Original Research *Highlight article*

Hepatitis B infection outcome is associated with novel human leukocyte antigen variants in Ghanaian cohort

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Impact statement

Genetic association studies can determine the effect size of gene loci on disease outcomes. In the arena of HBV infections, HLA alleles that associate with HBV outcomes can be used in clinical management decisions. This potential translational utility can shape the future management of HBV infections by identifying at-risk individuals and tailoring medical interventions accordingly. This precision medicine motif is currently only a nascent idea. However, it has stakes that may well override the current "wait and see" approach of clinical management of HBV infections. Here, we have identified HLA alleles associated with HBV outcome in a Ghanaian cohort. Our findings support the motif that HLA alleles associate with HBV outcome along geo-ethnic lines. This buttresses the need for further population pivoted studies. In the long term, our findings add to efforts towards the development of an HLA molecular-based algorithm for predicting HBV infection outcomes.

Abstract

Chronic hepatitis B infection is an important medical problem in sub-Saharan Africa. With increasing concerns of dwindling access to needed care, increasing cost of treatment, and rising prevalence of dire outcomes like liver cirrhosis and hepatocellular cancer, the need to determine the genetic associations underpinning hepatitis B virus persistence or clearance in a population comes to the fore. Genetic association studies have suggested a variation in human leukocyte antigen alleles associated with hepatitis B virus outcome along geo-ethnic lines. We investigated the association of human leukocyte antigen alleles to hepatitis B virus outcome against this backdrop. We used targeted next generation sequencing to type the human leukocyte antigen class I and II alleles of 173 study participants. These comprised of 92 cases with chronic hepatitis B infection and 81 healthy controls with serological evidence of naturally cleared hepatitis B virus infection. We have identified human leukocyte antigen alleles associated with hepatitis B virus clearance and persistence for the first time in a Ghanaian population. The class 1 allele C*16:01 (odds ratio (OR) = 3.4, confidence interval (CI) = 1.6–7.0, *P*-value = 0.01) was associated with hepatitis B virus persistence. Four class I alleles and one class II allele: A*34:02 (OR = 0.1, CI = 0.04–0.2, *P*-value = 3.4e-05), A*74:01 (OR = 0.3, CI = 0.2–0.7, *P*-value = 0.0135), B*13:02 (OR = 0.04, CI = 0.01–0.2, *P*-value = 0.000172), C*08:04 (OR = 0.06, CI = 0.01–0.2, *P*-value = 7.83e-05), and DRB1*08:04 (OR = 0.2, CI = 0.03–0.27, *P*-value = 0.000252) were associated with hepatitis B virus clearance. Our data show that previously reported human leukocyte antigen alleles associations to hepatitis B virus outcome are not found in this Ghanaian study. This study has therefore identified human leukocyte antigen types that are associated with either hepatitis B virus persistence or clearance and highlights the importance of geo-ethnic pivoted studies in determining the genetic associations to acute hepatitis B virus infection outcome.

Keywords: Human leukocyte antigen, chronic hepatitis B infection, association, genetics, virology, immunology

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Introduction

Chronic hepatitis B (CHB) is still important in the disease morbidity and mortality books of many countries, especially those in sub-Saharan Africa. In 2015, the World Health Organization (WHO) estimated that 257 million people were living with CHB worldwide; 887,000 deaths were reported in 2015 from CHB-related cirrhosis and hepatocellular cancer (HCC). As of 2016, only 4.5 million (16.7%) of persons diagnosed with CHB were on treatment (WHO Hepatitis B fact sheet, 2017).

The plurality of outcomes of CHB ranges from asymptomatic chronic infection to chronic hepatitis B disease, hepatocellular cancer, and liver cirrhosis.[1] Acute hepatitis B virus (HBV) infection in adults results in viral persistence in 5–10% of cases.[2] The determinants of outcome following HBV infection are not well understood.[3] Age, sex, and immune status have been reported to be associated with outcome following HBV infection.[4] The immune response has been conceptually implicated as vital to the determination of outcome following HBV infection.

The key immunological determinant is thought to be the potency of the CD8+ T cell response. This leads to clearance of the virus (resolution) if sufficient; or persistence of the virus (chronic hepatitis B) if not sufficient. This is undergirded by the adequacy of the HLA-restricted antigen presentation to CD4+ T helper cells, and failure or success to mount an effective antibody response to the core and surface antigens on HBV.[1,5–10] HLA proteins execute the vital function of presenting foreign antigens to CD8+ cytolytic T cells (class 1) and CD4+ helper T cells (class 2).[11] HLA genes are the most polymorphic loci known in the human genome. Thus, they serve as ideal candidates for genetic association studies into the determinants of outcome following acute HBV infection.[3]

Previous studies have investigated the association of HLA alleles with HBV persistence or clearance.[12–14] Thursz *et al.*[15] demonstrated that MHC class II allele DRB*1302 was associated with reduced risk of chronic HBV infection in the Gambia. This finding seems to vary among chronic HBV-infected patients in different parts of the world. Wang *et al.*[14] proposed that these seeming discordant conclusions can be brought closer to harmony by conducting HLA polymorphism association studies in different ethnic groups around the world.

With the rising importance of precision medicine, it will benefit future clinical management of CHB to be able to predict the outcome of CHB individually. This will require a molecular prediction algorithm that includes population-specific independent factors that are predictive of CHB outcome. One important step to this goal will be to identify population-specific HLA alleles associated with HBV persistence and clearance. This study sought to interrogate the association between HLA alleles and HBV infection outcome in a Ghanaian study population.

Materials and methods

Study participants recruitment

Ninety-two chronic HBV cases were recruited from the Department of Medicine's Liver/Gastroenterology clinic. This clinic is run every Tuesday at the old medical block of KBTH. All cases were verbally counseled on the study and informed consent was documented. Cases were identified as patients with at least six months history of persistent hepatitis B surface antigen (HBsAg) positivity. Cases were not stratified according to outcome and included asymptomatic CHB, persistently active CHB, liver cirrhosis, and hepatocellular cancer from CHB. A data collection tool was used to document demographic details, general medical history, and some laboratory results.

Eighty-one controls were recruited from replacement blood donors of the KBTH blood bank. Replacement blood donors are family or friends of patients who have received blood transfusions that must be replaced. It is a system utilized by the KBTH to maintain stocks in the blood bank. All controls were verbally counseled on the study and informed consent was documented. Controls have had previous hepatitis B infection and have cleared the virus. Controls were identified as replacement donors with HIV (human immunodeficiency virus) and HBsAg-negative serological tests, and IgG positive for HBcAb (hepatitis B core antibody) and/or HBsAb (hepatitis B surface antibody). Measurement of HBsAg, HBcAb, and HBsAb was done using an immunochromatographic-based rapid diagnostic kit (Dia Spot®) and following the manufacturer's instruction. Over 200 replacement donors were screened. A data collection tool was used to document demographic details.

Exclusion criteria for recruitment included participants who refused to consent for the study, cases with diagnosed co-morbidities such as alcoholic liver disease, hepatitis C, and HIV infections.

Sample collection and HLA assignment

A buccal swab was taken for each participant. Buccal swabs were shipped to Georgetown University Medical Center for HLA typing by targeted next generation sequencing.

Isolation of DNA using magnetic beads (KingFisher Flex Purification Instrument, ThermoFisher) was followed by long-range amplification of HLA loci in separate polymerase chain reactions.[16] The class I (HLA-A, -B, -C) amplicons included all exons and introns including portions of the 5′ and 3′ untranslated regions. The DRB1 amplicon includes exons 2–3. The DQB1 amplicon includes exons 2–4. DRB1 and DQB1 analysis includes only exons; introns are not evaluated.

The pooled amplicons were purified and sheared to an average size of 700 base pairs by sonication. A DNA library was constructed using an Accel-NGS 2S Plus DNA library kit (Swift Biosciences, Ann Arbor, MI, USA). DNA fragments were tagged with one unique dual index combination (Swift Biosciences). Libraries from 96 individuals were combined, purified, and 600–1200 bp DNA fragments

selected. The pooled libraries were sequenced simultaneously in a single 500 cycle (V2) paired-end run using an Illumina MiSeq (Illumina, San Diego, CA, USA).

Data analysis used the AssignTM for TruSightTM HLA software (version 2.1.0.934, Illumina Inc., San Diego, CA, USA). Sequencing data were interpreted using the July 2018 IPD-IMGT/HLA database 3.33.0.[17]

Data analysis

Data cleaning was done using Excel. Exploratory data analysis was done in R version 3.6. Association analysis was done using the python script for HLA association analysis, PyHLA.[18] Odds ratios were calculated in R using both 2×2 contingency tables and univariate logistic regression. Multivariate logistic regression models used all HLA alleles with a mean allele frequency greater than 5% as independent variables. Haplotype construction and analysis were done using the software Hapl-o-Mat.[19]

Results

Characteristics of study participants recruited

A total of 173 study participants were recruited; 92 cases and 81 controls. Both groups were similar in gender distribution (Chi-square = 0, *P*-value = 1). However, the case group was significantly older (median age = 36) compared to the controls group (median age = 33, Mann–Whitney U test statistic = 4428, *P*-value = 0.03). Both groups also differed in ethnicity distribution (Fisher's test, *P*-value = 0.03) (Table 1). Class I and II HLA distribution between the two groups was similar (Chi-square, *P*-value = 0.62 and 0.85, respectively, Table 1).

Table 1. Characteristics of study participants recruited.

Characteristic	Case	Control	*P*-value
Number (*n*)	92	81	
Gender (*n*, %)			
Male	52 (30)	69 (40)	**5.33e-05**[a]
Female	40 (23)	12 (7)	
Age	36	33	**0.03**[b]
Ethnicity (*n*, %)			
Akan	49 (30.6)	46 (26.6)	**0.003**[a]
Ewe	10 (6.9)	14 (8.1)	
Ga-Adangbe	11 (7.5)	20 (11.6)	
Others	14 (8.1)	1 (0.6)	
HLA Class I (*n*)			
A	184	156	0.62[c]
B	184	152	
C	182	158	
HLA Class II (*n*)			
DPB1	80	160	0.85[c]
DQB1	182	160	
DRB1	184	160	

[a]Fisher's exact test for count data.
[b]Mann–Whitney U test.
[c]Chi-square test.
Note: Statistically significant *P*-values are in bold. The HLA Class I and II data presented here represent count summaries of the respective alleles for cases and controls.

HLA haplotype frequencies for both cases and controls were determined using Hapl-o-Mat.[19] An expectation maximization (EM) algorithm was used that resolved for phase ambiguity in the HLA data. The algorithm uses a maximum likelihood estimation to compute the most probable set of haplotypes that explain the unphased genotype input.[20] The HLA haplotype frequencies from the Ghanaian study population (Supplementary Table 1) were compared with other populations from the US National Marrow Donor Program (NMDP) using principal components analysis (PCA).[21] The first principal component accounted for over 90% of the variance in the Ghanaian haplotype frequencies (Supplementary Figure 1). The first and second PCAs demonstrate that the Ghanaian haplotype frequencies do not have close genetic proximity to any group in the NMDP database (Supplementary Figure 2).

Tests for Hardy Weinberg equilibrium were done in Arlequin version 3.5.2.2.[22] HLA haplotype frequencies for cases met with Hardy Weinberg equilibrium expectations. HLA haplotype frequencies for controls did not meet Hardy Weinberg equilibrium expectations (Supplementary Figure 3).

HLA alleles associated with HBV persistence

Two alleles were associated with HBV persistence following 2×2 contingency table and univariate logistic regression analysis (Table 2, Figure 1). The class I HLA allele, C*16:01 (OR = 3.4, CI: 1.6–7.0, *P*-value = 0.005) and the class II HLA allele, DQB1*05:01 (OR = 3.3, CI = 1.5–7.5, *P*-value = 0.03). The risk of both alleles seemed additive, as increasing the presence of the allele was associated with increasing odds of HBV persistence (cases) (Cochrane Armitage trend test (CATT) C*16:01 = −3.25, *P*-value = 0.001); DQB1*05:01 = −2.73, *P*-value = 0.006) (Supplementary Table 8).

For the class I HLA allele C*16:01, 39 study participants had this allele. Five were homozygous—all cases. C*16:01 was heterozygous in 24 cases and 10 controls. A multivariate logistic regression analysis of the effect size of C*16:01 on HBV outcome was done using all HLA alleles as independent variables. This showed C*16:01 was associated with HBV persistence (OR=3.1, CI: 1.5–6.5, *P*-value = 0.01). However, when the model was adjusted for age and ethnicity as covariates, the association of C*16:01 allele with HBV persistence was lost (OR = 0.21, CI = 0.09–0.48, *P*-value = 0.00002) (Table 3, Figure 2).

For the class II HLA allele DQB1*05:01, 33 study participants had this allele, 5 were homozygous—all of whom were cases. DQB1*05:01 was heterozygous in 17 cases and 8 controls. A multivariate logistic regression analysis of the effect size of DQB1*05:01 on HBV outcome was done using all HLA alleles as independent variables. This, however, did not identify DQB1*05:01 as an allele associated with HBV persistence or clearance.

HLA alleles associated with HBV clearance

Four class I and four class II alleles were associated with HBV clearance following 2×2 contingency table analysis

Table 2. Alleles associated with HBV outcome following 2 × 2 contingency table analysis.

Allele	Number		Odds ratio (OR)	Confidence interval	*P*-value
	Case	Control			
C*16:01	29	10	3.4 (3.3)	1.6–7.0	0.005 (0.004)
DQB1*05:01	27	8	3.3 (2.9)	1.5–7.5	0.03 (0.02)
A*34:02	6	31	0.14 (0.12)	0.06–0.34	2.57e-05 (6.86e-6)
A*74:01	15	32	0.38 (0.3)	0.2–0.34	3.24e-02 (0.0008)
B*13:02	2	27	0.05 (0.04)	0.01–0.22	2.02e-06 (3.53e-05)
C*08:04	3	27	0.08 (0.07)	0.02–0.27	6.28e-06 (2.01e-5)
DQB1*04:02	18	40	0.36 (0.25)	0.2-0.64	4.30e-03 (5.4e-5)
DQB1*06:02	22	43	0.41 (0.2)	0.3-0.8	2.33e-02 (2.4e-8)
DRB1*08:04	17	39	0.31 (0.24)	0.2-0.6	1.60e-03 (5.25e-5)
DRB1*15:03	20	43	0.38	0.2-0.7	4.60e-03

*Results for univariate logistic regression outputs placed in brackets for ORs and p-values

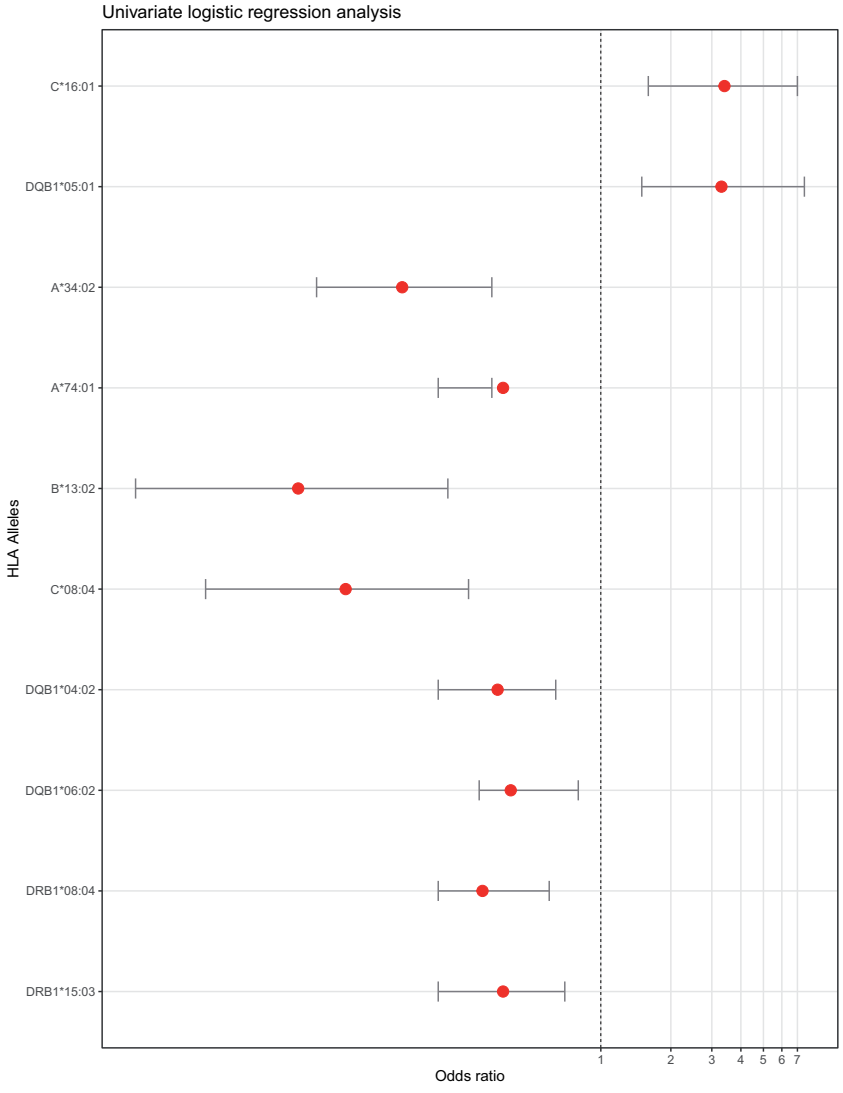

Figure 1. HLA alleles with association to HBV outcome following univariate logistic regression analysis. HLA alleles with odds ratios less than 1 are associated with HBV clearance; and alleles with odds ratios greater than 1 are associated with HBV persistence. (A color version of this figure is available in the online journal.)

and univariate logistic regression analysis (Table 2, Figure 1). Following multivariate logistic regression, all four class I and only one class II allele remained significantly associated with HBV clearance (Table 2, Figure 1).

Table 3. Alleles associated with HBV outcome following multivariate logistic regression analysis.

Allele	Odds ratio (OR)	Confidence interval	P-value
C*16:01	3.1	1.5–6.5	0.01
A*34:02	0.1	0.04–0.2	3.35e-05
A*74:01	0.3	0.2–0.7	0.0135
B*13:02	0.04	0.01–0.2	0.0002
C*08:04	0.07	0.01–0.2	7.83e-5
DRB1*08:04	0.2	0.1–0.4	0.00003

*An additive model with Bonferroni's correction for multiple testing was used.

Class I HLA alleles associated with HBV clearance

The class I alleles, A*34:02 (OR = 0.14, CI: 0.06–0.34, *P*-value = 2.57e-05); A*74:01 (OR = 0.3, CI: 0.2–0.7, *P*-value = 0.01), HLA supertype association A03[23]; B*13:02 (OR = 0.05, CI: 0.01–0.22, *P*-value = 2.02e-06), and C*08:04 (OR = 0.08, CI: 0.02–0.27, *P*-value = 6.28e-06) were associated with HBV clearance. The effect of all these HLA alleles seemed to be additive, as decreasing the presence of this allele was associated with increasing odds of HBV persistence (CATT: A*34:02, z = 5, *P*-value = 5.4e-7; A*74:01, z = 3.2, *P*-value = 0.002; B*13:02, z = 5.5, *P*-value = 4.4e-8; C*08:04, z = 5.2, *P*-value = 1.49e-7) (Supplementary Table 8).

Thirty-seven study participants had the A*34:02 allele. None were homozygous for it. A*34:02 was heterozygous in 6 cases and 31 controls with the allele. Forty-seven study participants had the A*74:01 allele; only one case was homozygous for it. A*74:01 was heterozygous in 14 cases and 32 controls with the allele. Of the 29 study participants

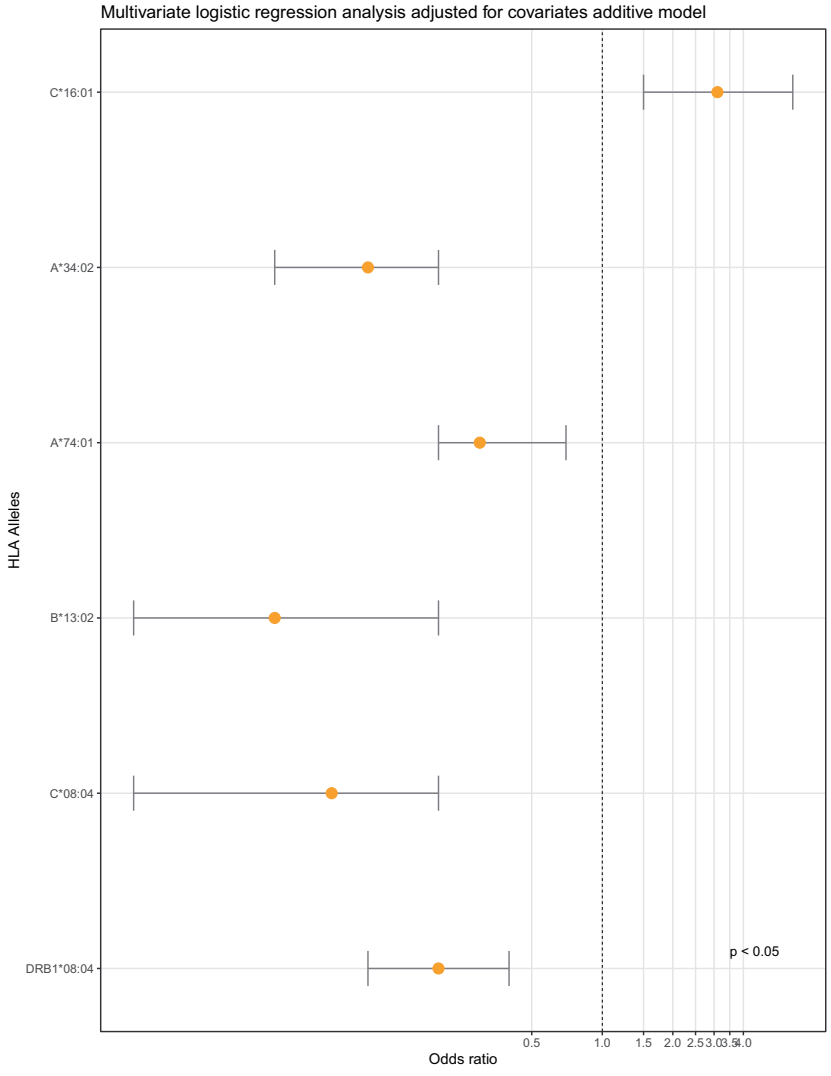

Figure 2. HLA alleles with association to HBV outcome following multivariate logistic regression analysis. All HLA alleles with allele frequency greater than 5% were used as independent variables in this model. HLA alleles with odds ratios less than 1 are associated with HBV clearance; and alleles with odds ratios greater than 1 are associated with HBV persistence. (A color version of this figure is available in the online journal.)

with the B*13:02 allele, none were homozygous for it. B*13:02 was heterozygous in 2 cases and 27 controls with the allele. Of the 30 study participants with the C*08:04 allele, none were homozygous for it. C*08:04 was heterozygous in 3 cases and 27 controls with the allele. Therefore, these protective alleles were more frequent in controls than cases.

A multivariate logistic regression analysis of the effect size of the HLA alleles was done using all HLA alleles as independent variables. This showed A*34:02 (OR = 0.1, CI: 0.04–0.2, *P*-value = 3.4e-5); A*74:01 (OR = 0.3, CI = 0.2–0.7, *P*-value = 0.0135); B*13:02 (OR = 0.04, CI = 0.01–0.2, *P*-value = 0.000172); and C*08:04 (OR = 0.06, CI = 0.01–0.2, *P*-value = 7.83e-05) were still associated with HBV clearance. When the model was adjusted for age, sex, and ethnicity as confounding covariates, the association of A*34:02, A*74:01, B*13:02, and C*08:04 alleles with HBV clearance was still protective (Table 3, Figure 2).

Class II HLA alleles associated with HBV clearance

The class II alleles, DQB1*04:02 (OR = 0.36, CI: 0.2–0.64, *P*-value = 4.3e-3), DQB1*06:02 (OR = 0.4, CI: 0.3–0.8, *P*-value = 2.33e-2), DRB1*08:04 (OR = 0.3, CI: 0.2–0.6, *P*-value = 1.6e-3), and DRB1*15:03 ((OR = 0.38, CI: 0.2–0.7, *P*-value = 4.6e-3) were associated with HBV clearance. The effect of all these HLA alleles seemed to be additive, as decreasing the presence of this allele was associated with increasing odds of HBV persistence (CATT: DQB1*04:02, z = 3.7, *P*-value = 0.0002; DQB1*06:02, z = 3.1, *P*-value = 0.002; DRB1*08:04, z = 4.2, *P*-value = 3.2e-5; DRB1*15:03, z = 5.4, *P*-value = 6.7e-8).

Fifty-eight study participants had the DQB1*04:02 allele. Two cases and one control were homozygous for this allele. DQB1*04:02 was heterozygous in 16 cases and 39 controls with the allele. Sixty-five study participants had the DQB1*06:02 allele. Four cases and one control were homozygous for this allele. DQB1*06:02 was heterozygous in 18 cases and 42 controls with the allele. Of the 56 study participants with the DRB1*08:04 allele, none were homozygous for it. DRB1*08:04was heterozygous in 17 cases and 39 controls with the allele. Of the 30 study participants with the DRB1*15:03 allele, none were homozygous for it. DRB1*15:03 was heterozygous in 3 cases and 27 controls with the allele. Therefore, these class II protective alleles were also more frequent in controls than cases.

A multivariate logistic regression analysis of the effect size of the class II HLA alleles was done using all HLA alleles as independent variables and adjusted for age, sex, and ethnicity as confounding covariates. This showed DQB1*04:02, DQB1*06:02, and DRB1*15:03 alleles were no longer associated with HBV clearance. Only DRB1*08:04 (OR = 0.2, CI = 0.03–0.27, *P*-value = 0.000252) were still associated with HBV clearance in the multivariate logistic model (Table 3, Figure 2).

Haplotype analysis shows A*34:02∼B*13:02, A*34:02∼DRB1*08:04, and A*74:01∼C*08:04 are associated with HBV clearance

Supplementary Table 2 shows the top 10 most common one field haplotypes among all study participants. Supplementary Tables 3 and 4 show the most frequent haplotypes for cases and controls, respectively. The most frequent one field haplotype among all study participants were A*34∼B*13∼C*08∼DQB1*06∼DRB1*08 (16.25%) and A*74∼B*53∼C*04∼DQB1*04∼DRB1*15 (16.25%). Both haplotypes were found exclusively among the controls. The most common haplotype among the cases were A*30∼B*42∼C*07∼DQB1*04∼DRB1*03 (2.71%) and A*02∼B*53∼C*04∼DQB1*06∼DRB1*15 (1.6%).

Supplementary Table 5 shows the top 10 most frequent two field haplotypes for all study participants. Supplementary Tables 6 and 7 show the most frequent haplotypes for cases and controls, respectively. The most common two field haplotypes among all study participants were A*34:02∼B*53:01∼C*04:01∼DQB1*06:02∼DRB1*15:03 (7.9%) and A*74:01∼B*13:02∼C*08:04∼DQB1*04:02∼DRB1*08:04 (7.6%). Both haplotypes were found exclusively among the controls. The most common two field haplotype among cases was A*30:01∼B*42:01∼C*17:01∼DQB1*04:02∼DRB1*03:02 (3.4%).

Table 4. HLA haplotypes significantly associated with outcome (clearance) following acute HBV infection.

Haplotype	Case number	Control number	OR	*P*-value
A*34:02∼B*13:02	0	27	0.01	0.00000000007991*
A*34:02∼DRB1*08:04	1	28	0.02	0.0000000005546*
A*74:01∼C*08:04	0	26	0.01	0.0000000002136*

*Statistically significant.

Table 5. HLA loci zygosity for all study participants.

Loci	Zygosity	Case (number)	Control (number)	Total
A	Homozygous	7	1	8
	Heterozygous	85	78	163
B	Homozygous	9	3	12
	Heterozygous	83	76	159
C	Homozygous	15	5	20
	Heterozygous	77	74	151
DPB1	Homozygous	17	43	60
	Heterozygous	20	23	43
DQB1	Homozygous	22	6	28
	Heterozygous	69	74	143
DRB1	Homozygous	7	3	10
	Heterozygous	85	77	172

Table 6. Association between HLA loci heterozygosity and HBV persistence.

HLA loci	Odds Ratio	*P*-value
A	0.2	0.9
B	0.9	0.8
C	0.4	0.05
DPB1	0.3	0.06
DQB1	0.3	0.005*
DRB1	0.5	0.3

*Statistically significant.

Next, we investigated the relationship between haplotypes and HBV outcome. We used HLA alleles with significant association with HBV outcome in a multivariate logistic regression adjusted for age, sex, and ethnicity as covariates (Table 3). Combinations of these HLA alleles were used to form two, three, and four block haplotypes. These haplotypes were then tested for association with outcome after HBV infection using a univariate logistic model. Haplotypes A*34:02∼B*13:02, A*34:02∼DRB1* 08:04 and A*74:01∼C*08:04 were the only haplotypes significantly associated with HBV clearance (Table 4).

Heterozygosity at class II locus DQB1 is associated with reduced odds of HBV persistence

Next, we interrogated the association between heterozygosity at an HLA class loci and outcome following HBV infection. Heterozygosity at an HLA loci is conceptually associated with a higher odd for antigen recognition, binding, and presentation to immune cells.[24,25] This in turn may lead to a higher odds of HBV clearance. Therefore, we tested the hypothesis that HLA loci heterozygosity will be associated with increased odds of HBV clearance or reduced odds of HBV persistence.

Table 5 summarizes the zygosity for the HLA loci typed in all the study participants. The Cochran–Mantel–Haenszel test of independence showed that there was an association between zygosity and outcome of HBV infection across all HLA loci (P-value = 2.2e-16; Woolf test P-value = 0.6). Table 6 shows the summary of the effect size of the association between HLA loci heterozygosity and HBV persistence. Heterozygosity at all HLA loci was associated with a reduced odds of HBV persistence (OR < 1). However, only heterozygosity at the class 2 HLA DQB1 loci was significantly associated with a reduced odds of HBV persistence (OR = 0.3, P-value = 0.005).

Discussion

Genetic association studies (candidate gene) are powered to identify the effect size of a genetic locus on a disease. The current body of scientific evidence suggests that HLA allele types influence HBV infection outcome along geo-ethnic lines.[14] This tacit consensus in the field of HLA/HBV associations highlights the need for population-tailored study designs. Here, we show the importance of this paradigm. We set out to determine the HLA alleles associated with HBV outcome in a Ghanaian study population. We have identified HLA alleles never reported as associated with outcomes following acute HBV infection.

The HLA class I allele C*16:01 was associated with a 3-fold increase in HBV persistence. A*34:02, A*74:01, B*13:02, and C*08:04 were associated with HBV clearance. Overall, the HLA class 1 loci had the most significant associations similar to the findings by Thio *et al*.[3] This implicates the role of CD8+ T cells as pivotal in determining outcome following acute HBV infection. This finding also agrees with a study that showed that chimpanzees infected with HBV could not clear the virus after CD8+ T cell depletion.[8] Of the class II HLA alleles, only DRB1*08:04 was significantly associated with HBV clearance.

C*16:01 was significantly associated with HBV persistence. This has never been reported. The HLA molecule encoded by this allele may be poor at antigen presentation to CD8+ T cells; therefore, its association with HBV persistence. However, functional studies are yet to implicate this allele in poor antigen presentation to CD8+ T cells.

The molecules encoded by A*34:02, A*74:01, B*13:02, and C*08:04 are likely to be very efficient at viral epitope presentation to CD8+ T cells. This may explain their association with HBV clearance. Functional studies are yet to validate this. This is the first study to report on this association. This may be on account of this allele's frequency in other populations compared to the Ghanaian population.

The association of heterozygosity with outcome following HBV infection was significant for only the DQB1 loci. This was similar to findings in the Gambian cohort[15] and a Caucasian cohort.[3] The inability to detect a significant association between heterozygosity in class I HLA loci and HBV outcome may stem from inadequate statistical power of this study design.

We recognize some limitations of this study. This study only focused on HLA alleles as the predictors of outcome following HBV infection. Factors such as other genetic determinants (cytokine genes and expression profiles, for example), nutritional status, and socioeconomic strata were completely ignored. Our application of Bonferroni correction in the multivariate analysis may have excluded otherwise significant allele associations. This may have increased type 2 errors. Our reporting of novel HLA associations requires further studies to investigate biological plausibility of these alleles. Compared to other studies, our relatively small sample size may have limited the power of the experimental design to detect allele associations. We may therefore have missed significant alleles with low frequency on account of this. Replication of this study in different populations in Ghana and elsewhere will help to validate the findings. Notwithstanding these limitations, the data generated here bring into perspective the potentially important role of HLA type differences as determinants of HBV infection outcome.

In summary, our data show that HLA class I molecules are significantly associated with HBV infection outcome in the Ghanaian population. This suggests that the role of cytolytic CD8+ T cells may be pivotal to acute HBV infection persistence or clearance. Further functional studies to interrogate this hypothesis are needed. The HLA heterozygous advantage is most significant at the class II HLA DQB1 loci. Overall, the data highlight the importance of geo-ethnic pivoted studies in determining the genetic factors associated with acute HBV infection outcome.

AUTHOR'S CONTRIBUTIONS

KZT, KAK, and OQ conceived the study; KZT, TA and IB performed study participants recruitment, data and sample collection; KZT, KAK, and OQ analyzed the data and drafted the manuscript; OQ and KAK supervised the study. All authors critically reviewed and edited the manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL STATEMENT

Ethical clearance for this study was obtained from the Institutional Review Boards of the Noguchi Memorial Institute of Medical Research (NMIMR, Study Number: 074/17–18) and Korle-Bu Teaching Hospital (KBTH, Reference Number 00080/2018); and the research and development office of the National Blood Services of Ghana (Research Protocol NBSGRD/11110/11).

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

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