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Evaluation of *IL10, IL19*, and *IL20* gene polymorphisms and chronic hepatitis B infection outcome

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Summary

Hepatitis B viral infection remains a serious global health problem despite the availability of a highly effective vaccine. Approximately 5% of HBV-infected adults develop chronic hepatitis B, which may result in liver cirrhosis or hepatocellular carcinoma. Variants of interleukin-10 (*IL10*) have been previously associated with chronic hepatitis B infection and progression to hepatocellular carcinoma. Single nucleotide polymorphisms (SNPs, n = 42) from the *IL10*, *IL19*, and *IL20* gene regions were examined for an association with HBV infection outcome, either chronic or recovered, in a nested case-control study of African Americans and European Americans. Among African Americans, three nominally statistically significant SNP associations in *IL10*, two in *IL20*, and one haplotype association were observed with different HBV infection outcomes ($P = 0.005–0.04$). The SNP, rs1518108, in *IL20* nominally deviated significantly from Hardy-Weinberg equilibrium in African Americans, with a large excess of heterozygotes in chronic HBV-infected cases ($P = 0.0006$), which suggests a strong genetic effect. Among European Americans, a nominally statistically significant SNP association in *IL20*, as well as an *IL20* haplotype were associated with HBV recovery ($P = 0.01–0.04$). These results suggest that *IL10* and *IL20* gene variants influence HBV infection outcome and encourage the pursuit of further studies of these cytokines in HBV pathogenesis.

Keywords

Interleukin-10; Inflammation; African American; Immunogenetics; Hepatitis b; HIV co-infection

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Introduction

Worldwide, 350 million people have chronic hepatitis B virus (HBV) infection; approximately 1.25 million of these people live in the United States of America (WHO, 2000). Approximately 5% of all hepatitis B infected adults will develop chronic hepatitis B (WHO, 2000). Nearly 5,000 people in the United States die each year from hepatitis B-related complications like liver cirrhosis and hepatocellular carcinoma (HCC). Treatment of hepatitis B-related liver disease results in annual health care costs and lost wages equaling $700 million (CDC, 2002).

HBV is transmitted via contact with infected body fluids, including blood, saliva, and semen. In high endemicity areas, including much of Asia and most of sub-Saharan Africa, HBV is usually acquired perinatally or during childhood, however in low endemicity regions, such as the United States and Europe, the virus is usually contracted in adulthood via high-risk sexual behaviors or percutaneously through injection drug use. Co-infection of HBV and the human immunodeficiency virus (HIV) is common given both viruses share similar routes of transmission in adults (Rodriguez-Mendez *et al*., 2000; Salmon-Ceron *et al*., 2005).

The natural history of chronic HBV infection is altered by HIV co-infection, with significantly more liver-related deaths among HIV-infected individuals (Thio *et al*., 2002; Konopnicki *et al*., 2005; Yachimski & Chung, 2005). While highly active anti-retroviral therapy (HAART) has improved the survival of HIV-infected people, unfortunately, many of those co-infected with HBV now go on to develop end-stage liver disease (Palella *et al*., 1998).

The normal cellular immune response of an HBV infection leads to liver damage that may result in cirrhosis and HCC (Lai *et al*., 2003). The pro-inflammatory T-helper 1 (Th1) and anti-inflammatory T-helper 2 (Th2) cells regulate this cellular immune response. Interleukin-10 (IL-10) is one of the critical modulators of this balance by suppressing the host Th1 immune response. IL-10 is produced by Th2 cells and inhibits expression of other pro-inflammatory cytokines such as IFN-gamma, IL-2 and TNF-alpha in Th1 cells (Pestka *et al*., 2004). IL-10 influences the natural history of HBV infection and other viral diseases such as Epstein-Barr, herpes zoster, HIV, and hepatitis C (Shin *et al*., 2000; Vicari & Trinchieri, 2004; Oleksyk *et al*., 2005).

Polymorphisms in the *IL10* proximal promoter region affect production of IL-10 (Crawley *et al*., 1999; Edwards-Smith *et al*., 1999) and have been examined extensively for associations with HBV infection and progression. Among populations throughout the world there are three classic promoter haplotypes, GCC, ACC, and ATA (Turner *et al*., 1997). The GCC haplotype produces high levels of IL-10, ACC medium, and ATA low (Crawley *et al*., 1999; Edwards-Smith *et al*., 1999). The three proximal promoter SNPs and haplotypes have been associated with hepatitis B infection outcomes in Chinese (Zhu *et al*., 2005; Peng *et al*., 2006), Korean (Shin *et al*., 2003; Cheong *et al*., 2006), and Japanese (Miyazoe *et al*., 2002; Migita *et al*., 2005) populations. These reports suggest an important role for *IL10* variation in HBV infection outcome, which we sought to examine further. Additionally, the gene paralogs, *IL19* and *IL20*, located in the 100 kb 1q31-q32 region telomeric to *IL10* (Pestka *et al*., 2004), have been recently identified as having early pro-inflammatory cytokine and immunoregulatory activities (Liao *et al*., 2002; Parrish-Novak *et al*., 2002; Wolk *et al*., 2002).

We examined 42 single nucleotide polymorphisms (SNPs) located in the *IL10*, *IL19*, and *IL20* genes in European Americans and African Americans for host genetic differences between individuals with chronic hepatitis B infection or serologic evidence of recovery. Host genetic variation was evaluated by differences in allele, genotype, and haplotype frequencies using a nested case-control study design.

Materials and Methods

Patients

The null hypothesis that host genetic differences did not exist between chronic hepatitis B infected and recovery individuals was tested in a nested case-control study among African Americans (AA) and European Americans (EA). Individuals selected for this study were participants in several HIV infection and progression cohorts: AIDS Link to the Intravenous Experience (ALIVE, number of AA (n_{AA}) = 93, number of EA (n_{EA}) = 3), Multicenter Hemophilia Cohort Study (MHCS, n_{AA} = 7, n_{EA} = 36), Hemophilia Growth and Development Study (HGDS, n_{AA} = 7, n_{EA} = 55), and the Multicenter AIDS Cohort Study (MACS, n_{AA} = 14, n_{EA} = 398) for HIV-1/AIDS (demograpic characteristics are described in Karacki *et al*., 2004). Cases had chronic hepatitis B, which was defined by testing positive for HBsAg at two visits separated by a minimum of six months. Controls were individuals who spontaneously recovered without treatment and had serologic evidence of prior infection (antibodies against hepatitis B core antigen anti-HBc, and against HBsAg, anti-HBs) with HBsAg undetectable at two time points separated by a minimum of six months (Lok & McMahon, 2004). Each chronic hepatitis B case was matched with at least one, and when possible, two recovery controls for age, gender, race, and HIV status, the demographic factors known to be associated with HBV infection outcome (Karacki *et al*., 2004). A total of 121 African Americans (45 chronic cases, 76 recovery controls) and 492 European Americans (179 chronic cases, 313 recovery controls) were studied. The majority of subjects were HIV infected (68.5% in European Americans, 76.9% in African Americans), which was one of the very significant factors matched between cases and controls.

This study was conducted following the principles of the Helsinki Declaration of 1975 (1996). Study participants provided written informed consent to be included in the parent cohorts, which were approved by their respective Institutional Review Boards.

Study design

We examined SNPs located in and around the *IL10, IL19*, and *IL20* genes for differences in allele, genotype, and haplotype frequencies for African Americans and European Americans. A total of 42 SNPs were chosen for genotyping, 25 from *IL10*, 10 from *IL19*, and seven from *IL20*. The 25 SNPs genotyped in *IL10* resulted in saturated coverage of the gene. For the *IL19* and *IL20* genes, haplotype tagging or functionally important SNPs were chosen. SNPs were genotyped using multiplexed length-modified single-base extension and 5′ exonuclease assays (Oleksyk *et al*., 2005). The forward and reverse primers, as well as the probe sequences for each SNP are reported in Supplementary Table 1. SDS v2.2 software (Applied Biosystems, Foster City, CA) was used to call genotypes generated with the 5’ exonuclease assays. GeneScan v3.5 and Genotyper v3.6 software programs (Applied Biosystems, Foster City, CA) were used to call genotypes from the single-base extension assays. Both standard Qiagen (Hilden, Germany) and phenol-chloroform extraction methods were used to isolate DNA from EBV transformed B-cell lines established from each individual (Dean *et al*., 1994).

Given the departure from Hardy-Weinberg equilibrium (HWE) observed at rs1518108 in the *IL20* gene (Fig. 1), genotypes were confirmed by sequencing all 45 African American cases and 10 controls. The forward primer TCAAGTCCTTATCTTTGTGTCCAA and reverse primer TGTTGGGCAGCTGTTACTTG were used for sequencing with Big Dye v.1.1 terminator kit and the AB3730 sequencer (Applied Biosystems, Foster City, CA). Sequencher v.4.5 software (Gene Codes, Ann Arbor, MI) was used to align the sequences and to validate the genotyping results.

Statistical analyses

The statistical package SAS v9.1 and SAS/Genetics (SAS Institute Inc., Cary, NC) was utilized for the statistical analysis. The P values reported throughout this paper are nominal (uncorrected for multiple testing). African Americans and European Americans were analyzed separately for allele, genotype, and haplotype associations. Seven loci in *IL10* (rs3024510, rs3024506, rs5743625, rs3024489, rs1800895, rs5743623, and rs7349077) had a minor allele frequency of less than 5% in cases and controls among both racial populations and were not considered in further SNP or haplotype analyses. Chi-square tests (χ^2) were performed to determine whether an association existed for alleles and genotypes. If a nominally significant allelic or genotypic association was present ($P < 0.05$) or suggestive ($P < 0.10$) at a locus, then conditional logistic regression (CLR) was performed. The most frequent homozygote allele in the control group was chosen as the referent, which allowed for separate evaluation of heterozygotes and rare allele homozygotes. A sub-analysis of individuals stratified by HIV status was also performed to examine whether the effect of any associations between the two groups differed.

Haplotypes were characterized based on SNPs in the two regions of linkage disequilibrium present around *IL10*, *IL19*, and *IL20*. One LD block contains the *IL10* gene and the other both *IL19* and *IL20* (Oleksyk *et al*., 2005). A haplotype trend regression (HTR) model was used to assess associations between haplotypes and HBV recovery (Shrestha *et al*., 2006). Briefly, haplotype frequencies were calculated first, using the expectation-maximum (EM) algorithm methods (Excoffier & Slatkin, 1995) modeled after the SNPHAP software program (Clayton). Statistical methods were then used to estimate a posterior probability matrix of values based on all possible haplotypes for each individual, given their genotypes at each locus (Schaid *et al*., 2002; Zaykin *et al*., 2002) that were then included in a conditional logistic regression analysis (Breslow & Day, 1980). A stepwise regression approach identified the most parsimonious model. Rare haplotypes, with frequencies < 3% were combined into one haplotype. Odds ratios (OR), 95% confidence intervals (CI), and probabilities were calculated for haplotypes with $P < 0.10$.

Results

Allele and genotype frequencies as well as p-values for the allelic and genotypic χ^2 tests for chronic HBV-infected cases and recovery controls for all 35 SNP loci are shown for African Americans in Supplementary Table 2A and for European Americans in Supplementary Table 2B. The CLR results for SNPs with $P < 0.05$ from either the allelic or genotypic χ^2 analysis are shown in Table 1.

Among African Americans, a χ^2 analysis revealed three nominally statistically significant allelic associations with HBV infection outcome in the *IL10* region (rs1800893, $P = 0.04$; rs1800896, $P = 0.01$; and rs1518110, $P = 0.04$) and one in the *IL20* region (rs1518108, $P = 0.01$). There were three nominally statistically significant genotypic associations for African Americans; one at *IL10* (rs1800896, $P = 0.02$) and two at *IL20* (rs1400986, $P = 0.03$, and rs1518108, $P = 0.002$). Among European Americans, rs3024517 in *IL20* was nominally statistically significantly associated for alleles and genotypes ($P = 0.03$, $P = 0.02$, respectively). This SNP was found to be in strong LD ($r^2 = 0.974$, $D' = 0.993$) with rs1400986, which also had nominally statistically significant allelic and genotypic associations, ($P = 0.04$ and $P = 0.03$, respectively).

Odds ratios (OR) and 95% confidence intervals (CI) were computed using CLR for SNPs with $P \leq 0.05$ for either the allelic or genotypic χ^2 tests (Table 1). Three genotypic associations were observed in African Americans and two genotypic associations in European Americans with HBV infection outcome. African American recovery controls were more likely to have the TT

genotype compared to the GG referent genotype at rs1518110 with a protective OR_{TT} = 0.11 (0.01–0.93), *P* = 0.04. Chronic HBV infected cases were more likely to have the CT or CC genotypes at rs1518108 in *IL20* compared to the referent TT with OR_{CT} = 7.58 (2.14–26.89), *P* = 0.0017 and OR_{CC} = 5.45 (1.31–22.77), *P* = 0.02. Among European Americans, recovery controls were more likely to have the CC genotype at rs1400986 and the AA genotype at rs3024517 due to strong LD, compared to the referent genotypes, TT and GG. The protective odds ratios were OR_{CC} = 0.23 (0.07–0.80), *P* = 0.02 for rs1400986 and OR_{AA} = 0.16 (0.04–0.70), *P* = 0.01 for rs3024517. These associations were examined further in an analysis stratified by HIV status. The direction of the associations reported above among HIV positive and negative European Americans, as well as HIV positive African Americans were consistent with those reported above (analysis not shown, and the African American HIV negative sample had insufficient numbers for examination).

All 35 loci genotyped were tested for HWE. African American cases deviated from HWE at one locus, rs1518108 in *IL20*, based on allele frequencies from African American controls (Fig. 1, P=0.0006). As noted above, genotypes at this locus have a strong association with susceptibility to chronic hepatitis B infection in African Americans (*P* = 0.0017). Genotypes for rs1518108 were confirmed by two independent methods, both genotyping and sequencing, and using multiple replications.

Haplotypes were analyzed in the two blocks of LD that encompass *IL10* and the *IL19/IL20* combination (Oleksyk *et al*., 2005) for an association with hepatitis B infection outcome. The LD block containing *IL10* was analyzed using the proximal promoter SNPs (Turner *et al*., 1997), the distal promoter SNPs (Gibson *et al*., 2001), and the SNPs that covered the length of the gene (Oleksyk *et al*., 2005). In the *IL19/IL20* block, haplotypes for each individual gene as well as both genes combined were tested separately.

Associations were evident in the *IL20* gene among African Americans and European Americans (Table 2). Haplotype results for all three genes are shown in Tables 2 and 3. Differences in haplotype frequencies existed between cases and controls in the *IL20* region, which were reflected with a global *P* = 0.005 for African Americans and *P* = 0.036 for European Americans. In African Americans, the *IL20* haplotype GTGTC was susceptible with OR = 14.41 (2.23 – 93.28), *P* = 0.005. In European Americans, the *IL20* haplotype GCATT was protective with OR = 0.38 (.15 – .97), *P* = 0.04.

Discussion

Our study provides new insights into the pathogenesis of chronic hepatitis B in regards to the *IL10, IL19* and *IL20* candidate genes. There was evidence that HBV infection outcome in African Americans was influenced by SNP variants of the *IL10* and *IL20* genes, whereas recovery in European Americans was influenced solely by SNP variants in *IL20*. Our strongest findings were among variants of *IL20*, especially the SNP rs1518108, which had a very strong association in African Americans. Additionally, an *IL20* haplotype in African Americans and a different *IL20* haplotype in European Americans were associated with differences in HBV infection outcome. This is an important finding since *IL20* variants have not been implicated with HBV pathogenesis before, although they have been associated with hepatitis C recovery in African Americans (Oleksyk *et al*., 2005).

The *IL20* gene is a paralog of *IL10* and belongs to the *IL10* family of cytokine genes (Xu, 2004). Two highly correlated SNPs, rs1400986 and rs3024517 (r^2 = 0.974), were associated with HBV recovery in European Americans (OR_{CC} = 0.23 [0.07–0.80], *P* = 0.02 and OR_{AA} = 0.16 [0.04–0.70], *P* = 0.01 respectively). In African Americans the association observed with rs1518108 is the most striking and significant (*P* = 0.0017). This intergenic SNP that lacks a

known functional role and creates no obvious deleterious genetic change is positioned nearly 1Kb downstream from the *IL20* polyadenylation site and 28 Kb upstream from *IL24* in an area of moderate conservation. In European Americans, the minor allele (T) had a frequency of 49% in both cases and controls. However, in African Americans, the T allele was more common in recovery controls (64%) than chronic HBV-infected cases (48%, allelic $P = 0.01$). We saw a departure from HWE among African American cases (more CT and less TT genotypes than expected at rs1518108, $P = 0.0006$, Fig. 1). At this locus, chronic HBV-infected African American cases were more likely to have the CT genotype than were those who had recovered (who were more likely to have TT). The heterozygous disadvantage observed in African American cases could be due to very recent admixture between stratified populations. However, this is very unlikely because excess heterozygotes are not observed in controls at rs1518108, or at the other 34 loci examined in this study, or at additional loci examined among African Americans from the same cohort (Duggal *et al*., 2005;Oleksyk *et al*., 2005;Javanbakht *et al*., 2006;Shrestha *et al*., 2006). The TC genotype of rs1518108 has also been associated with reduced susceptibility to psoriasis in Estonians (Kingo *et al*., 2004). Taken together, these results suggest that heterozygotes at rs1518108 (or a haplotype it tags) are more susceptible to HBV infection in African Americans, and that polymorphisms at the locus have a role in other inflammatory disorders.

Several studies indicate that the role of *IL10* variants, especially those in the promoter region, are important in HBV infection and progression to liver disease (Miyazoe *et al*., 2002; Shin *et al*., 2003; Zhu *et al*., 2005). We tested these promoter region variants of *IL10* for an association with HBV infection outcome. A positive association was observed between the proximal promoter SNP *IL10*-1082, known for its effects on IL-10 production (Turner *et al*., 1997; Eskdale *et al*., 1998), among allelic ($P = 0.01$) and genotypic tests ($P = 0.02$) with chronic hepatitis B infection in African American cases. Associations with the other proximal promoter SNP, *IL10*-592, also involved in IL-10 production, were not observed in either population. Two additional SNPs in *IL10*, rs1800893 and rs1518110, were associated with HBV infection outcome in allelic tests ($P = 0.04$) among African Americans. The effect of *IL10* polymorphisms on HBV infection outcome is consistent with the role of IL-10 as an immunomodulatory cytokine.

SNP associations alone often cannot represent the complexity of the variation among hosts. Rather, haplotype analysis offers more power and further insight when one studies multifactorial diseases such as HBV infection (de Bakker *et al*., 2005). Two blocks of LD are present in the genomic region examined: the *IL10* gene and the *IL19/IL20* genes. These LD blocks have been reported in our previous study using the same SNP markers (Oleksyk *et al*., 2005). The SNP, rs1518108 was found to be in weak LD ($|D'|$ between 0.07 and .25) with other SNPs of *IL20* in an Estonian population (Koks *et al*., 2004), and is located in an area with elevated rates of recombination based on HapMap data (Altshuler *et al*., 2005). We have included rs1518108 in the *IL20* haplotype analysis based on the $|D'|$ value of 0.72 we observed in African Americans with one of its neighboring SNPs in *IL20*, rs1109461.

Our study is the first to demonstrate an association between haplotypes of *IL20* and susceptibility to chronic hepatitis B infection among African Americans and European Americans (Table 2, $P = 0.005 – 0.04$). Haplotypes of *IL19* and *IL20* have been associated with hepatitis C clearance (Oleksyk *et al*., 2005) and susceptibility to psoriasis (Kingo *et al*., 2004;Koks *et al*., 2004;Koks *et al*., 2005). *IL20* may be a common link between these diseases. In transgenic mice, IL-20 over-expression causes a shiny skin appearance and neonatal lethality, possibly due to skin-barrier defects (Otkjaer *et al*., 2005). In humans, IL-20 stimulates immune cells in the skin effecting keratinocyte proliferation that can result in psoriasis (Stenderup *et al*., 2007). The observed altered epidermal differentiation and hyper-proliferation resembles human psoriatic abnormalities. In humans, IL-20 is known to induce production of

IL-6 and TNF-alpha in monocytes; stimulate expression of keratinocyte growth factor, IL-6, TNF-alpha, and proto-oncogene tyrosine-protein kinase ROS in $CD8^+$ T cells, and likely functions as an early pro-inflammatory cytokine (Xu, 2004). Recently, IL-19 and IL-20 were identified as modulators of the Th1/Th2 balance, likely acting through receptor subunits shared with IL-10 (Oral *et al*., 2006). Lastly, the emergence or reappearance of psoriasis is a common side effect of interferon-alpha treatment, a cornerstone of therapy for patients with chronic hepatitis infection (Seckin *et al*., 2004;Kartal *et al*., 2005;Ketikoglou *et al*., 2005).

There are some limitations to this study. We have examined a subset of the variation known to be present in the *IL10*, *IL19* and *IL20* gene regions. Although genotyping additional SNPs and other genetic variants could yield additional associations, our selection of SNPs was designed to encompass the major known haplotypes in the gene region. Another limitation is the lack of functional studies of the association between serum levels of cytokines and the genetic polymorphisms assayed in this specific group of patients. Also, about half of the patients examined are co-infected with HIV, which was taken into account in two ways. First, this is a nested case-control study where those with and without HBV recovery were matched based on HIV infection status. Second, stratified analyses that were undertaken based on HIV infection and race showed no difference in associations for rs1518110, rs1518180, rs3024517 and rs1400986. Third, HBV infection generally occurs before HIV infection; thus, HBV recovery or persistence is usually determined prior to HIV infection (Kingsley *et al*., 1990; Levine *et al*., 1996). The inclusion of samples from HIV positive individuals complicates interpretation of the study, but we believe the correction for the complication of HIV infection makes results reported valid albeit more difficult to interpret..

In our study we applied association tests to 35 different SNPs. Using the Bonferroni correction, our significance levels would have to be lowered from $P < 0.05$ to $P < 0.0014$ to identify positive associations with certainty when analyzing the two racial groups separately for each of the allelic, genotypic, and HWE tests (Bonferroni, 1935). Nominally the most statistically significant associations we observed were $P = 0.0006$ for genotypic deviations from HWE (Fig. 1) and $P = 0.0017$ for CLR of genotypes for the locus rs1518108 in African Americans (Table 1), essentially meeting these Bonferroni criteria. None of the nominally significant P-values meets the strictest criteria of Bonferroni correction for 6 times 35 tests ($P < 0.00024$), but this cut-off is too strict because the comparisons are not independent due to haplotype structure and the statistical tests are related by common data and hypotheses under evaluation. Some of the allelic and genotypic χ^2 tests in Table 1 ($0.05 < P < 0.0017$) among both racial populations are also likely to be true statistically significant associations, but are confounded by a number of false discoveries (Supplementary Table 2A and 2B footnote) (Jung *et al*., 2005). Given the importance of IL-10 in regulating the immune response and its impact on the natural history of many diseases (Moore *et al*., 2001), along with the newly discovered roles of IL-19 and IL-20 in regulating the Th1/Th2 balance (Oral *et al*., 2006), exploring the associations we observed with rs1518108 as well as with SNPs with a $P < 0.05$ provides a reasonable approach to choosing targets for future research in samples from the same and different human racial and ethnic groups.

In summary, we found evidence to support the hypothesis that host genetic variability in *IL10* and *IL20* influence the outcome of acute HBV infection. Populations at higher risk of acquiring HBV should be the focus for replication of these results and furthering our understanding of the complex relationship between host genetics and HBV infection outcome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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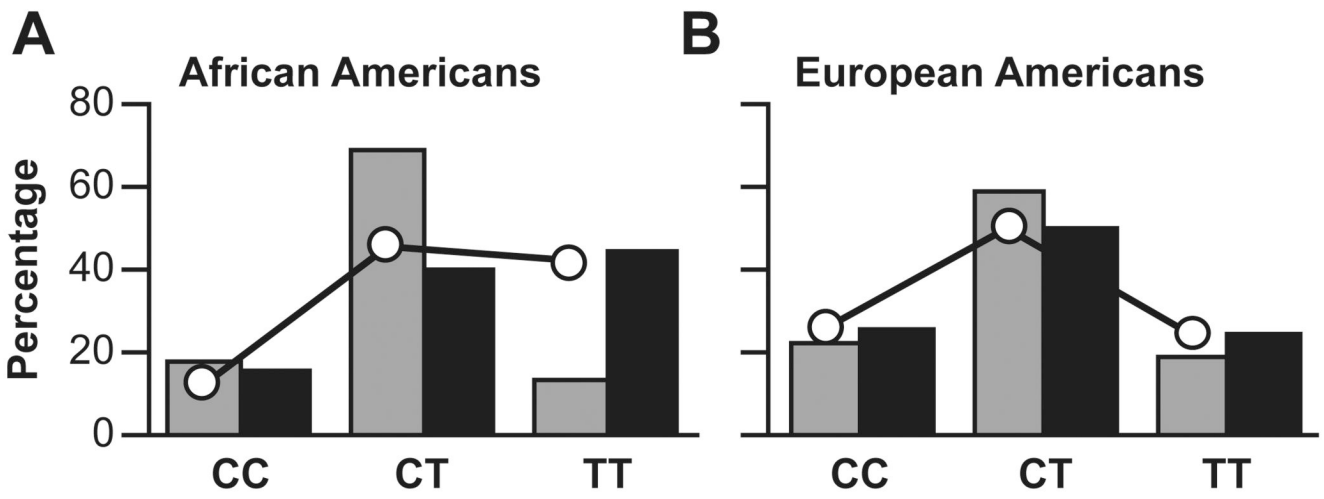

Fig. 1.

Deviations from Hardy-Weinberg equilibrium (HWE) for rs1518108. Chronic HBV-infected cases are associated with a SNP in *IL20*, rs1518108, which deviates from HWE in African Americans. The genotypes CC, CT, and TT are shown for chronic cases (grey bar) and recovery controls (black bars) in A) African Americans and B) European Americans. The open circles represent genotypic frequencies expected by HWE based on recovery control allele frequencies. The observed genotypic frequencies for African American chronic cases deviate from HWE, with less TT and more CT genotypes ($\chi^2 = 14.8$, d.f. = 2, $P = 0.0006$). Deviations from HWE remained in African Americans even after utilizing a different set of control allele frequencies (C = 44%, T = 56%) from 1,480 African Americans ($\chi^2 = 8.148$, d.f. = 2, $P = 0.017$). Support for a similar association in Europeans is suggestive but not statistically significant ($\chi^2 = 5.83$, d.f. = 2, $P = 0.054$).

Table 1

Allelic and genotypic associations of single *IL10* and *IL20* SNPs with chronic hepatitis B

	SNPs			Frequency n (%)			χ^2		CLR	
Sample	**Gene**	**NCBI ID**	**Position**	**Genotype**	**Cases**	**Controls**	**Allelic *P***	**Genotypic *P***	**OR (95% CI)**	***P***
African American										
	IL10	*rs1800893*	Promoter −1353	GG	12(27)	35(46)	0.04	0.10	1[+]	-
				AG	26(58)	34(45)			2.03 (0.78–5.31)	0.15
				AA	7(15)	7(9)			3.02 (0.64–14.15)	0.16
		rs1800896	Promoter −1082	AA	15(33)	38(50)	0.01	0.02	1	-
				AG	24(53)	32(42)			1.56 (0.69–3.55)	0.29
				GG	6(13)	6(8)			2.35 (0.59–9.41)	0.23
		rs1518110	Intronic +954	GG	16(48)	22(35)	0.04	0.08	1	-
				GT	16(48)	29(46)			0.81 (0.32–2.06)	0.66
				TT	1(3)	12(19)			0.11 (0.01–0.93)	0.04
	IL20	*rs1400986*	Intergenic 1807	CC	8(18)	25(33)	0.76	0.03	1	-
				CT	16(36)	45(59)			0.34 (0.10–1.13)	0.08
				TT	21(47)	6(8)			0.63 (0.19–2.15)	0.46
		rs1518108	Intergenic 6296	TT	6(13)	34(45)	0.01	0.001 0.03	1	-
				CT	31(69)	30(39)			7.58 (2.14–26.89)	0.0017
				CC	8(18)	12(16)			5.45 (1.31–22.77)	0.02
European American										
	IL20	*rs1400986*	Intergenic 1807	TT	124(69)	196(63)	0.04		1	-
				CT	52(29)	97(31)			0.87 (0.57–1.31)	0.49
				CC	3(2)	20(6)			0.23 (0.07–0.80)	0.02
		rs3024517	Intronic 3374	GG	125(70)	200(64)	0.03	0.02	1	-
				AG	52(29)	93(30)			0.90 (0.60–1.35)	0.60
				AA	2(1)	20(6)			0.16 (0.04–0.70)	0.01

[+]The referent group is the most common homozygote among the recovery controls. SNP locations given for *IL10* are derived from the start position of the first exon and match previous reports (Turner *et al.*, 1997; Gibson *et al.*, 2001). SNP locations for *IL20* are from AF402002.1 at NCBI, which contains the complete gene sequence.

Table 2

Haplotype Trend Regression analysis for SNPs in *IL19* and *IL20*

	African Americans			European Americans		
Gene	**Frequency (%)**			**Frequency (%)**		
Haplotype	**Cases**	**Controls**	**Global *P***	**Cases**	**Controls**	**Global *P***
IL20			0.005			0.036
GTGCT	12.8	24.3				
GTGTC[1]	35.0	18.1		35.7	37.0	
GTGTT	9.5	16.3		34.9	28.6	
GCGTT	4.3	15.9				
GCGTC	8.1	9.8				
GCATT[2]	7.8	6.2		8.3	13.6	
GCATC	7.2	4.6		7.0	7.6	
CTGTT	5.8	0.0		5.3	6.4	
GCGCT[3]	6.1	0.0				
GTGCC	3.1	0.0				
CTGTC				8.6	6.8	
IL19			> 0.10			> 0.10
ACC	57.1	57.5		75.9	75.8	
TCT	10.8	15.6		7.8	8.1	
TCC	13.6	15.3				
ATC	9.2	6.8				
ATT	6.4	3.1		13.5	12.4	
IL19/IL20			0.053			0.055
ACCGTGCT	9.3	21.2				
ACCGTGTT	12.4	15.2		31.0	26.5	
ACCGTGTC[4]	19.9	12.6		31.8	30.9	
TCCGCGTT	0.0	11.5				
TCTGTGTC	6.9	7.5		3.3	6.7	
TCTGTGCT	4.1	7.2				
TCCGCGTC	13.0	5.5				

	African Americans			European Americans		
Gene	**Frequency (%)**			**Frequency (%)**		
Haplotype	**Cases**	**Controls**	**Global *P***	**Cases**	**Controls**	**Global *P***
ACCGCGTC	0.0	5.4				
ACCGCATT[5]	5.5	5.2		8.5	14.0	
ACCGCATC	5.2	4.6		7.7	7.7	
ACCGCACT	4.7	0.0				
ATCGCGTT	3.8	0.0				
ATTCTGTT	3.1	0.0		5.1	5.9	
TCTGTGTT				4.9	0.0	
ATTCTGTC				0.0	6.6	

[1] GTGTC : OR = 14.41 [2.23 – 93.28], *P* = 0.005 for African Americans

[2] GCATT : OR = .38 [.15 – .97], *P* = 0.04 for European Americans

[3] GCGCT : OR = 51.16 [0.88 – 2977.64], *P* = 0.06 for African Americans

[4] ACCGTGTC : OR = 6.13 [.90 – 41.83], *P* = 0.06 for African Americans

[5] ACCGCATT : OR = .41 [.16 – 1.05], *P* = 0.06 for European Americans

The SNPs typed in the *IL19* and *IL20* genes were analyzed for haplotype associations using the haplotype trend regression method (Shrestha *et al*., 2006). For each haplotype with *P* (χ^2) < .10, an odds ratio (OR), 95% confidence ratio (CI), and CLR *P* value was determined. The global *P* value is based on the likelihood ratio test between cases and controls. *IL20* haplotypes include the ordered SNPs: rs1713239, rs1400986, rs3024517, rs1109461, and rs1518108. Two SNPs in *IL20*, rs2981573 and rs2232360, were not included because of some missing genotypes between essential cases and matched controls. *IL19* haplotypes include the ordered SNPs: rs2243168, rs2243176, and rs2243191. The SNPs comprising the *IL19* haplotypes were chosen because they contained the most complete set of data for the most individuals and may not fully represent the biological impact *IL19* given the presence of other variants which were not included in this analysis. The combined *IL19/IL20* extended haplotype analysis includes the ordered SNPs: rs2243168, rs2243176, rs2243191, rs1713239, rs1400986, rs3024517, rs1109461, and rs1518108. Nucleotide substitutions and positions for SNP loci are presented in Supplemental Table 1.

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Table 3

Haplotype Trend Regression analysis for SNPs in *IL10*

	African Americans			European Americans		
Gene	**Frequency (%)**			**Frequency (%)**		
Haplotype	**Cases**	**Controls**	**Global *P***	**Cases**	**Controls**	**Global *P***
IL10 Proximal Promoter			> 0.10			> 0.10
ATA	34.4	44.0		22.3	24.0	
GCC	40.0	28.9		45.6	45.0	
ACC	25.5	27.0		32.0	31.0	
IL10 Distal Promoter			0.057			> 0.10
AGC[1]	48.8	62.4		60.8	59.3	
TAA	20.9	13.7		18.1	18.2	
AGA	5.0	8.1				
AAA	14.2	7.3				
TGA	11.0	6.5		14.0	12.5	
TAC				3.1	0.0	
TGC				3.9	3.4	
IL10 Gene			> 0.10			> 0.10
AGCCGAATTGGTTIAGC	24.2	39.1		18.6	19.6	
TAACAGCGCCGTCIGGC	12.1	10.9		18.1	19.6	
AGCCGAATTGATTIAGC	7.6	8.7				
AGCCGACGCGGTCDAAT	6.1	8.7				
AAACGACGCGGTTIAGC	4.5	6.5				
AGCCAGCGCCGTCIAGC	7.6	6.5				
AGATAGCGCGGTTIAGC	6.1	5.4				
TAACAGCGCCGTCIAGC	7.6	5.4				
AGCCGACGCGGTTIAGC	9.1	4.3		34.3	30.8	
TGACAGCGCCGTCIAGC	6.1	4.3		13.7	14.0	
AAACAACGCGGTCIAGC	6.1	0.0				
TGACGACGCGGTCDAAT	3.0	0.0				
AGCCAGCGCCGCCIAGC				6.4	8.9	
TGCCAGCGCCGTCIAGC				4.9	3.8	

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	African Americans			European Americans		
Gene	**Frequency (%)**			**Frequency (%)**		
Haplotype	**Cases**	**Controls**	**Global *P***	**Cases**	**Controls**	**Global *P***
AGCCGAATCGGTTIAGC				0.0	3.3	
TACCAGCGCCGTCIGGC				4.1	0.0	

[1] AGC : OR = .28 [.07 – 1.12] *P* = 0.07 in African Americans

The distal and proximal promoter SNPs, a subset of the total variants typed in *IL10*, were analyzed for haplotype associations using the haplotype trend regression method (Shrestha *et al.*, 2006). For each haplotype with *P* (χ^2) < .10, an odds ratio (OR), 95% confidence ratio (CI), and CLR *P* value was determined. The global *P* value is based on the likelihood ratio test between cases and controls. Distal promoter haplotypes include the ordered SNPs: rs1800890, rs6703630, and rs6693899. Proximal promoter haplotypes include the ordered SNPs: −1082 (rs1800896), −819 (rs1800871), and −592 (rs1800872). The *IL10* gene includes the ordered SNPs: rs1800890, rs6703630, rs6693899, rs5743624, rs1800893, rs1800896, rs1800872, rs1518110, rs1554286, rs1878672, rs3024494, rs3024509, rs3024496, rs9282739, rs3024498, rs6697497, and rs6687786. Nucleotide substitutions and positions for SNP loci are presented in Supplemental Table 1.