, the SNP would have a greater effect in HCV infection. In support of this hypothesis, it was shown that IFN-λ1 (IL29) induced transcription of antiviral genes in 2 hepatoma cell lines, but in 1 cell line there was no effect on HBV replication and in the other there was a marginal decrease [14]. Furthermore, in the HBV transgenic mouse, IFN-λ2 (IL28A) was unable to induce intrahepatic ISG expression or to decrease HBV replication [11]. Additional work is needed to determine whether these findings can be extrapolated to IL28B. It is also plausible that the IL28B and IFN-α pathways are synergistic and that the synergism more strongly enhances the immune response to HCV than to HBV or HIV.

One limitation of this study was that it could not determine whether the *IL28B* SNP influences the response of chronic hepatitis B treatment to IFN-α. Another limitation is that IL28B may still have a role in the immune response to hepatitis B or HIV that is not affected by this SNP.

In conclusion, the SNP upstream of *IL28B* that has the strongest genetic association with HCV recovery to date has no effect on HBV recovery, HIV infection, or HIV disease progression. These data are consistent with HIV genome-wide association studies in which the *IL28B* SNPs were not associated with HIV RNA levels [15]. Thus, the effects of this SNP cannot be generalized to these chronic viral infections in which IFN-α is important. Additional studies are needed to understand the mechanisms underlying the beneficial effect of this SNP in HCV infection but not in HIV or HBV infection.

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