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Regulatory polymorphisms in the interleukin-18 promoter are associated with hepatitis C virus clearance

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Abstract

The immune response is critical in determining the outcome of hepatitis C virus (HCV) infection. Interleukin (IL)-18 is a pivotal mediator of Th1/Th2 driven immune response. Two IL-18 promoter polymorphisms (-607 C/A and -137 G/C) and their haplotypes were known to affect the IL-18 expression. We examined the role of these polymorphisms in determining HCV clearance or persistence. Genotyping was performed among African American injecting drug users (IDUs) with HCV clearance (n = 91) and HCV persistence (n = 182), and among European American hemophiliacs mainly infected through plasma transfusion. Among IDUs, *IL18* -607A (Odds ratio [OR], 3.68; 95% confidence interval [CI],1.85–7.34) and *IL18* -137C (OR, 2.33; 95% CI, 1.24–4.36) were significantly associated with HCV clearance. A haplotype carrying -607A and -137C (OR, 4.53, 95% CI, 1.77–11.6) was also strongly associated with viral clearance. No association was found among hemophiliacs. These results suggest that *IL18* promoter polymorphism may affect the outcome of HCV infection in certain groups.

Keywords

Interleukin-18; Hepatitis C virus; Single nucleotide polymorphisms

Hepatitis C virus (HCV) infection is one of the most common chronic viral infections in the world. Approximately 80 to 90% of acutely infected individuals develop persistent infection, a major risk for developing liver cirrhosis and liver cancer, while a small portion of patients (10–20%)clear the virus. The interaction of the host defense system and HCV is not well understood, nor are the factors involved in determining the viral clearance. It is generally believed that the outcome from acute HCV infection is determined by the competence of the

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host innate and adaptive immune responses. HCV clearance is associated with vigorous HCV-specific $CD4^+$ and $CD8^+$ T-cell responses [1–3]. By contrast, lack of a sustained HCV-specific T-cell response is associated with development of persistent infection [3]. It is therefore plausible that host genetic factors that control the immune responses may play a critical role in determining HCV outcomes.

Host genetic factors that have been implicated in HCV infection or persistence mainly include certain alleles in *HLA* class I and II and cytokine genes [4–9]. Cytokines play an indispensable role in regulating immune responses that control HCV clearance or persistence and resulting pathogenesis. Genetic polymorphisms of the cytokine genes *IFNG* (interferon gamma), *TNFα* (tumor necrosis factor-alpha), *IL-10*, and *IL-19/20* (interleukin 10 and 19/20) have been implicated in determining the outcome of HCV infection [7–9].

IL-18, a proinflammatory cytokine, is an important regulator of innate and acquired immune response. IL-18 is involved in both T-helper type 1 (Th1) and Th2 immune responses, depending on the context of the immunological milieu. In the presence of IL-12, IL-18 stimulates *IFNG* expression, promoting Th1-mediated immune response, whereas, without IL-12, IL-18 stimulates Th2 responses. IL18 plays a critical role in the host defense against infection with intracellular microbes, and on the other hand, in inducing autoimmune diseases and propagating inflammatory process [10,11]. IL-18 is significantly upregulated in HCV chronically infected persons compared to healthy persons or asymptomatic carriers and its higher level is correlated with hepatic injury [12–15], indicating a key role of IL-18 in HCV pathogenesis. Two single nucleotide polymorphisms (-607 C/A and -137 G/C) in the promoter region of the *IL-18* gene have repeatedly been found to be associated with the *IL-18* promoter transcription activity [16–19]. Lower promoter activity was observed for the minor alleles -607A and -137C compared to the more common alleles -607C and -137G, respectively. Haplotypes carrying these alleles also correlated with IL-18 levels in PBMC or plasma [16, 19,20]. Moreover, these haplotypes capture the majority of genetic variation of *IL18*, due to the presence of strong linkage disequilibrium among polymorphisms in the gene [21,22]. These SNPs have been implicated in various immune disorders such as Type I diabetes, asthma, and rheumatoid arthritis [21,23,24].

Since IL-18 is an important immune regulator involved in HCV pathogenesis, we hypothesized that promoter polymorphisms known to modulate *IL-18* expression and protein levels may influence the outcome of HCV infection. In this study, the role of the *IL-18* promoter polymorphisms -607 C/A and -137 G/C and their haplotypes was examined in European-American and African-American individuals with well-defined outcomes of HCV clearance or persistence.

METHODS

Study Participants

Study participants were enrolled in three USA-based natural history HIV-1 cohorts: 1) the AIDS Link to the Intravenous Experience (ALIVE) cohort is a community-based cohortof intravenous injection drugusers in Baltimore, MD, enrolled in 1988–1989 [25]; 2) the Hemophilia Growth and Development Study (HGDS) isa multicenter prospective studythat enrolled children withhemophilia whoreceived blood products between1982 and 1983 [26]; 3) The Multicenter Hemophilia Cohort Study (MHCS) isa prospective followed cohort of persons with hemophilia enrolled in 1982–1986 [27]. Informed consent was obtained from all participants and the study was approved by the institutional review boards at all participating institutions.

Study Design

A nested case-control design was used in which one person with viral clearance (case) was matched to two or one persons from the same cohort with viral persistence (controls) [7]. The matching criteria were HIV-1 status, gender, geographic location, and ethnicity. The mean age at study entry was similar between case and control groups (Table 1). In AA, all cases had two matched controls, while in EA, some cases only one matched control was available. All participants tested for HCV in the cohorts who cleared HCV were included as the cases. Prior infection was defined as detection of HCV antibody (anti-HCV) by enzyme immunoassay (EIA) and recombinant immunoblot assay (RIBA). Case subjects with HCV clearance were those had cleared viremia without any HCV-specific treatment, demonstrated by detection of anti-HCV (confirmed by RIBA) and undetectable HCV RNA in the serum for a minimum of 6 months. Persistently infected individuals had detectable anti-HCV and HCV RNA in serum for a minimum of 6 months.

Serologic testing

Participants who tested positive for anti-HCV by second generation Ortho HCV 2.0 EIA (Ortho Diagnostic Systems, Raritan, NJ, USA) had one sample, assessed for HCV RNA by a branched DNA (bDNA) assay (Quantiplex HCV RNA 2.0 assay, Chiron Corporation, Emeryville, CA, USA). Participants with a negative bDNA assay had a second sample tested, separated by a minimum of 6 months from the first, for HCV RNA with the HCV COBAS AMPLICOR system (Roche Diagnostics, Branchburg, NJ, USA) and their antibody status confirmed by a RIBA (RIBA 3.0, Chiron Corporation, Emeryville, CA, USA). The limits of viral detection for the bDNA and the COBAS assays are approximately 200,000 equivalents/ml (30,000 IU/ml) and 100 copies/ml (50 IU/ml), respectively. Only subjects with a negative HCV RNA by COBAS were defined as viral clearance. Participants with two positive bDNA assays were defined as having a persistent HCV infection. Individuals with a negative bDNA and a positive COBAS were not included in this study. HIV-1 testing was carried out by EIA and positive specimens were confirmed by Western blot, as previous described [25–27].

Genotyping

SNPs at position -607C/A (dbSNP accession number, rs1946518) and -137G/C (rs187238) in the promoter region of *IL-18* gene were genotyped by PCR-restriction fragment length polymorphism (PCR-RFLP) assays. The primers were designed based on the GenBank sequence AB015961. The -607 site was amplified using primers 5′-ttctgttgcagaaagtgtaaaattTt-3′ and 5′-aaaggatagttgatacaggccatt-3′, and the -137 site was amplified using primers 5′-tgcttctaatggactaaggaggtg-3′ and 5′-cttcttttaatgtaatatcactattttcatgaGa-3′, respectively (The capitalized nucleotides were artificially introduced to create the restriction enzyme site). PCR reactions were performed in a final volume of 20 μl consisting of 50mM KCL, 10mM Tris-HCL (pH 8.3), 2.5 (for -607) or 3.5 (for -137) mM $MgCl_2$, 0.25 mM dNTP, 25 ng DNA, 1.25 U TaqGold polymerase (Applied Biosystems, Foster City, CA) and 0.20 μM of each forward and reverse primer. At the first PCR cycling step, denaturation at 95°C for 10 min was performed. This was followed by 35 cycles of 94°C for 30s, 58°C for 30s and 72°C for 45s; finally 72°C for 7 min. The PCR products were digested with respective restriction enzymes (New England Biolabs, Beverly, MA) overnight and then separated on 4% agarose gels. For the -607 site, the product sizes digested with Dra I is 154 bp for the C allele and 125 and 28 bp for the A allele. For the -137 site, the product sizes digested with Bgl II is 105 and 36 bp for the G allele and 141 bp for the C allele. The samples from cases and controls were randomly distributed in the plates. Two water controls were included on each 96-well plate. About 70 % of all samples were independently genotyped twice. Persons performing genotyping were blinded to the clinical data. The

genotypes obtained were free of water contamination or of inconsistencies between duplicate DNA samples.

Statistical Analyses

Statistical testing was conducted using the SAS package v9.1 (SAS Institute Inc., Cary, NC). Haplotype frequencies and linkage disequilibrium estimates between the two SNPs were evaluated by a maximum likelihood method using the expectation maximization algorithm [28]. For the SNP and haplotype analyses, a conditional logistic regression was used for the matched case-control comparison for the dominant genetic model, comparing those carrying one or two minor alleles to those homozygous for the major frequency allele. The association with HCV viral load at study entry was evaluated by the Student's t test. Odds ratios (ORs) and confidence intervals (CIs) were calculated. OR > 1 indicates a protective association with HCV clearance. The *P* values are two-sided.

RESULTS

To determine the effect of IL-18 on HCV clearance, we examined the two *IL-18* promoter SNPs -607 and -137 in two case-control groups, consisting of either African Americans (91 HCV clearance cases and 182 chronically infected controls) and European Americans (106 clearance cases and 192 chronically infected controls) (Table 1). Since the allele frequencies of these two SNPs differ in the two populations, the analysis was performed separately for AA and EA. Genotype frequencies for each SNP conformed to Hardy-Weinberg expectations in the control groups.

In AA, both promoter SNPs -607 C/A and -137 G/C showed differences in frequency distributions between HCV clearance and persistence groups (Table 2). The -607A allele occurred more frequently in the clearance group (43.9%) than in the persistence group (33.5%). The -137C allele was also more frequently observed in the clearance group (26.9%) than in the persistence group (19.5%). In a dominant genetic model, the genotypes carrying one or two copies of -607A (OR = 2.92, 95% CI, 1.59–5.36; *P* = 0.0005) or -137C (OR = 2.09, 95% CI, 1.20–3.65; *P* = 0.009) were associated with clearance of HCV infection. In EA, genotypes containing these variant alleles did not differ in frequency distribution between the two groups. Since HBsAg was more common in HCV clearance than in persistence group (Table 1), the HBsAg status was adjusted for in the logistic regression analysis. After adjustment for HBsAg status, the effects of -607A (OR = 3.68, 95% CI, 1.85–7.34, P = 0.0002) or -137C (OR = 2.33, 95% CI, 1.24–4.36, P = 0.024) became stronger (Table 2).

As a means of validation of the observed frequencies, we checked the allele frequency of IL-18 SNPs in the general population. Among 48 AA and 40 EA chromosomes genotyped in the coriell human variation panel available from dbSNP database, the allele frequency of -607A is 0.354 and 0.425 and that of -137 is 0.15 and 0.30, respectively. The frequencies in this unselected population are mostly similar to the observed in the HCV persistent group, consistent with the expectation that HCV persistence is much more common than clearance.

Haplotypes formed by variants -607 C/A and -137 G/C were reconstructed by the expectation maximization method. A near-complete linkage disequilibrium (LD) exists between these two variants ($D' = 0.99$, $r^2 = 0.49$). Three 2-locus haplotypes (CG, AC and AG) were present in both clearance and persistence groups (Table 3). Compared to the reference homozygous GC haplotype, AG and AC haplotypes were elevated in the HCV clearance group. AC was found to be strongly associated with HCV clearance (OR = 3.15, 95% CI, 1.45–6.87; *P* = 0.004). AG was also associated with HCV clearance with marginal significance (OR = 2.50, 95% CI, 1.04–6.04; *P* = 0.04). With adjustment of HBsAg status, the effect of AC became stronger (OR = 4.53, 95% CI, 1.77–11.6, P = 0.0016), while the effect of AG became nonsignificant (OR =

2.48, 95% CI, 0.99–6.23, P = 0.054). These results suggest that haplotype AC, composited by -607A and -137C, affords the major effect on HCV clearance. Nevertheless, as both of AG and AC haplotypes carry -607A, -607A appears to have a broader effect than -137C.

In EA, complete LD between two variants was observed (D′= 1, $r^2 = 0.54$). All three haplotypes in EA were similarly distributed between clearance and persistence groups. With adjustment of HBsAg, OR of AC or AG haplotypes was 1.23 or 1.01 (P > 0.5), respectively, in the same direction as in AA (Table 3).

To assess the potential confounding effect of HIV-1 infection on HCV clearance, analyses in AA were also done separately for HIV-1 positive and negative groups. The SNP effects on HCV clearance were similar in HIV-1 positives (-607: OR = 3.40, CI, 1.23–9.41; -137: OR =2.03, CI, 0.87–4.77) and in HIV-1 negatives (-607: OR = 2.67, CI, 1.25–5.71; -137: OR = 2.13, CI, 1.02–4.46), with broadly overlapped 95% CIs. Similar results were observed for the haplotypes (haplotype AC: OR = 3.96, CI, 1.07–14.61; OR = 2.75, CI, 1.04–7.29; haplotype AG: OR =2.26, CI, 0.54–9.37; OR = 2.57, CI, 0.86–7.67; HIV-1 positives or negatives, respectively). These explanatory analyses indicate that the observed effects were not confounded or influenced by HIV-1 infection.

The potential impact of *IL-18* SNPs on HCV viral load was assessed in the HCV persistence control group. In AA, the HCV RNA level at study entry was 6.85 ± 0.72 (mean ± standard deviation) $\log_{10}$ copies/ml in the referent homozygous CG haplotype group, while was nonsignificantly slightly lower in the haplotype AC (6.75 ± 0.81, $P = 0.44$) or AG (6.68 ± 0.87, $P = 0.26$) groups, respectively. In EA controls, the HCV RNA level was measured at 6.44 ± 0.74, 6.38 ± 0.73, 6.53 ± 0.75 $\log_{10}$ copies/ml in these 3 groups (P >0.5), respectively.

DISCUSSION

Clinical outcomes of hepatitis C virus infection are determined by the interplay among host immune response, viral, and environmental factors. By comparing patients who either had persistent HCV infection or had cleared HCV infection, the present study demonstrated that two functional promoter variants (-607 C/A and -137 G/C) in the *IL-18* gene were associated with HCV clearance of HCV infection in AA but not EA. A haplotype (AC) carrying both -607A and -137C was strongly associated with HCV clearance (OR, 4.53). This finding points to a critical role of IL-18 in determining the outcome of HCV infection.

From individual SNP analysis, both -607A and -137C were associated with clearance of HCV infection, with the effect of -607A being stronger. Among three haplotypes observed, haplotype AC, was significantly associated with HCV clearance, while AG showed a tendency of association with clearance, also suggesting a broader effect of -607A than -137C. Whether the -137C allele confers an independent effect is difficult to know since -137C is tracking -607A.

Promoter SNPs -607 and -137 modulate *IL-18* gene expression through alteration of nuclear factor binding site. A change from C to A at site -607 disrupts a binding site of cAMP-responsible element (CRE), which mediates transcriptional activation in response to cAMP [29], resulting in low IL-18 production. The -137 G/C SNP is located on the binding sites of GATA-3, H4TF-1 and hepatocyte nuclear factor-3beta (HNF-3b), a liver-enriched transcription factor [30,31], suggesting potential differential regulation of -137 binding site by H4TF-1 in hepatocytes. Zhou et al. demonstrated that the promoter sequence with haplotype AG exhibits lower promoter activity than that with CG and that -607A was associated with lower serum IL-18 levels than -607C [19]. Monocytes from -137C carriers produce a lower amount of IL-18 than that from -137G carriers [18]. Giedraitis found that haplotypes CG and AG had higher transcription activity than haplotype AC. Based on these promoter activity studies, the more common alleles, -607A and -137C or haplotype AC associated with HCV

clearance would be predicted to exhibit low promoter activity. The AC haplotype has been associated with atopic eczema and asthma, which are classified as Th2-dominant diseases [21,32,33]. Our finding of the AC haplotype association with viral clearance suggests that the same genetic variation may increase risk to one group of disorders but protect against other types of diseases such as infection.

The genetic epidemiological results point to an important role of IL-18 in the pathogenesis of HCV infection. The mechanisms may involve both the immunoregulation function and the inflammation inducing effect of IL-18. It is believed that the dynamics of TH1/Th2 response determine the outcome of HCV infection and IL-18 is an important mediator of TH1/TH2 balance. Thus, it is plausible to reason that down-regulation of IL-18 by these promoter SNPs help achieve an optimal balance of TH1/TH2 in favor of HCV clearance. There is evidence that low production of IL-18 contributes to the viral clearance [34]. Through studying mice after pulmonary infection with influenza A virus, *IL-18* deficiency was found to be associated with accelerated viral clearance of influenza A virus and enhanced activation of CD4+ T-cells [34]. Furthermore, by comparing patients with chronic hepatitis B and normal controls, haplotype AC of *IL-18* was found to be associated with protection against HBV infection [35]. This corroborates our finding of haplotype AC association with HCV clearance and suggests a common role of these *IL-18* SNPs in viral hepatitis. A related issue is whether IL-18 SNPs affect HCV viral load. A nonsignificant reduction of 0.1 or 0.17 $\log_{10}$ copies/ml HCV RNA was observed in the AC and AG haplotype groups compared to CG haplotype group in the AA patients with HCV persistence, suggesting a possible HCV inhibition effect. However, as the duration of HCV infection of these patients is unknown, further studies on this aspect are needed to draw any conclusion.

On the other hand, IL-18, as a proinflammatory cytokine, directly induces inflammation in liver cells. In animal experiments, IL-18 along with TNF-α have been identified as essential mediators of T cell-mediated liver injury [36]. The simultaneous neutralization of TNF-α and IL-18 fully protected the mice against hepatic damage induced by exotoxin [37]. A neutralizing antibody to IL-18 completely prevented LPS-induced hepatic damage [38]. In clinical studies, IL-18 was found to be upregulated in chronic hepatitis C and related with hepatic injury [12–15]. A lower level of IL-18 production modulated by -607A may help protect hepatocytes from persistent inflammation induced by HCV.

In this study, the impact of IL-18 variants on HCV clearance was only observed in AA but not in EA. In a recent reviewing of meta-analyses of 43 validated genetic associations, Ioannidis et al found that 15 analyses achieved statistical significance in two different racial groups. Regardless of whether achieving significance, in 32 studies the genetic effects, as estimated by odds ratio, were in the same direction across different racial groups [39]. In this study, the lack of positive association in EA may be related with the initial HCV infection dosage, immune response or genetic background. More than 90% of the AA were exposed to HIV-1 through needle sharing and presumably received low-dose HCV inocula, whereas more than 90% of EA were hemophiliacs exposed to high-dose HCV inocula by plasma infusion. It is possible that high-dose inocula of HCV may overwhelm the host's immune system such that the beneficial role of genetic *IL-18* modulation is minimized. Alternatively, it is possible that low-dose inocula leads to a more efficient immune response. Khakoo et al. reported a similar differential effect for HLA-KIR genotypes, where KIR2DL3:HLA-C1 homozygosity was strongly protective in patients infected through needle injection but not in patients infected through blood transfusions [4,40]. The distinctive genetic effects may also be due to the difference in genetic background. It is well known that AA and EA differ in the rate of HCV clearance and treatment response [41,42]. Recent studies presented biological evidence that may explain the differential epidemiological effects observed in different racial/ethnic groups. Through immunologic analysis of a large number of patients who resolved HCV infection or

had persistent HCV infection, EA and AA were found to differ in their CD4 T-cell responsiveness to HCV antigens [41]. The ability of HCV proteins to induce IL-10 production differed in mononuclear cells from AA or EA donors carrying the same IL-10 promoter variants that predict IL-10 levels [43]. Thus, it is not unlikely that the production of IL-18 and induction of immune response from the same genetic variation may differ among groups with different genetic backgrounds. Future replication in other independent samples and evaluation of functional relevance will help address these issues.

The strengths of this study include the use of nested case-control design of cases and controls selected from well-characterized longitudinal cohorts, controlling for factors known to influence outcomes by case-control matching on age and HIV-1 status, as well as the robust association with the clearance phenotype of SNPs with known function. There are limitations to the study. First, the positive association was found only in the African American group. Second, we cannot exclude the possibility that the -607 and -137 effects are tracking other alleles in the *IL-18* gene, since there is strong LD within the gene.

In summary, this study suggests that two promoter polymorphisms in the *IL-18* gene influence the outcome of HCV infection, with the -607A and -137C alleles and their haplotype favoring HCV clearance. These findings highlight a critical role of IL-18 in resolving HCV infection. Since these SNPs are quite common, the impact on the HCV epidemic may be significant. These two alleles have been associated with lower IL-18 expression level, suggesting that higher level of IL-18 promotes persistence of HCV infection. If these results are validated, IL-18 could be considered as a target for therapeutics.

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Table 1

Demographic characteristics of case patients and control subjects, stratified by cohort and ethnicity.

	HCV Clearance	HCV Persistence
ALIVE:		
Number	86	172
Mean age (years)	34.5	34.9
Male (%)	70.3	70
AA (%)[a]	88.5	89
EA (%)[a]	10.3	5.8
HIV-1 (% positive)	40	40
HBsAg (% positive)	7.4	3.9
MHCS:		
Number	89	172
Mean age (years)[b]	21.9	26.1
Male (%)	98	98
AA (%)[a]	13.3	14.5
EA (%)[a]	75.6	74.4
HIV-1 (% positive)	30	30
HBsAg (% positive)[b]	16.9	6.6
ALIVE and MHCS, AA:		
Number	91	182
Mean age (years)	32.6	32.9
Male (%)	70	70
HIV-1 (% positive)	39	39
HBsAg (% positive)[b]	11.8	4.1
ALIVE and MHCS, EA:		
Number	106	192
Mean age (years)	20	23
Male (%)	95	96
HIV-1 (% positive)	32	32
HBsAg (% positive)[b]	18.1	5.4

Note. AA, African American; ALIVE, AIDS Link to the Intravenous Experience; EA, European American; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HGDS, Hemophilia Growth and Development study; MHCS, Multicenter Hemophilia Cohort Study.

[a] Other ethnic groups comprise the percentage needed to reach 100%.

[b] $P<0.05$.

Table 2

Comparison of IL-18 promoter polymorphisms between persons with HCV clearance and persistence*

Group	SNP	Allele Frequency		HBsAg adjustment	OR	95% CI	*P*
		Clearance	Persistence				
AA		n = 182	n = 364				
	-607A	0.439	0.335	Unadjusted	2.92	1.59–5.36	0.0005
				Adjusted	3.68	1.85–7.34	0.0002
	-137C	0.269	0.195	Unadjusted	2.09	1.20–3.65	0.009
				Adjusted	2.33	1.24–4.36	0.024
EA		n = 212	n = 384				
	-607A	0.41	0.42	Unadjusted	0.89	0.54–1.47	0.64
				Adjusted	1.17	0.67–2.05	0.57
	-137C	0.276	0.29	Unadjusted	0.89	0.55–1.45	0.65
				Adjusted	1.08	0.64–1.83	0.78

Note. AA, African American; EA, European American; n, number of alleles.

*OR is the odds ratio of having HCV cleared compared to having persistent HCV infection when carrying one or two copies of -607A or -137C. Analyses were performed using conditional logistic regression, with or without addjustment of HBsAg status.

Table 3

Haplotype distribution of two *IL-18* promoter polymorphisms in persons with HCV clearance and persistence.

Haplotype	-607C/A	-137G/C	Haplotype Frequency		HBsAg adjustment	OR[a]	95% CI	*P*
			Clearance	Persistence				
AA								
CG	C	G	0.561	0.663		1		
AC	A	C	0.267	0.193	unadjusted	3.15	1.45–6.87	0.0038
					adjusted	4.53	1.77–11.6	0.0016
AG	A	G	0.172	0.144	unadjusted	2.5	1.04–6.04	0.038
EA					adjusted	2.48	0.99–6.23	0.054
CG	C	G	0.580	0.575		1		
AC	A	C	0.277	0.288	unadjusted	0.94	0.51–1.73	0.84
					adjusted	1.23	0.69–2.41	0.55
AG	A	G	0.134	0.136	unadjusted	0.9	0.37–2.22	0.82
					adjusted	1.01	0.40–2.54	0.98

Note. AA, African American; EA, European American; OR, Odds Ratio; CI, Confidence interval.

[a]OR is the odds ratio of having HCV cleared compared to having persistent HCV infection for the specific haplotype; OR and CI were calculated for each of two haplotypes (AC and AG) excluding the other haplotype, in relative to the homozygous CG haplotype, using conditional logistic regression, with or without adjustment for HBsAg status.