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Genetic Variation in *CLDN1* and Susceptibility to Hepatitis C Virus Infection

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Abstract

Claudin-1 is a recently discovered co-receptor for hepatitis C virus (HCV) that is required for late-stage binding of the virus. Because variants in the gene that encodes claudin-1 (*CLDN1*) could play a role in HCV infection, we conducted a 'whole gene association study' among injection drug users (IDUs) to examine whether *CLDN1* genetic variants were associated with the risk of HCV infection or with viral clearance. In a cross sectional study, we examined genotype results for 50 single nucleotide polymorphisms (SNPs) across the *CLDN1* gene region, comparing genotypes among participants with chronic HCV (n=658) to those in IDUs who had cleared HCV (n=199) or remained HCV-uninfected (n=68). Analyses were controlled for racial ancestry (African American or European American) by stratification and logistic regression modeling. We found that participants who remained uninfected more often carried *CLDN1* promoter region SNPs *-15312C* [odds ratio (OR), 1.72; 95% confidence interval (CI) 1.00-2.94; p=0.048], *-7153A* (OR, 2.13; 95% CI, 1.25-3.62; p=0.006) and *-5414C* (OR, 1.78; 95% CI, 1.06-3.00; p=0.03). HCV-uninfected participants less often carried *CLDN1 IVS1-2983C* (OR, 0.55; 95% CI, 0.31-0.97; p=0.04), which lies in intron 1. *CLDN1 -15312C*, *-7153A* and *-5414C* formed a haplotype in both the African American and European American participants and a haplotype analysis supported the association of *CLDN1 -7153A* in the HCV-uninfected participants. The analyses of HCV clearance revealed no associations with any SNP. These results indicate that genetic variants in regulatory regions of *CLDN1* may alter susceptibility to HCV infection.

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Conflicts of interest

The authors report no conflicts of interest.

Keywords

claudin-1; epidemiology; genetic; susceptibility; viral receptor

Introduction

Transmission of hepatitis C virus (HCV) is primarily through exposure to infected blood. With repeated exposure, as can occur among injection drug users (IDUs), most people become infected with HCV and 75-80% of HCV-infected people develop chronic infection. In the United States and in many other parts of the world, chronic HCV infection is a leading cause of end stage liver disease, hepatocellular carcinoma and liver transplantation. (1-3) An effective HCV vaccine is not yet available and current therapy for chronic HCV infection results in a sustained virological response in only about half of patients (4). A better understanding of HCV infection is required, therefore, to decrease the incidence of chronic liver disease and hepatocellular carcinoma.

Cellular entry is a potential point of intervention in viral infections, but entry of HCV into hepatocytes is complex and incompletely understood. Scavenger receptor class B member I (SR-BI) and CD81 appear to be required, but not sufficient, for HCV entry. (5) Recently, claudin-1 was discovered as an additional co-receptor required for late-stage binding of HCV (6). Claudin proteins are important components of tight junctions(7) and claudin-1 is highly expressed in liver (8). Mutations introduced into first extra cellular loop of this protein, which is critical for virus entry, make cell-lines less permissive to HCV infection (6).

In vitro evidence for a key role of claudin-1 in HCV infection has focused our attention on its gene, *CLDN1*. Previous studies of this gene have examined its role in cancer (8,9) and the neonatal sclerosing cholangitis syndrome. Two *CLDN1* variants that result in complete loss of function of claudin-1 have been linked to this rare syndrome, but each appears to be very uncommon and geographically isolated (10,11). Because more subtle, but more common, *CLDN1* variants found in coding or gene regulatory regions could play a role in HCV infection, we used a set of assays targeting single nucleotide polymorphisms (SNPs) to interrogate the *CLDN1* gene region for variants that alter HCV susceptibility or clearance among IDUs who were at high risk of infection with HCV.

Materials and methods

Subjects

This study was conducted in IDUs who enrolled in the Urban Health Study (UHS) between 1998 and 2000. Every month UHS investigators recruited IDUs from street settings in one of six inner-city San Francisco Bay area neighborhoods that were visited in rotation (12). Persons 18 years of age or older were eligible for enrollment if they either had injected illicit drugs within the past 30 days or previously participated in the UHS. New participants were screened for visible signs of recent or chronic injection (i.e., recent venipuncture sites or scars). Trained staff obtained informed consent, interviewed participants using a standardized instrument, counseled them on reducing infection risks, and referred them to appropriate medical and social services. Participants were asked about sociodemographic factors, including race/ethnicity, and their injection drug history, including age at first injection. The questionnaire did not inquire about treatment for HCV infection, but because these IDUs had very limited access to anti–viral treatment for HCV infection at the time of subject recruitment (1,13) it is highly likely that the HCV seropositive, HCV RNA negative subjects in this study had recovered spontaneously. Blood samples were collected by a

trained and certified phlebotomist. Study participants received modest monetary compensation. Further details about UHS are provided elsewhere (12,14). Study procedures were approved by the Committee on Human Subjects Research at University of California, San Francisco and an Institutional Review Board of the National Cancer Institute.

The present study was restricted to subjects who described themselves as either white, but not Hispanic, or black. Participants of other racial/ethnic groups were too few in number to permit a meaningful analysis. We used this information to control for potential confounding caused by genetic differences between participants of different ancestry (i.e., population stratification). Because of our focus on genetic ancestry, we refer to participants herein as European Americans or African Americans consistent with other recent genetic studies. (15,16) Subjects were divided into three outcome groups based on the results of HCV antibody and HCV RNA testing: 1) negative for both antibody and RNA (uninfected); 2) positive for both antibody and RNA (chronic); 3) positive for antibody and negative for RNA (cleared). Participants were tested for HCV antibody and RNA only once. To increase the likelihood that HCV-uninfected participants had been exposed to HCV we restricted this group to subjects who had used drugs ≥10 years. All subjects with cleared infection were included in the study and selected subjects with chronic HCV were frequency matched (maximum 4:1 ratio) to those with cleared HCV on the basis of self-reported ethnic background and duration of IDU. We assessed possible repeat enrollment by comparing demographic information and through DNA testing (described below).

To identify rare variants that might have protected the uninfected IDUs from infection with HCV we sequenced the four exons of *CLDN1* and the flanking intronic regions for 25 IDUs who were negative for both HCV antibody and HCV RNA and who had injected drugs for at least 10 years. Sequence analysis of 50 chromosomes has a 99% probability of discovering a variant which will have an estimated minor allele frequency (MAF) greater than 5% (17). For comparison, we also sequenced this region in the SNP500Cancer population, which consists of 102 individuals of four self-described ethnic groups in the United States (18) [Bekker et al. in press], as well as in 50 demographically matched UHS participants with chronic or cleared infection.

Viral Assays

We tested for HCV antibodies by HCV version 3.0 ELISA Test System (Ortho-Clinical Diagnostics, Raritan, NJ). Participants who were negative by HCV EIA and HCV RNA were considered to be uninfected with HCV. Those who were positive by HCV EIA were considered to have been infected with HCV and were tested for HCV viremia using a branched-chain DNA assay [VERSANT® HCV RNA 3.0 Assay (bDNA), Bayer-Diagnostics, Tarrytown, NY; analytical sensitivity, 2.5×103 copies/ml].

We classified each participant's HBV infection status based on serologic testing for antibody to hepatitis B core antigen (HBc ELISA Test System, Ortho-Clinical Diagnostics, Raritan, NJ), hepatitis B surface antigen (Genetic Systems HBsAg EIA version 3.0, Bio-Rad Laboratories, Redmond, WA), IgM anti-HBc (ETI-Core-IgMK plus, DiaSorin, Stillwater, MN) and antibody to HBsAg (ETI-AB-AUK plus for anti-HBs, DiaSorin, Stillwater, MN) as previously described (Tseng, 2007). Plasma from each participant was tested for antibodies to human immunodeficiency virus type 1 (HIV-1) by Genetic Systems™ rLAV EIA (Bio-Rad Laboratories, Redmond, WA) and reactive samples were confirmed by HIV-1 Western Blot.

Genotyping

We defined the gene region for *CLDN1* as beginning 35 kb upstream of the transcription start site and ending 10 kb downstream of the polyA tail. To efficiently 'tag' *CLDN1* haplotypes ($r^2 \geq 0.8$) for populations of European and African origin, we used the multi-population approach of Tagzilla software (http://tagzilla.nci.nih.gov/) to select 63 SNPs with a MAF≥0.05 from the International HapMap Project phase II database (19,20). In addition, resequencing the *CLDN1* region in the SNP500 population (18) yielded four additional SNPs with a MAF ≥0.05 [Bekker et al., submitted]. Of these 67 SNPs, 12 either could not be designed or failed genotyping. Thus, study subjects were successfully genotyped for 55 SNPs (Table 1). The nomenclature we used to describe genetic sequence variations was adapted from Antonarakis SE and the Nomenclature Working Group and a description can be found at: http://snp500cancer.nci.nih.gov/terms_snp_region.cfm.

We extracted genomic DNA from cryopreserved lymphocytes using a modified salt precipitation-extraction method (Gentra Systems, Minneapolis, MN) or from granulocytes using a silica membrane binding method (Qiagen Inc., Valencia, CA), based on availability of specimens. Genotyping was performed at the NCI Core Genotyping Facility using the 5′ nuclease allelic discrimination (TaqMan) assay on an Applied Biosystems (ABI) PRISM 7900HT Sequence Detection System. Details for these assays are available at: http://snp500cancer.nci.nih.gov/terms_snp_region.cfm. The assay completion rate ranged from 93.2% to 98.2% with a median of 94.4%.

We used the AmpFℓSTR® Identifiler® PCR Amplification Kit (Applied Biosystems, Foster City, CA) to type 15 tetranucleotide short tandem repeat loci and the Amelogenin locus. These results were used to verify duplicate specimens that had been included for quality control purposes, as well as to identify subjects who were unintentionally included in duplicate (due to multiple anonymous enrollments). Results from such duplicate specimens were eliminated from the database.

DNA Sequencing

Genomic DNA was sequenced from DNA of the 25 HCV-uninfected IDUs who are described above. We used Primer3 software (http://fokker.wi.mit.edu) to design four primer pairs to sequence the four exons of *CLDN1* and the immediate flanking intronic regions. Bi-directional sequence analysis was performed using the Dye Terminator method (ABI Perkin-Elmer, Foster City, CA). Sequence tracings were analyzed with Sequence Analysis 3.7 software and Seqscape software version 2.5 (Applied Biosystems).

Statistical Analyses

Genotype and Haplotype Data

Genotype results are presented for all subjects with DNA available for all assays. All analyses were conducted separately among European American and African American participants. We examined departure from Hardy-Weinberg proportions for each locus among the participants who were positive for HCV RNA and then evaluated differences in genotype frequencies by HCV status. We estimated the odds ratio (OR) and corresponding 95% confidence interval (CI) with logistic regression models that used subjects with chronic infection as the referent group. The Pearson χ^2 test was used to calculate p-values; $p \leq 0.05$ (two-tailed) was considered statistically significant.

For selected linked variants, we used Haplo Stats software (version 1.3.8; http://mayoresearch.mayo.edu/mayo/research/schaid_lab/software.cfm) to infer haplotype frequencies and calculate measures of association. Haplotype frequencies were estimated

from the genotype data by an expectation-maximization progressive insertion algorithm. Using the most common haplotype as the referent, ORs for minor haplotypes (adjusted for racial ancestry), as well as accompanying 95% CIs and p-values, were estimated with generalized linear models that assume a co-dominant (additive) relationship.

Pairwise Linkage Disequilibrium

In order to compare the patterns of pair-wise linkage disequilibrium among SNPs genotyped as a part of this study, the coefficient of correlation (r^2) between all SNPs was estimated for African-Americans and Caucasians, separately, using the GLU software package (http://cgf.nci.nih.gov/development/tooldev.html).

Results

Descriptive Data

The study population is described in Table 2. For subjects of either African American or European American background, age was very similar for participants with chronic or cleared infection, but HCV-uninfected IDUs were younger and had used injection drugs for fewer years than other subjects. Regarding recent drug use, the percentage of participants who reported having shared injection equipment during the 30 days prior to enrollment was similar for participants with chronic infection (22.9%) or cleared infection (20.7%) or those who were HCV-uninfected (23.5%).

Males were predominant in all six groups, but especially among the HCV-uninfected participants. UHS participants with chronic HCV were less likely to have chronic HBV than those with cleared HCV infection (21) and HCV–uninfected participants were considerably less likely to have ever been infected with HBV (African Americans, 35.9%; European Americans, 27.6%). The HB vaccination rate among HCV-uninfected participants was 7.7% among African Americans and 3.5% among European Americans. Among African American participants, HIV infection rates were similar among those with chronic (12.8%) or cleared (13.3%) infection, but lower among the uninfected (5.1%). Among the European Americans, HIV infection was lower among IDUs with cleared infection (3.7%) than those with chronic infection (11.2%) or those who were uninfected with HCV (6.9%).

Genotype Results

Among the 55 *CLDN1* variants (Table 1), we found departure from Hardy-Weinberg proportions among the participants with chronic HCV infection for four SNPs, three among African Americans (*-21778T>A*; p<0.0001; *-15054G>A*, p=0.01; *IVS3-426C>A*, p=0.005) and one among European Americans (*IVS1+4405T>C*, p=0.005). In addition, one SNP (*-8927A>C*) had a MAF <0.05 for both African Americans and European Americans UHS participants with chronic HCV infection. None of these SNPs were associated with HCV outcomes (data not shown). Our statistical analysis focused on the remaining 50 SNPs, of which 47 had a MAF ≥5% among African Americans and 44 had a MAF ≥5% among European Americans.

HCV-Uninfected Participants

For African American participants, complete genotype results comparing HCV-uninfected participants to those with chronic HCV infection are found in Supplemental Table 1. Among African Americans, the frequency of three SNPs differed between uninfected participants and those with chronic HCV infection (Table 3). Assuming a dominant genetic model, HCV-uninfected African American IDUs more often carried *CLDN1 -7153A* (OR, 2.23; 95% CI, 1.13-4.39; p=0.02] and *CLDN1 -5414C* (OR, 2.41; 95% CI, 1.18-4.91 p=0.02) and less often carried *CLDN1 IVS1-2983C* (OR, 0.27; 95% CI, 0.09-0.79; p, 0.02). *CLDN1*

-7153A is linked with *CLDN1 -5414C* (r^2=0.73), as well as with *CLDN1* -24218 (r^2=0.63) and -15312 (r^2=0.87) (Figure 1a). *CLDN1* -24218C and *CLDN1*-15312C were also more common among African American HCV-uninfected participants (OR, 1.51 and 1.59, respectively; Table 3), but these results were not statistically significant. In a co-dominant (i.e., per allelic copy) genetic model, the OR for *CLDN1 -7153A* was 1.83/copy (95% CI, 1.06-3.17; p=0.03) and for *CLDN1 -5414C* the OR was 1.97/copy (95% CI, 1.18-3.29; p=0.01). The data were too sparse to determine which genetic model fit the data better.

Figure 1b shows the linkage disequilibrium pattern among the UHS participants of European origin. In this sub-population, *CLDN1 -7153A* was strongly linked with -15312 (r^2=1.00) and *CLDN1* -24218 (r^2=0.93), and more weakly with *CLDN1 -5414C* (r^2=0.58). Among the European-American IDUs, those who were HCV-uninfected carried *CLDN1*-24218C, -15312C and -7153A about twice as frequently as those with chronic HCV infection (Table 3), but these differences was not statistically significant (p=0.1 for each comparison. In contrast to the finding among African-American participants, among IDUs of European ancestry *CLDN1 IVS1-2983C* was not less frequent among HCV-uninfected participants (OR, 0.89; 95% CI, 0.39-2.01; p=0.77) and there were no other notable genotype results among HCV-uninfected European American participants (Supplemental Table 2).

Because the odds ratios for the associations with the linked SNPs *CLDN1 -24218C, -15312C, -7153A* and *-5414C* were consistent among HCV-uninfected participants of both ancestral groups, we combined these data in a logistic regression analysis that controlled for ancestry (Table 3). A strong association was found for *CLDN1 -7153A* (OR, 2.13; 95% CI, 1.25-3.62; p=0.006) and there were also statistically significant associations with *CLDN1 -15312C* (OR, 1.72; 95% CI, 1.003-2.94; p= 0.048) and *CLDN1 -5414C* (OR, 0.78; 95% CI, 1.06-3.00; p=0.03). The association with *CLDN1 -24218C* (OR, 1.7; 95% CI, 0.99-2.91) was of borderline statistical significance (p=0.05). Logistic regression models that controlled for gender, age, HBV infection and HIV-1 infection status in addition to ancestry yielded ORs that did not differ meaningfully from those adjusted for ancestry alone. For *CLDN1 -7153A*, the fully adjusted model yielded an OR of 2.02 (compared to 2.13 in the ancestry-adjusted model) and full adjustment had no effect on results for *CLDN1 -24218C, -15312C* and *-5414C*. Similarly, logistic regression models that combined participants who had either chronic or cleared HCV infection in the referent group yielded very similar results to those reported above (which are based on comparison to participants with chronic HCV infection).

An analysis of the haplotypes comprised of *CLDN1 -24218, -15312, -7153* and *-5414* supported an association of the *CLDN1 -7153A* variant among those who were uninfected with HCV. Using the haplotype with the four major variants as the referent (haplotype frequency, 77.5%), uninfected participants more often carried the haplotype that included all four minor alleles (ancestry adjusted OR, 1.78; 95% CI, 1.12-2.82; p=0.01; haplotype frequency, 13.5%) or one of three rare haplotypes (combined haplotype frequency, 1.5%) that included *CLDN1 -7153A* plus one or more other minor variants (ancestry adjusted OR, 2.98; 95% CI, 1.07-8.34; p=0.04). There was no association for haplotypes in which the sole minor variant was either *CLDN1 -24218* (ancestry adjusted OR, 0.90; p=0.9; haplotype frequency, 1.5%) or *CLDN1 -5414* (ancestry adjusted OR, 1.01; p=1.0; haplotype frequency, 6.0%). Haplotypes in which the sole minor variant was either CLDN1-*7153* or *CLDN1 -15312* were not found.

CLDN1 Sequencing among HCV-Uninfected IDUs

The *CLDN1* coding region sequences from 25 HCV-uninfected IDUs revealed no novel variants compared to those found in the SNP500Cancer population, and the distribution of SNPs among HCV-uninfected subjects did not differ from that found in participants with

chronic or resolved infection. All exonic variants present among the HCV-uninfected participants caused a synonymous amino acid substitution.

HCV Clearance

The comparison of genotype frequencies among participants who had cleared HCV to those with chronic HCV revealed no associations among either African American (Supplemental Table 3) or European American (Supplemental Table 4) participants. Specifically examining *CLDN1 -7153A*, the SNP most strongly associated with HCV-uninfected participants, the OR for cleared HCV infection compared to chronic HCV infection (dominant genetic model) was 1.27 (95% CI, 0.78-2.07; p=0.33) among African Americans and 1.12 (95% CI, 0.64-1.97; p=0.69) among European Americans. *CLDN1 IVS1-2983C*, which was less frequent among HCV-uninfected African American participants, was equally frequent among participants with chronic or cleared HCV infection (African Americans: OR, 1.14; 95% CI, 0.69-1.9; p=0.61 European Americans: OR, 0.96; 95% CI, 0.60-1.54; p=0.86).

Discussion

In this study of IDUs at high risk of HCV infection, we found several linked *CLDN1* variants, as well as associated haplotypes, to be more common in African Americans and European Americans who had remained uninfected with HCV despite long-term (>10 years) drug use. These variants reside in the 5′ untranslated region of *CLDN1*, where promoter or other gene regulatory elements may be present, and the most strongly associated SNP was found ~7000 base pairs upstream of the transcription start site. One of these SNPs, or a genetic variant in linkage disequilibrium with them, may decrease susceptibility to HCV infection by reducing the level of claudin-1 in the liver. To our knowledge no results from previous studies of *CLDN1* genetic variation and HCV infection are available for comparison with our findings.

Two factors support the plausibility of this association. First, the results were consistent in separate analyses of African American and European American participants, although none of the associations reached statistical significance in the latter group. Second, it is biologically plausible that variants which may regulate a gene encoding a viral co-receptor could alter susceptibility to infection with that virus. Evidence that the identified variants alter *CLDN1* expression in the liver would provide important support for the association, but liver tissue was not collected as part of the present study.

There is evidence that HCV-uninfected IDUs in the UHS population are exposed to HCV. Although the prevalence of antibody to HCV in UHS participants who had injected drugs for <10 years fell over time in parallel with prevention efforts, the vast majority of IDUs enrolled in UHS who injected drugs >10 years had antibodies to HCV (22). In another study of subjects enrolled in UHS, about half of those who had injected drugs >10 years yet were negative by both HCV EIA and HCV RNA PCR assays exhibited T-cell reactivity to non-structural viral elements, indicating that cell entry by HCV had occurred (23). The lower prevalence of both HBV and HIV among HCV-uninfected participants suggests that behavioral factors, such as safer injection practices may have reduced the risk of blood borne infections in this group. To the extent that is so, it would have reduced our ability to detect differences in HCV infection risk due to genetic factors.

We used ‘tag SNPs’ drawn from the International HapMap II database to interrogate *CLDN1*. This approach, which is similar to that employed in some ‘genome wide association studies’, allowed us to identify several SNPs [and a haplotype] of interest that lie in a potential promoter region. However, the number of SNPs that we analyzed (50) raises the question of whether the associations we observed are chance findings resulting from

multiple statistical comparisons. Because some of the SNPs we genotyped are linked to one another the number of independent associations is fewer than the number of SNPs that were analyzed. The application of a Bonferroni correction to adjust for multiple testing is much too conservative, especially considering that the purpose of this study was to discover novel genetic associations. Expert groups have concluded that replication of results is the most appropriate way to evaluate genetic associations (24,25). As we noted above, the consistency of our findings in separate analyses of African American and European American IDUs enrolled in UHS provides an internal replication of the association between *CLDN1* promoter variants and HCV infection status, but our findings should be evaluated in independent studies.

Further studies are also needed to elucidate whether any of these SNPs (or a linked variant) have a functional role in liver tissue. Such studies are challenging because they require access to a fairly large number of liver tissue specimens that are suitable not only for genotyping but also for functional studies to assess gene expression or protein levels. However, if a suitable collection of such specimens were defined, it could be used to systematically examine associations between SNPs and functional markers.

In summary, SNPs located in a promoter region of *CLDN1* and a related haplotype are associated with protection against HCV infection. If confirmed, this finding would support the importance of claudin-1 *in vivo* and its potential as a target for intervention against HCV infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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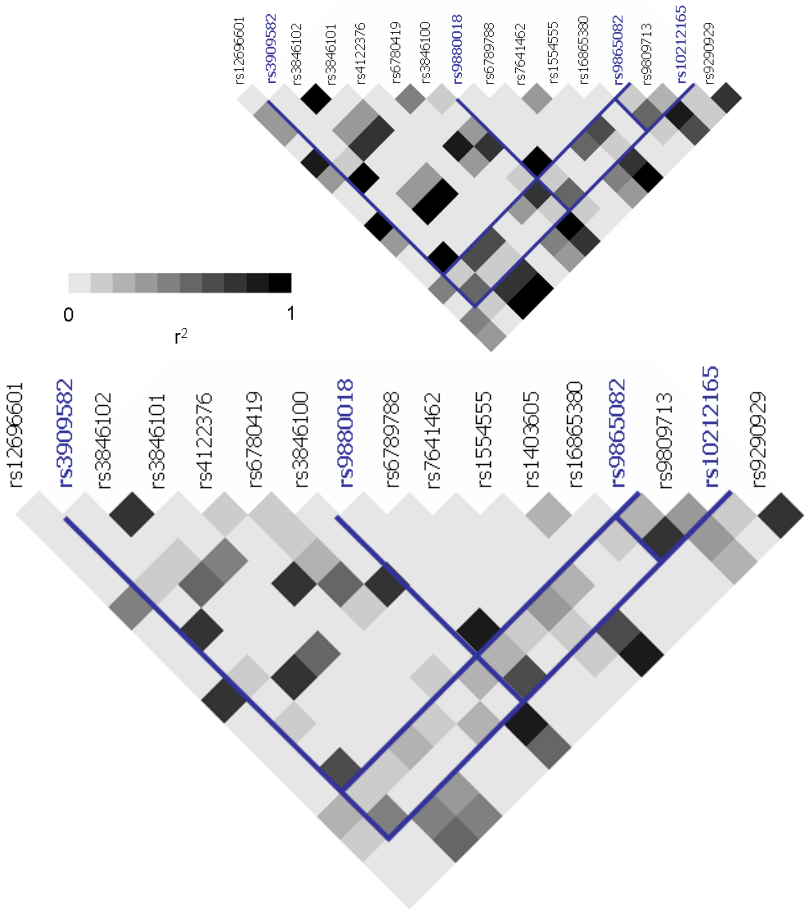

Figure 1.

Linkage disequilibrium (r^2) between *CLDN1* promoter region tag SNPs rs3909582 (-24218T>C), rs9880018 (-15312T>C), rs9865082 (-7153G>A) and rs10212165 (-5414T>C) among African American (1a) and European American (1b) UHS participants.

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Table 1

Single nucleotide polymorphisms examined with dbSNP identifier, genomic systematic designation, protein changes, observed minor allele frequency (MAF) and test for Hardy-Weinberg (P_{HW}) proportions. Based on Human Genome Build 35.1 and dbSNP build 126.

			African Americans		European Americans	
dbsnpid[†]	**Genomic Systematic**[*]	**Protein Systematic**	**MAF**[‡]	**P_{HW}**[§]	**MAF**[‡]	**P_{HW}**[§]
rs9290930	g.-31219C>T		0.10	0.30	**0.04**	0.50
rs1527958	g.-31293T>A		0.07	0.67	0.07	0.16
rs16865409	g.-33166A>T		0.22	0.71	0.08	0.95
rs13340153	g.-35089C>T		0.12	0.69	0.22	0.20
rs1425101	g.-35673C>T		0.13	0.33	0.07	0.15
rs11922773	g.-37032C>G>T		0.25	0.93	0.47	0.64
rs12696601	g.-25676T>C		0.24	0.32	0.30	0.53
rs3909582	g.-24218T>C		0.20	0.71	0.09	0.28
rs3846102	g.-23624G>A		0.14	0.62	0.43	0.53
rs3846101	g.-21778T>A		0.16	**0.00**	0.43	0.90
rs4122376	g.-19066G>A		0.10	0.06	**0.002**	0.98
rs6780419	g.-16326G>A		0.42	0.33	0.35	0.62
rs3846100	g.-16164G>C		0.21	0.37	0.48	0.85
rs9880018	g.-15312T>C		0.18	0.76	0.09	0.40
rs6789788	g.-15054G>A		0.15	**0.01**	**0.046**	0.39
rs7641462	g.-13780T>G		0.28	0.57	0.30	0.68
rs1554555	g.-11216C>T		0.17	0.13	0.43	0.81
rs1403605	g.-8927A>C		**0.03**	0.62	**0.00**	
rs16865380	g.-7793T>C		0.12	0.77	**0.01**	0.89
rs9865082	g.-7153G>A		0.20	0.73	0.09	0.39
rs9809713	g.-7056G>A		0.47	0.14	0.55	0.95
rs10212165	g.-5414T>C		0.25	0.99	0.15	0.94
rs6781278	g.-4021T>C		0.25	0.32	0.50	0.87
rs9290929	g.-3743T>C		0.21	0.25	0.43	0.82
rs16865352	g.-1784T>C		0.12	0.77	0.11	0.60
rs16865347	g.-1401G>A		0.10	0.49	0.12	0.34

			African Americans		European Americans	
dbsnpid[†]	**Genomic Systematic**[*]	**Protein Systematic**	**MAF**[‡]	**P_{HW}**[§]	**MAF**[‡]	**P_{HW}**[§]
rs16865346	g.-918T>A		0.10	0.06	0.07	0.60
rs56235513	g.-460C>T		0.11	0.10	0.10	0.35
rs55641411	g.-443G>T		0.11	0.25	0.11	0.34
rs12696600	g.Ex1+9G>T	p.R61L	0.24	0.18	0.49	0.43
rs893051	g.IVS1+172C>G		0.49	0.78	0.59	0.35
rs9835663	g.IVS1+3015G>A		0.26	0.45	0.27	0.55
rs3774024	g.IVS1+3959T>C		**0.049**	0.36	0.06	0.55
rs11717803	g.IVS1+4405T>C		0.10	0.05	**0.02**	**0.005**
rs6793070	g.IVS1-4145G>A		0.14	0.53	0.10	0.34
rs3732923	g.IVS1-3884A>T		0.51	0.50	0.40	0.20
rs3732924	g.IVS1-3782A>C		0.27	0.54	0.28	0.47
rs9848283	g.IVS1-2983C>T		0.16	0.68	0.46	0.88
rs9866788	g.IVS1-2295A>G		0.22	0.99	0.49	0.71
rs6809986	g.IVS1-2044T>C		0.12	0.78	**0.001**	0.98
rs6809685	g.IVS1-1769T>C		0.22	0.09	0.12	0.66
rs6776378	g.IVS2+135C>T		0.29	0.32	0.25	0.44
rs6800425	g.IVS2+337T>C		0.19	0.90	0.39	0.69
rs3774025	g.IVS2-905A>G		0.19	0.98	0.39	0.97
rs3774032	g.IVS3-632G>A		0.09	0.80	0.20	0.92
rs17501010	g.IVS3-426C>A		0.21	**0.005**	0.12	0.62
rs9847792	g.IVS3-268T>C		0.16	0.15	**0.01**	0.91
rs55724972	g.Ex4+552G>T		**0.01**	0.84	0.09	0.28
rs9290926	g.*3581C>A		0.06	0.94	0.13	0.46
rs7632915	g.*4617T>G		0.24	0.55	0.39	0.81
rs13092700	g.*6505T>C		**0.02**	0.71	0.07	0.58
rs12629245	g.*8429A>C		0.13	0.23	0.12	0.18
rs9810521	g.*8608A>G		0.11	0.24	**0.004**	0.93
rs6776530	g.*10365C>A		0.10	0.39	0.30	0.47

			African Americans		European Americans	
dbsnpid[†]	**Genomic Systematic**[*]	**Protein Systematic**	**MAF**[‡]	**P_{HW}**[§]	**MAF**[‡]	**P_{HW}**[§]
rs9878318	g.[*]11360G>A		0.29	0.61	0.45	0.75

[†] dbSNP database, http://www.ncbi.nlm.nih.gov/SNP

[*] See http://snp500cancer.nci.nih.gov/terms_snp_region.cfm for a description of the genetic variant nomenclature; rs9290930, rs1527958, rs16865409, rs13340153, rs1425101, and rs11922773 are defined in relation to *CLDN16*, which is adjacent to *CLDN1* and read on the opposite chromosomal strand

[‡] Minor allele frequency (%)

[§] P-values for Hardy-Weinberg proportions

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Table 2

Demographic and clinical characteristics of the study population.

	African Americans			*European Americans*		
	Chronic (n=320)	**Cleared (n=90)**	**Uninfected (n=39)**	**Chronic (n=338)**	**Cleared (n=109)**	**Uninfected (n=29)**
Years of injection drug use (median, IQR)	27, 22-32	25, 20-30	17, 12-23	24, 17-29	24, 18-30	17, 12-20
Age (median, IQR)	46, 43-51	46, 42-49	43, 35-47	43, 37-49	43, 38-48	33, 31-39
Male (%)	63.1	65.6	79.5	74.9	68.8	82.8
HBV infection						
Acute (%)	0.3	1.1	0.0	0.3	4.6	0.0
Chronic (%)	2.5	13.3	0.0	3.6	5.5	0.0
Resolved (%)	82.3	76.7	35.9	75.7	74.3	27.6
Never infected (%)	10.6	7.8	56.4	15.1	12.8	69.0
Vaccinated (%)	2.8	0.0	7.7	4.7	1.8	3.5
Unknown (%)	1.3	1.1	0.0	0.6	0.9	0.0
HIV-1- Infected (%)	12.8	13.3	5.1	11.2	3.7	6.9

Table 3

Genotype frequency for selected single nucleotide polymorphisms among IDUs with chronic HCV and IDUs who were uninfected with HCV, odds ratios (OR), 95% confidence interval and p-values. Results are presented separately for African Americans and European Americans, as well as for both groups combined, adjusted for ancestry.

Genomic Systematic		African Americans				European Americans				Combined Adjusted for Ancestry		
	Genotype	Chronic	Uninfected	OR	p-value	Chronic	Uninfected	OR	p-value	OR	95% CI	p-value
-24218T>C	TT	199	21			277	20					
	CT	99	15	1.44	0.31	49	7	1.98	0.14			
	CC	14	3	2.03	0.29	4	1	3.46	0.28			
	CT or CC	113	18	1.51	0.23	53	8	2.09	0.10	1.70	0.99-2.91	0.05
-15312T>C	CC	208	22			273	20					
	CT	92	15	1.54	0.23	52	7	1.84	0.19			
	TT	9	2	2.1	0.36	4	1	3.41	0.28			
	CT or TT	101	17	1.59	0.18	56	8	1.95	0.13	1.72	1.00-2.94	0.048
-7153G>A	GG	200	17			276	20					
	AG	100	19	2.24	0.02	52	7	1.86	0.18			
	AA	11	2	2.14	0.35	4	1	3.45	0.28			
	AG or AA	111	21	2.23	0.02	56	8	1.97	0.13	2.13	1.25-3.62	0.006
-5414T>C	TT	175	13			233	19					
	CT	115	19	2.22	0.04	85	8	1.15	0.74			
	CC	19	5	3.54	0.03	8	1	1.53	0.69			
	CT or CC	134	24	2.41	0.02	93	9	1.19	0.69	1.78	1.06-3.00	0.03
IVS1-2983C>T	TT	226	35			96	9					
	CT	87	4	0.3	0.03	169	13	0.82	0.66			
	CC	7	0	0	0.99	72	7	1.04	0.95			
	CT or CC	94	4	0.27	0.02	241	20	0.89	0.77	0.55	0.31-0.97	0.04