Received: 21 September 2016 | Accepted: 27 February 2017

DOI: 10.1111/liv.13405

ORIGINAL ARTICLE

WILEY Liver INTERNATIONAL

Role of HLA-DP and HLA-DQ on the clearance of hepatitis B virus and the risk of chronic infection in a multiethnic population

Julieta Trinks[1,2] | Nao Nishida[3,4] | María Laura Hulaniuk[1] | Mariela Caputo[2,5] | Takayo Tsuchiura[3] | Sebastián Marciano[6] | Leila Haddad[6] | Jorgelina Blejer[7] | Sonia Bartoli[8] | Beatriz Ameigeiras[9] | Silvia E. Frías[9] | Cecilia Vistarini[9] | Fabiana Heinrich[10] | Carlos Remondegui[11] | Susana Ceballos[11] | Gustavo Echenique[12] | Miguel Charre Samman[13] | Claudia D'Amico[14] | Amalia Rojas[14] | Alfredo Martínez[15] | Ezequiel Ridruejo[15] | Roberto J. Fernández[7] | Leandro Burgos Pratx[16] | Horacio Salamone[16] | Félix Nuñez[16] | Omar Galdame[6] | Adrián Gadano[6] | Daniel Corach[2,5] | Masaya Sugiyama[3] | Diego Flichman[2,17] | Katsushi Tokunaga[4] | Masashi Mizokami[3]

[1]Basic Sciences and Experimental Medicine Institute (ICBME), University Institute of the Italian Hospital of Buenos Aires, Buenos Aires, Argentina

[2]Scientific and Technological Research Council (CONICET), Buenos Aires, Argentina

[3]Department of Hepatic Disease, Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Chiba, Japan

[4]Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

[5]Genetic Fingerprints Unit, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina

[6]Hepatology Unit, Italian Hospital of Buenos Aires, Buenos Aires, Argentina

[7]"Hemocentro Buenos Aires" Foundation, Buenos Aires, Argentina

[8]Hemotherapy Unit, "Pablo Soria" Hospital, San Salvador de Jujuy, Argentina

[9]Hepatology Unit, "José María Ramos Mejía" General Hospital, Buenos Aires, Argentina

[10]"San Antonio" Hospital, Gualeguay, Argentina

[11]Infectology and Tropical Medicine Unit, "San Roque" Hospital, San Salvador de Jujuy, Argentina

[12]"Nuestra Señora del Rosario" Clinic, San Salvador de Jujuy, Argentina

[13]Central Public Health Laboratory, San Salvador de Jujuy, Argentina

[14]Ambulatory Medical Specialities Center (CEMA), Mar del Plata, Argentina

[15]Center for Medical Education and Clinical Research "Norberto Quirno" (CEMIC), Buenos Aires, Argentina

[16]Transfusional Medicine Unit, Italian Hospital of Buenos Aires, Buenos Aires, Argentina

[17]Virology Department, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina

Correspondence
Julieta Trinks, MD, PhD, Basic Sciences and Experimental Medicine Institute (ICBME), University Institute of the Italian Hospital of Buenos Aires, Buenos Aires, Argentina.
Email: julieta.trinks@hospitalitaliano.org.ar

Abstract

Background & Aims: HBV infection exhibits geographical variation in its distribution in South America. While HBV rates are low in central Argentina, the north-western region exhibits intermediate HBV rates. Unfortunately, the reasons that could explain this difference are still unknown.

Abbreviations: AIMs, ancestry informative markers; ALT, alanine aminotransferase; anti-HBc, total anti-HBV core; CI, confidence interval; GWAS, genomewide association study; HBeAg, hepatitis B e antigen; HBsAg, HBV surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HLA, human leucocyte antigen; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; SNPs, single nucleotide polymorphisms.

Julieta Trinks and Nao Nishida contributed equally to this study.

Funding information
This work was supported by Grants from Allende Foundation, Italian Hospital of Buenos Aires, PICT 2014-1675 to Julieta Trinks and by a Grant from the National Center for Global Health and Medicine (25-shi-202) to Masashi Mizokami.

Handling Editor: Gabriele Missale

Methods: A total of 1440 Argentines were recruited and grouped into HBV patients, HBV-resolved individuals and healthy controls. Genetic ancestry was assessed by analysis of biparental lineages and ancestry autosomal typing. SNPs of *HLA-DPA1* (rs3077), *HLA-DPB1* (rs9277542), *HLA-DQB1* (rs2856718) and *HLA-DQB2* (rs7453920) were determined, and HBV genotyping was performed by phylogenetic analysis in HBV patients.

Results: Native American ancestry prevailed in the north-western region when compared with central Argentina ($P<.0001$). However, no differences were observed among the three groups of each region. The distribution of HBV genotypes revealed significant differences ($P<.0001$). Three SNPs (rs3077, rs9277542 and rs7453920) showed a significant association with protection against chronic HBV and viral clearance in both regions. The remaining SNP showed a significant association with susceptibility to chronic HBV. The frequency rates of rs3077-T, related to protection against chronic HBV and viral clearance, were lower in north-western Argentina when compared with central Argentina. The same uneven frequency rates were observed for SNP rs9277542.

Conclusions: This is the first study addressing the associations between the *HLA-DP* and *HLA-DQ* loci and the protection against chronic HBV and viral clearance in a multiethnic South American population. The uneven distribution of *HLA-DP* and *HLA-DQ* supports the HBV epidemiological differences observed in these two regions of Argentina with dissimilar ancestry genetic background.

KEYWORDS
genotypes, HBV, HLA genes, Native American

1 | INTRODUCTION

Although the exact mechanism which determines hepatitis B virus (HBV) persistence or clearance is not completely understood, the reason for this difference in response is believed to be attributed to numerous inter-related factors, such as viral, host and extrinsic factors.[1]

Central and South-East Asia, Sub-Saharan Africa and the Amazon basin are high prevalence regions where the main route of infection is vertical transmission and horizontal transmission between children, whereby 90% will develop chronic hepatitis as infants or in early childhood. In contrast, Europe and USA are low prevalence areas where viral transmission mainly occurs during adulthood and 15% of those infected will develop chronic hepatitis.[1,2]

Ten genotypes of HBV have been identified so far (A-J), which are further subdivided into subgenotypes.[3] They have a distinct distribution. Genotype A is present in north-western Europe, North America, Central Africa and Asia, and genotype D has been found throughout the world. In contrast, genotypes F and H prevail in Native American populations.[3]

Key points

- While HBV rates are low in central Argentina, the north-western region exhibits intermediate endemicity. In this study, genetic ancestry, viral and host genetic factors were determined in 1440 subjects from these two distant geographical regions.
- SNPs rs3077 (*HLA-DPA1*), rs9277542 (*HLA-DPB1*) and rs7453920 (*HLA-DQB2*) were significantly associated with protection against chronic HBV and viral clearance in central and north-western Argentina.
- SNP rs2856718 (*HLA-DQB1*) was significantly associated with susceptibility to chronic HBV in both regions.
- The uneven distribution of *HLA-DP* and *HLA-DQ* support the HBV epidemiological differences observed in these regions of Argentina with dissimilar ancestry genetic background.

Cellular immune responses influenced by the highly polymorphic human leucocyte antigen (HLA) class I and II molecules are related to the outcome of any viral infection in acute or chronic infection.[4] In this regard, a genomewide association study (GWAS) revealed that 11 single nucleotide polymorphisms (SNPs) belonging to class II *HLA-DP* region are involved in HBV persistence or clearance among Japanese subjects.[5] However, upon independent validations, only two SNPs remained significant.[6] A second GWAS study has reported two SNPs (rs2856718 and rs7453920) within the *HLA-DQ* locus to be significantly associated with hepatitis B persistence.[7] Since then, several other studies conducted on other populations have investigated the role of HLA variants on development of persistent chronic HBV infection or its clearance.[8-12] However, no similar studies have been carried out in Latin America.

In the last thirty years, HBV has emerged as a problem among the Native American communities in the Amazon Basin and in other Amazon-related ecological systems from South America.[2]

Argentina is a South American country with a multiethnic population.[13] In the central and metropolitan region, the population consists mainly of European descendants, whereas in north-western Argentina the highest frequency of Native American ancestry has been observed.[13] With regard to HBV infection, the overall prevalence is low in the central region[14] with a high frequency of genotypes A and D.[15] In contrast, the north-western region exhibits intermediate HBV rates and a high prevalence of HBV genotype F,[15] particularly among those living in the area of the Yungas rainforest,[16,17] a transitional environment between the Andes highlands and the lowland tropical forest of the Amazonia. Unfortunately, the reasons that could explain the different features of HBV infection observed in two geographical regions of Argentina have not been explored so far.

Thus, this study aimed to determine the role of HLA-DP and HLA-DQ variants on the persistence or clearance of HBV infection and its potential association with HBV genotypes in two regions of Argentina with dissimilar ancestry genetic background.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

This study conformed to the 1975 Declaration of Helsinki and was approved by the Ethics Committee on Research from the Italian Hospital of Buenos Aires, "Ramos Mejía" Hospital, and the Health Ministry of Jujuy Province in Argentina.

2.2 | Characteristics of studied subjects

A total of 1440 Argentines were recruited in the period 2013-2015 in the central and north-western areas. All subjects signed an informed consent statement prior to their enrolment in this study and sample collection, and their basic demographic data were recorded.

Subjects were defined into three groups: (i) HBV-infected patients (201 from the central and 200 from the north-western region) who exhibited positivity for HBV surface antigen (HBsAg) and total anti-HBV core (anti-HBc) antibodies for more than 6 months, (ii) HBV-resolved individuals (318 from the central and 313 from the north-western region) who were negative for HBsAg but positive for total anti-HBc antibodies and (iii) healthy controls (207 from the central and 201 from the north-western region) who exhibited negativity for HBsAg and total anti-HBc antibodies.

HBV status was measured based on serological results for HBsAg and anti-HBc (ARCHITECT, Abbott, IL, USA). For clinical staging, inactive carrier state was defined by the presence of HBsAg with normal alanine aminotransferase (ALT) levels over 1 year and without evidence of cirrhosis. Chronic hepatitis was defined by elevated ALT levels persisting over 6 months. Cirrhosis was diagnosed by ultrasonography, platelet counts <100 000/cm^3 or a combination thereof. Histological confirmation by fine-needle biopsy of the liver was performed as required. Hepatocellular carcinoma (HCC) was diagnosed by ultrasonography, computerized tomography, magnetic resonance imaging, angiography, tumour biopsy or a combination thereof.

2.3 | Molecular evaluation of ancestry

In all samples, ethnicity was assessed in both maternal and paternal lineages by analysis of the presence or absence of Native American haplogroups in mitochondrial DNA (A2, B2, C and D1) and Y-chromosome SNPs (Q1a3a).[18]

In order to further increase the analysis of genetic ancestry, ancestry informative markers (AIMs) were characterized.[19] The parental populations used as reference groups were obtained from the Genome Diversity Project—Centre des etudes de polymorphisms humane (CEPH).

2.4 | HBV genotyping

Partial amplification of HBV *S* gene was carried out in all HBV-infected patients, and PCR products were bidirectionally sequenced. HBV genotypes were determined by phylogenetic analysis.[20]

The GenBank/EMBL accession numbers for the sequences reported in this study are KX631743-KX631919, KX641714-KX641745, KJ810920, KJ810961, KJ810930, KJ843166-KJ843195, KJ843209 and KJ843217.

2.5 | HLA-SNPs genotyping assays

SNPs of *HLA-DPA1* (rs3077), *HLA-DPB1* (rs9277542), *HLA-DQB1* (rs2856718) and *HLA-DQB2* (rs7453920) were determined by commercial TaqMan PCR assays (Applied Biosystems, Foster City, CA, USA).

2.6 | Statistical analyses

Hardy-Weinberg equilibrium (HWE) was performed on each SNP with a cut-off *P* value of .01. The chi-square test of independence and the odds ratio (OR) in two-by-two cross tables were used for comparisons

TABLE 1 Demographics of the recruited subjects from the two geographical regions of Argentina

Group	Healthy control (HBsAg−/Anti-HBc−)		Resolved individuals (HBsAg−/Anti-HBc+)		Persistent HBV infection (HBsAg+/Anti-HBc+)	
Region	Central	North-western	Central	North-western	Central	North-western
Number of samples	207	201	318	313	201	200
Gender (% male)	57%	55%	66%	71%	73%	67%
Age (average±SD)	36.7 ± 9.3	34.6 ± 9.9	55.4 ± 6.7	50.2 ± 7.2	57.2 ± 10.9	37.6 ± 5.8

SD, Standard Deviation.

of cases and control groups, using R-program (www.r-project.org). Statistical significance was defined by $P<.05$. Linkage disequilibrium was analysed with the r^2 statistic using Haploview software.[21] Logistic regression analyses adjusted for sex (used as independent variable) were performed to assess associations between the viral genetic factors, age, the place of birth and host genetic factors in the group of HBV-infected patients. HBV genotypes were grouped taking into consideration their worldwide geographical distribution (HBV/A and HBV/D vs HBV/F and HBV/H). Patients were classified into four age groups: 18-30, 31-40, 41-50 and ≥51 years.

Moreover, univariate and multivariate logistic regression analyses were performed to assess the effect of age, place of birth and host genetic factors on the risk of chronic infection. HBV genotypes were not included in these analyses as genotyping was performed in chronic HBV-infected patients, and not in spontaneous resolvers.

Meta-analysis was performed using the random-effects model (DerSimonian-Laird method) or fixed-effects model to calculate pooled OR and its 95% confidence interval (95% CI).

3 | RESULTS

3.1 | Characteristics of the study population

The demographics of the recruited subjects are shown in Table 1. When the age and gender distribution was compared between the same group from both areas, no significant differences were observed, except among the HBV-infected patients. In this group, the HBV-infected patients from north-western Argentina were significantly younger than those from the central region (37.6±5.8 years vs 57.2±10.9 years; $P=.0002$).

With regard to the group of HBV-infected patients, statistically significant differences were observed in the prevalence of HBV inactive carriers between central and north-western Argentina (35% vs 46%; $P=.0226$). Moreover, the HBeAg-positivity rate was significantly higher among HBV chronic patients from the central region (52% vs 10%; $P<.0001$) (Table 2).

3.2 | Molecular evaluation of ancestry

The prevalence of Native American maternal and paternal haplogroups was the lowest for the central region when compared with north-western Argentina (Figure 1A-D). However, no significant differences were observed in the distribution of the maternal and paternal haplogroups among the three groups of each geographical region (Figure 1A-D).

TABLE 2 Characteristics of the HBV patients included in this study

	Central region	North-western region
Inactive HBV carriers	70 (35%)*	92 (46%)*
Chronic hepatitis	115 (57%)	96 (48%)
	60 (52.2%) HBeAg [+]**	9 (9.4%) HBeAg [+]**
Cirrhosis	14 (7%)	12 (6%)
HCC	2 (1%)	N.D.
Total	201 (100%)	200 (100%)

N.D., Not determined due to the lack of diagnostic methods in the area.
*$P=.0226$; **$P<.0001$.

Furthermore, the major ancestry component as revealed by STRUCTURE analysis was as follows: 86.3% European for central Argentines, 88.2% Native American for north-western Argentines. The minor component (<2.8%) for these populations was the West African ancestry (Figure 2). In agreement with the results obtained for the analysis of matri and patrilineage, no significant differences were observed in the distribution of biparentally transmitted markers of the human genome among the three groups of each region (Figure 2).

3.3 | HBV genotyping

HBV *S* gene was successfully amplified in 356 out of 401 samples (88.8%): 186 samples (186/201; 92.5%) from the central and 170 samples (170/200; 85%) from the north-western region. Statistical significant differences were observed when the distribution of HBV genotypes was compared between the two studied regions (Figure 3).

3.4 | HLA-SNPs genotyping

In this case-control study, two SNPs in HLA-DP (rs3077 and rs9277542) and two SNPs in HLA-DQ (rs2856718 and rs7453920)

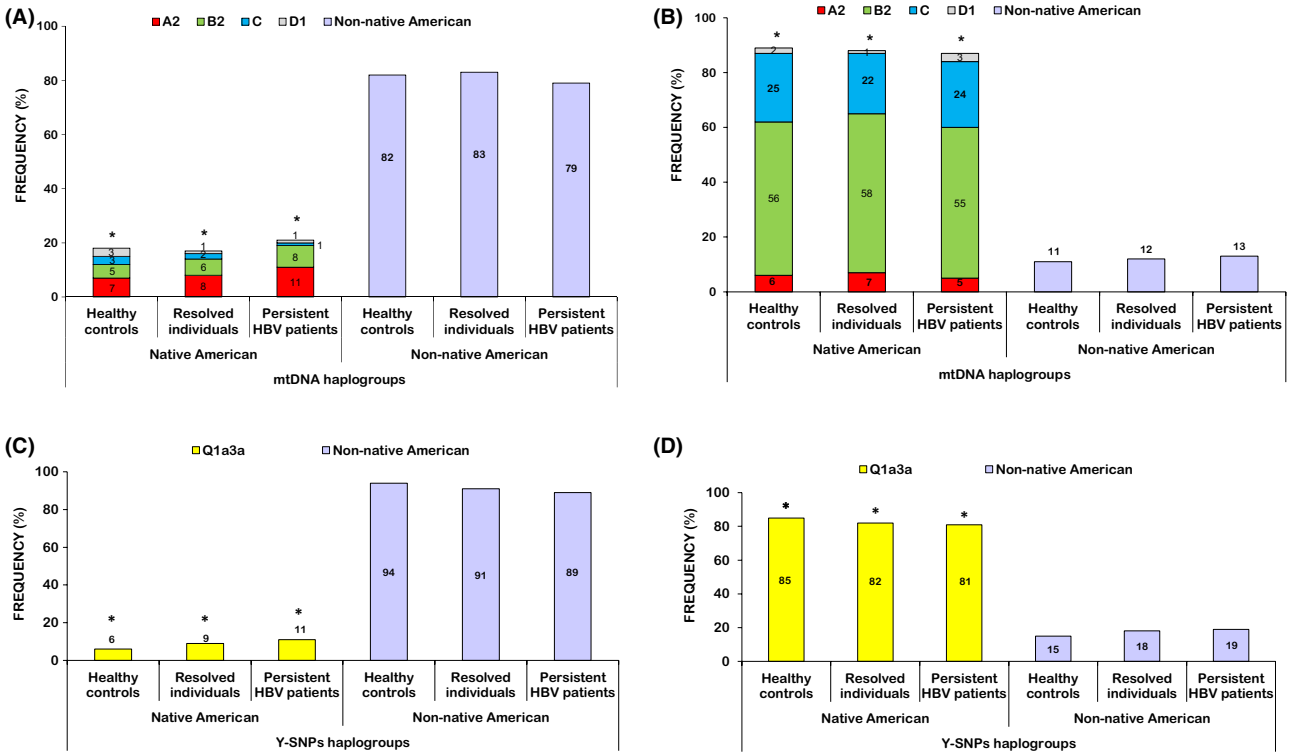

FIGURE 1 Prevalence of Native American mitochondrial DNA (mtDNA) haplogroups (A2, B2, C and D1) and Y-chromosome haplogroup (Q1a3a) among healthy uninfected controls, resolved HBV individuals and persistent HBV patients from central (A for mtDNA and C for Y-chromosome) and north-western Argentina (B for mtDNA and D for Y-chromosome). *P<.0001 when comparing central (A and C) with north-western Argentines (B and D respectively)

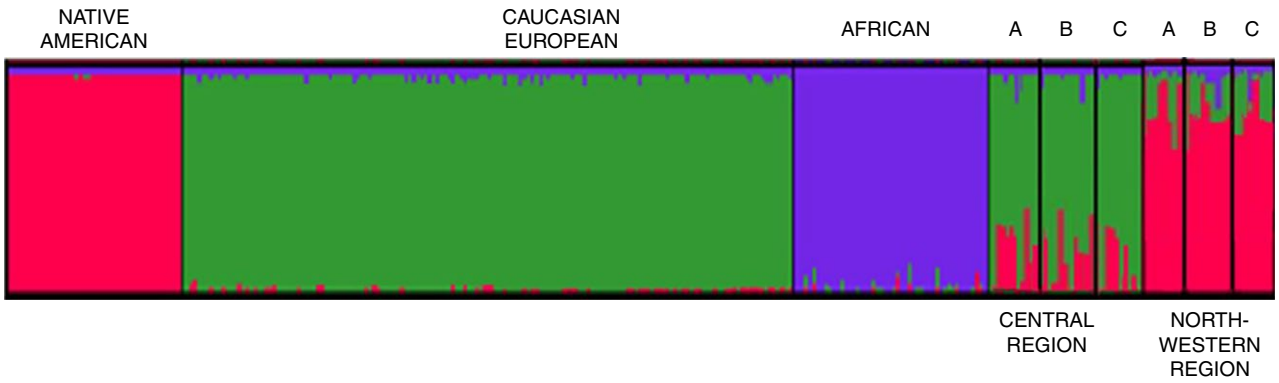

FIGURE 2 STRUCTURE Software Bar plot representation. Three parental populations were included (Native American indicated in red, European in green and finally West Sub-Saharan African in blue). Genetic admixture analysis and plotting was performed with Structure v 2.3.4 software assuming admixed model, INDEPENDENT allele frequencies, pop info and k=3. Samples were grouped as healthy uninfected controls (A), resolved HBV individuals (B) and persistent HBV patients (C)

were analysed. The HWE was assessed for the four SNPs using the chi-square test with one degree of freedom; however, only rs3077 deviated from HWE when analysed for the HBV carrier group in the central region. This SNP was found to be in HWE in healthy control subjects, and therefore, none of the SNPs were excluded from the analysis.

The genotypic distribution of the four SNPs among HBV-infected patients, resolved individuals and controls from the two geographical regions are shown in Tables 3 and 4. Uneven frequency rates in the two areas were observed for SNPs rs3077 and rs9277542 (Tables 3 and 4). The prevalence rates of rs3077-T and rs9277542-T, related to protection against chronic HBV infection and clearance, were lower among HBV-infected patients (0.35 for rs3077-T and 0.27 for rs9277542-T), resolved individuals (0.46 for rs3077-T and 0.39 for rs9277542-T) and uninfected controls (0.44 for rs3077-T and 0.4 for rs9277542-T) from north-western Argentina when compared to those from the central area (0.74, 0.83 and 0.82 for rs3077-T and 0.65, 0.72, 0.73 for rs9277542-T respectively) (Tables 3 and 4).

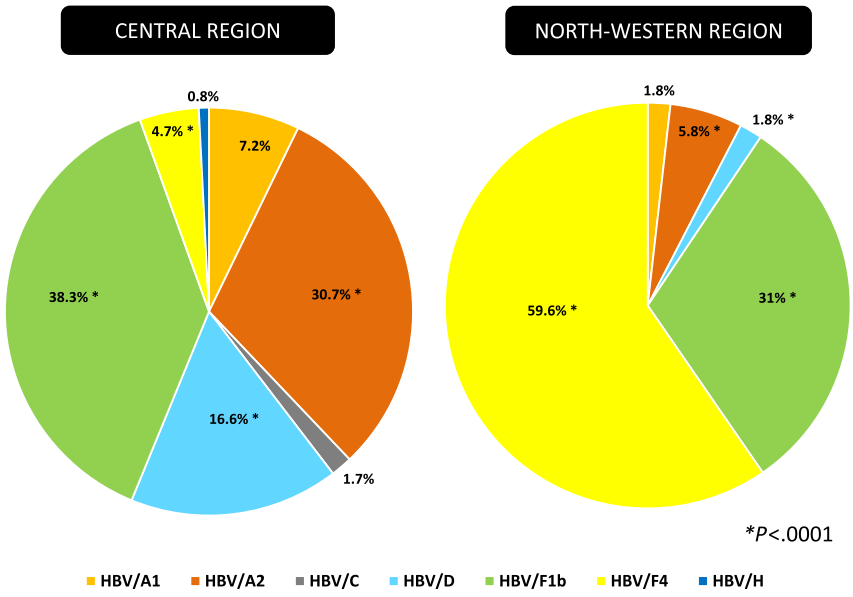

FIGURE 3 Distribution of HBV genotypes in central and north-western Argentina. *P<.0001 when comparing central with north-western Argentina

3.4.1 | Protective effects against chronic hepatitis B in central and north-western Argentina

In central and north-western Argentina, the genotype distribution in HBV carriers compared with healthy uninfected subjects showed that all analysed SNPs have a significant association (Table 3). With regard to the HLA-DP SNPs, rs3077-T and rs9277542-T were dominantly associated with protection against HBV infection with an OR of 0.37, 95% C.I. 0.17-0.80, and *P* value of .00939 for rs3077-T and an OR of 0.43, 95% C.I. 0.22-0.82 and *P* value of .00904 for rs9277542-T in central Argentina; and an OR of 0.55, 95% C.I. 0.37-0.84, and *P* value of .00467 for rs3077-T and an OR of 0.58, 95% C.I. 0.39-0.87 and *P* value of .00792 for rs9277542-T in the north-western region (Table 3). In contrast, HLA-DQ rs2856718-T exhibited a higher OR (1.93, 95% C.I. 1.25-2.99, *P* value=.00299 in the central region, and an OR of 2.18, 95% C.I. 1.35-3.50, *P* value=.00116 in north-western Argentina), indicating a dominant association with susceptibility to HBV infection, whereas rs7453920-A was dominantly associated with protection against HBV infection with an OR of 0.66, 95% C.I. 0.45-0.97, and *P* value of .03603 in the central region, and an OR of 0.60, 95% C.I. 0.39-0.93, and *P* value of .02291 in north-western Argentina (Table 3).

The results of the meta-analysis across 12 independent studies are shown in Table S1. In the case of rs7453920, Pmeta=<.0001, OR=0.56 was obtained only after results from Al Qahtani et al. were excluded (Table S1). As shown in Table S1, the odds ratios from the studies included in the meta-analysis were heterogeneous for HLA-DP SNPs.

3.4.2 | Clearance of hepatitis B virus in central and north-western Argentina

With regard to the genotype distribution in HBV carriers compared with subjects with resolved-HBV infection, all analysed SNPs in central and north-western Argentina exhibited a significant association (Table 4). When the HLA-DP SNPs were analysed, it was revealed that rs3077-T and rs9277542-T were dominantly associated with HBV clearance with an OR of 0.24, 95% C.I. 0.11-0.51, and *P* value of .00008 for rs3077-T and an OR of 0.47, 95% C.I. 0.27-0.82, and *P* value of .00684 for rs9277542-T in central Argentina; and an OR of 0.51, 95% C.I. 0.35-0.74, and *P* value of .00040 for rs3077-T and an OR of 0.52, 95% C.I. 0.37-0.75, and *P* value of .00043 for rs9277542-T in the north-western region (Table 4). In contrast, rs2856718-T exhibited a higher OR (1.78, 95% C.I. 1.19-2.67, *P* value=.00485 in the central region, and an OR of 2.03, 95% C.I. 1.31-3.16, *P* value=.00147 in north-western Argentina), indicating a dominant association with persistence of infection HBV; whereas rs7453920-A was dominantly associated with HBV clearance with an OR of 0.65, 95% C.I. 0.45-0.92 and *P* value of .01595 in the central region, and an OR of 0.47, 95% C.I. 0.32-0.71 and *P* value of .00020 in north-western Argentina (Table 4).

The results of the meta-analysis across 12 independent studies are shown in Table S2. In the case of rs3077, Pmeta=<.0001, OR=0.59 was obtained only after results from Wang et al. and Al Qahtani et al. were excluded (Table S2). In the case of rs7453920, Pmeta=<.0001, OR=0.64 was obtained only after results from Al Qahtani et al. were excluded (Table S2). As shown in Table S2, the odds ratios from the studies included in the meta-analysis were heterogeneous for HLA-DP SNPs.

3.4.3 | Estimation of linkage disequilibrium

Among the HLA class II loci analysed in this study, a weak linkage disequilibrium (r^2<.1) was observed between *HLA-DQB1/HLA-DQB2* locus and *HLA-DPA1/HLA-DPB1* loci in populations from central and north-western Argentina (Figure 4). However, the linkage disequilibrium was stronger between *HLA-DPA1* (rs3077) and *HLA-DPB1* (rs9277542) SNPs among north-western Argentines (Figure 4). Similar linkage

TABLE 3 Genotypic distribution for HLA gene polymorphisms when HBV patients were compared with healthy uninfected control group

		Central region					North-western region				
SNP	Genotype	HBV Carriers	Controls	OR (95% CI)	χ^2	*P* value	HBV carriers	Controls	OR (95% CI)	χ^2	*P* value
rs3077				0.63 (0.45-0.87)*	7.60	**.00584**			0.66 (0.50-0.88)*	8.03	**.00460**
	CC	24	10				88	61			
	CT	57	55				86	102			
	TT	120	142				26	38			
	CC+CT vs TT			0.68 (0.45-1.02)	3.51	.06088			0.64 (0.37-1.10)	2.61	.10642
	CC vs CT+TT			0.37 (0.17-0.80)	6.75	**.00939**			0.55 (0.37-0.84)	8.00	**.00467**
	HWE	0.0002	0.13				0.49	0.69			
rs9277542				0.67 (0.50-0.90)*	6.91	**.00858**			0.55 (0.41-0.75)*	15.26	**.00009**
	CC	31	15			103	77				
	CT	80	81			85	86				
	TT	90	111			12	38				
	CC+CT vs TT		0.70 (0.47-1.04)	3.19	0.07393			0.27 (0.14-0.54)	15.30	**0.00009**	
	CC vs CT+TT		0.43 (0.22-0.82)	6.82	**0.00904**			0.58 (0.39-0.87)	7.05	**0.00792**	
	HWE	0.07	0.97			.31	0.12				
rs2856718				1.81 (1.37-2.38)**	17.50	**.00003**			1.81 (1.37-2.40)**	17.43	**.00003**
	TT	62	31				73	42			
	TC	94	102				93	97			
	CC	45	74				34	62			
	TT+TC vs CC			1.93 (1.25-2.99)	8.81	**.00299**			2.18 (1.35-3.50)	10.55	**.00116**
	TT vs TC+CC			2.53 (1.56-4.11)	14.59	**.00013**			2.18 (1.39-3.40)	11.93	**.00055**
	HWE	0.41	0.67				0.64	0.72			
rs7453920				0.73 (0.54-0.99)**	4.20	**.04050**			0.65 (0.45-0.95)**	5.02	**.02508**
	AA	18	24				7	10			
	AG	67	85				41	59			
	GG	116	98				152	132			
	AA+AG vs GG			0.66 (0.45-0.97)	4.40	**.03603**			0.60 (0.39-0.93)	5.18	**.02291**
	AA vs AG+GG			0.75 (0.39-1.43)	0.77	.38050			0.69 (0.26-1.86)	0.54	.46354
	HWE	0.08	0.4				0.05	0.32			

CI, Confidence Interval; OR, odds ratio; χ^2, Chi-square.

**P* value and odds ratio of T allele from two-by-two allele frequency table.

***P* value and odds ratio of minor allele from two-by-two allele frequency table.

Bold values indicate statistically significant *P* values (*P*<.05).

TABLE 4 Genotypic distribution for HLA gene polymorphisms when HBV patients were compared with resolved individuals

		Central region					North-western region				
SNP	Genotype	HBV carriers	Resolved	OR (95% CI)	χ^2	*P* value	HBV carriers	Resolved	OR (95% CI)	χ^2	*P* value
rs3077				0.58 (0.43-0.79)	12.14	**.00049**			0.61 (0.47-0.79)*	14.04	**.00018**
	CC	24	10				88	90			
	CT	57	89				86	156			
	TT	120	219				26	67			
	CC+CT vs TT			0.67 (0.46-0.97)	4.57	**.03258**			0.55 (0.33-0.90)	5.81	**.0159**
	CC vs CT+TT			0.24 (0.11-0.51)	15.56	**.00008**			0.51 (0.35-0.74)	12.52	**.00040**
	HWE	0.0002	0.79				0.49	0.97			
rs9277542				0.72 (0.55-0.94)*	5.68	**.01721**			0.59 (0.45-0.78)*	14.11	**.00017**
	CC	31	25				103	112			
	CT	80	130				85	160			
	TT	90	163				12	41			
	CC+CT vs TT			0.77 (0.54-1.10)	2.07	.15013			0.42 (0.22-0.83)	6.64	**.0100**
	CC vs CT+TT			0.47 (0.27-0.82)	7.31	**.00684**			0.52 (0.37-0.75)	12.38	**.00043**
	HWE	0.07	0.9				0.31	0.17			
rs2856718				1.69 (1.31-2.18)**	16.83	**.00004**			1.61 (1.25-2.08)**	13.69	**.00022**
	TT	62	52				73	79			
	TC	94	158				93	142			
	CC	45	108				34	92			
	TT+TC vs CC			1.78 (1.19-2.67)	7.94	**.00485**			2.03 (1.31-3.16)	10.11	**.00147**
	TT vs TC+CC			2.28 (1.50-3.48)	15.09	**.00010**			1.70 (1.16-2.50)	7.42	**.00645**
	HWE	0.41	0.65				0.64	0.11			
rs7453920				0.73 (0.56-0.97)**	4.70	**.03021**			0.52 (0.37-0.73)**	14.62	**.00013**
	AA	18	34				7	22			
	AG	67	135				41	103			
	GG	116	149				152	188			
	AA+AG vs GG			0.65 (0.45-0.92)	5.81	**.01595**			0.47 (0.32-0.71)	13.87	**.00020**
	AA vs AG+GG			0.82 (0.45-1.50)	0.41	.52097			0.48 (0.20-1.14)	2.85	.09143
	HWE	0.08	0.68				0.05	0.14			

CI, Confidence Interval; OR, odds ratio; χ^2, Chi-square.

**P* value and odds ratio of T allele from two-by-two allele frequency table.

***P* value and odds ratio of minor allele from two-by-two allele frequency table.

Bold values indicate statistically significant *P* values (*P*<.05).

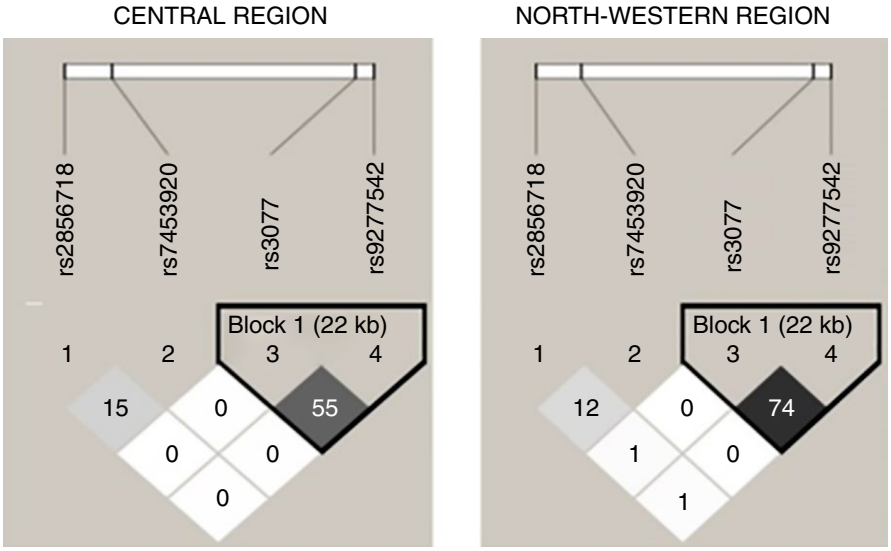

FIGURE 4 Estimation of linkage disequilibrium blocks in the populations from central and north-western Argentina. The LD blocks (r^2) were analysed using the Gabriel's algorithm

disequilibrium blocks were observed among the three subgroups of each geographical region (HBV carriers, HBV-resolved individuals and healthy controls).

3.5 | Association between host genetic factors and disease progression

Data analysis showed no association between the studied HLA-DP and -DQ SNPs across disease progression (chronic hepatitis and cirrhosis vs inactive carriers) in both geographical regions (data not shown).

3.6 | Logistic regression analyses

No significant association was observed between the viral and host genetic factors (*P*=.263); however, the place of birth was significantly associated with the HBV genotypes (OR=15.8, 95% CI=7.8-10.2, $P=1.41 \times 10^{-13}$) and the genotype of *HLA* SNPs (OR=14.1, 95% CI=7.6-15.3, $P=2 \times 10^{-16}$ for rs3077; OR=13.6, 95% CI=6.8-12.7, $P=2.8 \times 10^{-13}$ for rs9277542; OR=13.4, 95% CI=6.1-11.4, $P=5.9 \times 10^{-13}$ for rs2856718 and OR=2.85, 95% CI=2.3-4.7, $P=1.5 \times 10^{-9}$ for rs7453920).

Although age was not associated with the studied host genetic factors (*P*=.849), it was significantly related to HBV genotypes (OR=1.12, 95% CI=1.06-1.18, $P=1.18 \times 10^{-5}$) being the risk of infection with HBV/F and H higher among subjects aged 31-40 (OR=5.64; 95% CI=2.12-8.65; *P*=.00019) whereas the risk of infection with HBV/A and D was higher among subjects aged ≥51 (OR=2.81; 95% CI=1.83-6.79; *P*=.0029).

Multivariate logistic regression analysis was performed to determine the independent effects of these variables on the risk of chronic HBV infection. HBV genotypes were not included as viral genotyping was performed in chronic HBV-infected patients, and not in spontaneous resolvers. In this study, *HLA* SNPs were the only independent risk factors for chronic infection.

4 | DISCUSSION

In order to shed light on the different features of HBV infection observed in South America, 1440 HBV-infected patients, spontaneous resolvers and healthy uninfected controls were recruited in two distant geographical regions of Argentina.

In Argentina, the HBV epidemiological differences observed in the central and north-western regions could not be attributed to dissimilar viral transmission, but to a different age at the moment of infection. The transmission of HBV in Argentina occurs mostly through horizontal transmission.[14] In agreement to what has been observed in the Amazon Basin,[2] the higher prevalence of HBV infection in the north-western region does not respond to vertical dissemination, but to horizontal virus transmission—probably during childhood—through mechanisms that are not fully understood.

The population diversity in South America should be described by molecular approaches in order to avoid drawing false conclusions.[22] In admixed populations, it is essential to analyse matri and patrilineage as well as biparentally transmitted markers of the genome because both can reveal different geographical ancestry components. Furthermore, when the sample consists of subpopulations with various disease rates, the standard case-control analysis may result in spurious associations at any locus when allele frequencies differ among the subpopulations.[23] In this study, significant differences were observed in the ancestry components of the recruited subjects from both regions (Figures 1A-D and 2). These results are in agreement with those previously reported.[13,24] It is important to mention that similar ancestry genetic backgrounds were found among the three groups of each region (Figures 1A-D and 2), avoiding the risk of population substructure as a confounding factor in the genetic association study.

The distribution of HBV genotypes in Argentina displays striking differences, as observed in Figure 3. The higher prevalence of HBV/A2 and HBV/D in central Argentina and the almost absolute predominance of HBV/F1b and HBV/F4 in the north-western region are in line with previous studies.[15] Moreover, as observed in the results herein,

this distribution of HBV genotypes is related to the multiethnic origin of the population. While HBV/F is an indigenous genotype of the American continent, HBV/A2 and HBV/D are the signatures of the European colonization.[15] The presence of HBV/F in central Argentina is the result of the recent arrival of immigrants from areas with high proportion of Native American ancestry (Bolivia, Peru and even north-western Argentina) to the central area.[13]

The earliest observations recognizing HBV as a serious health problem among Native communities in the American continent were in Alaska and in north-western Colombia and the western Brazilian Amazon.[25,26] Later studies identified HBV as the most common cause of HCC among South American natives and genotype F1b together with hepatitis delta virus as responsible for epidemic outbreaks in indigenous communities in the Amazon basin.[2,27] In North America and the circumpolar Arctic, serologic surveys performed in the 1970s and 1980s have demonstrated that Alaskan Eskimos, Canadian Inuit and Greenland Inuit have very high prevalence rates of HBV.[28] In Alaska, a high incidence of HCC in Eskimos, especially males, has been reported, being HBV genotypes A, D and especially F1b related to higher or faster rates of HCC and fibrosis.[29] A recent report has demonstrated that the combination of Basal Core Promoter, preCore mutations and N51H mutations could enhance the carcinogenic potential of HBV/F1b which may explain the unusually high incidence of HCC development among Alaska Native persons chronically infected with this genotype.[30]

The results presented herein show that HBV/F4—followed by HBV/F1b—is the most prevalent genotype among populations with high Native American ancestry components in north-western Argentina. Moreover, although the lack of diagnostic methods for HCC in the region is a limiting factor for a proper analysis of the situation, inactive carrier state is the most common form of clinical presentation of this viral infection. In contrast to Alaskan natives, HBV/genotype F1b does not seem to be related to liver cancer in Argentina. Future studies should focus on performing comparative nucleotide and amino acid sequence analyses between Alaskan and Argentinian HBV/F1b isolates.

It has been reported that mutations in the Basal Core Promoter and preCore regions are implicated in the progression of the infection.[31] Interestingly, when compared with central Argentina, a lower hepatitis B e antigen (HBeAg) positivity rate was observed among patients from the north-western region, in spite of their younger age (Tables 1 and 2). This finding could be explained by the different seroconversion rate observed among genotypes and subgenotypes (D≫F4>A2>F1b),[32] considering that 59.6% of the cases in north-western Argentina were infected with HBV/F4.

The major finding of the present study was that host genetic factors could help explain the different features of HBV infection observed in these two regions of Argentina with dissimilar ancestry genetic background. Three of four SNPs (rs3077, rs9277542 and rs7453920) showed a significant association with protection against chronic HBV and viral clearance in both regions, whereas the remaining SNP rs2856718 exhibited a significant association with susceptibility to chronic HBV.

The *HLA-DQB2* SNP rs7453920 failed to show any association with susceptibility to HBV infection as observed by Mbarek et al.[7] Similar observations were made for the non-risk allele A of rs7453920, which was also dominantly associated with protection against chronic HBV and clearance in both regions of Argentina. However, these results are in contrast to those reported in Saudi Arabia where the allele G was dominantly associated with HBV clearance.[11] The dissimilar ancestry genetic background of Argentines and Saudi Arabian may help elucidate this difference in genotype distribution.

The SNP rs2856718, located in the intergenic region between *HLA-DQA2* and *HLA-DQB1*, was found to be dominantly associated with HBV infection in both areas, which was consistent with previous studies.[7,11]

In addition to the above two SNPs, *HLA-DPA1* SNP rs3077-T showed a protective effect against HBV infection and a significant association with HBV clearance in Argentina. The result obtained herein was consistent with studies conducted on different populations that reported the T allele to have a protective effect against chronic HBV infection,[5,7,10,11] while others also reported a significant association with HBV clearance.[6,9,12,33-36]

Among all evaluated alleles, only rs3077 deviated from HWE when analysed for the HBV carrier group in the central region. The most likely reason is that the immune response is greatly diminished in homozygous carriers who are therefore a selected population among carriers of persistent HBV infection. Familial relationship did not apply for the violation of the HWE as the patients had not been related with each other, and assay error is unlikely since the HWE was observed in the control group.

With regard to *HLA-DPB1* SNP rs9277542, similar observations to those obtained in this study were made for the non-risk allele T, which was also dominantly associated with protection against chronic HBV infection and HBV clearance among Japanese and Korean populations.[6] This SNP, which shows strong linkage disequilibrium with rs9277535 on the HapMap JPT, has been related to chronic HBV infection in Asian and Saudi Arabian populations,[7,9,10,35] but not in Caucasians, African and European Americans.[10,37] This could be explained by substantial variation in linkage disequilibrium patterns among different populations.

Meta-analysis revealed that these results were similar to those obtained among Asian populations. However, *P* values were lower in those reports, most likely as a consequence of the high number of samples enrolled in each Asian study.[5-9,12,33-36] Moreover, the fact that these genetic variants have such a strong impact on HBV infection among different worldwide populations may indicate that they have actually been under selection pressure. When early human populations migrated from Africa to Europe, Asia and the Americas—each with different climates and sources of food, they encountered markedly different pathogenic environments, likely resulting in population-specific selection on the immune response. Today, the functional consequences of the genetic variants that facilitated survival in ancestral Asian, European and American human populations might underlie the phenotypic differences reported between individuals and groups.[38]

The frequency rates of rs3077-T, related to protection against chronic HBV infection and clearance, were lower in north-western Argentina when compared to the central area. The same uneven frequency rates were observed for SNP rs9277542. This is consistent with previous data which show that the frequencies of the protective alleles for rs3077-T and rs9277542-T are markedly different between populations.[39] Taken together, all these findings of *HLA-DP* genomic variations may shed light on the difference in the geographical distribution of HBV infection: it is possible that the lower prevalence of chronic HBV infection in central Argentina is due to the higher prevalence of the protective *HLA-DP* alleles. Similarly, the high prevalence of chronic HBV in the north-western region is likely due to the lower prevalence of the protective *HLA-DP* alleles.

The results from the present study suggest that *HLA-DP* and *HLA-DQ* variations are probably some of the genetic factors which play an important role in the development of chronicity of HBV infection. However, it should be noted that other factors, such as viral, environmental and other host genetic factors, are also associated with chronicity of HBV infection. Although in this study logistic regression analyses showed no significant association between the viral and host genetic factors, both were independently associated with the geographical origin of the infected individual. Moreover, age was significantly related to HBV/genotypes, but not to HLA SNPs. Multivariate analysis confirmed that HLA SNPs are independent risk factors for chronic HBV infection. These results are in agreement with the dissimilar demographical characteristics and ancestry background of HBV-infected patients in Argentina, and help clarify the effects of the multiple overlapping factors that impact the different features of this viral infection observed in these distant geographical regions of the same country.

Although it would have been interesting to compare host genetic and viral factors related to HBV infection between indigenous populations residing in the different geographical areas, there are unfortunately no remaining autochthonous populations in the central Argentina. In the north-western and central regions of Argentina, historical differences in pattern of settlement and migration have resulted in a higher contribution of Native American ancestry among north-western Argentines due to the high population density of aboriginal in the area at the moment of the conquest; and a higher contribution of Caucasian European ancestry among central and metropolitan Argentines, due to the presence of sparse populations of semi-sedentary and nomadic natives in the region at the moment of the conquest and the arrival of a large number of European immigrants from 1856 to 1930 to the Port of Buenos Aires, the main port of Argentina.[13] Consequently, current population in central Argentina consists of European immigrants, their descendants and admixed people.[13]

This is the first study to report the associations between the *HLA-DP* and *HLA-DQ* loci and the protection against chronic HBV and viral clearance in a multiethnic South American population. These results suggest that comparative analyses of *HLA-DP* and *HLA-DQ* alleles and haplotypes are urgently needed in these populations as they would clarify key host factors of the susceptible and protective *HLA* alleles and haplotypes for chronic HBV and clearance.

ACKNOWLEDGEMENTS

We thank all the patients and families who contributed to this study and Tech. Noelia Bravo Cordeiro for technical assistance.

CONFLICT OF INTEREST

None of the authors have any relevant conflicts of interest to disclose.

REFERENCES

1. Tran TT, Martin P. Hepatitis B: epidemiology and natural history. *Clin Liver Dis*. 2004;8:255-266.
2. Echevarría JM, León P. Epidemiology of viruses causing chronic hepatitis among populations from the Amazon Basin and related ecosystems. *Cad Saude Publica*. 2003;19:1583-1591.
3. Norder H, Couroucé AM, Coursaget P, et al. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology*. 2004;47:289-309.
4. Singh R, Kaul R, Kaul A, Khan K. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. *World J Gastroenterol*. 2007;13:1770-1787.
5. Kamatani Y, Wattanapokayakit S, Ochi H, et al. A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. *Nat Genet*. 2009;41:591-595.
6. Nishida N, Sawai H, Matsuura K, et al. Genome-wide association study confirming association of HLA-DP with protection against chronic hepatitis B and viral clearance in Japanese and Korean. *PLoS One*. 2012;7:e39175.
7. Mbarek H, Ochi H, Urabe Y, et al. A genome-wide association study of chronic hepatitis B identified novel risk locus in a Japanese population. *Hum Mol Genet*. 2011;20:3884-3892.
8. Li J, Yang D, He Y, et al. Associations of HLA-DP variants with hepatitis B virus infection in southern and northern Han Chinese populations: a multicenter case-control study. *PLoS One*. 2011;6:e24221.
9. Wang L, Wu XP, Zhang W, et al. Evaluation of genetic susceptibility loci for chronic hepatitis B in Chinese: two independent case-control studies. *PLoS One*. 2011;6:e17608.
10. Vermehren J, Lötsch J, Susser S, et al. A common HLA-DPA1 variant is associated with hepatitis B virus infection but fails to distinguish active from inactive Caucasian carriers. *PLoS One*. 2012;7:e32605.
11. Al-Qahtani AA, Al-Anazi MR, Abdo AA, et al. Association between HLA variations and chronic hepatitis B virus infection in Saudi Arabian patients. *PLoS One*. 2014;9:e80445.
12. Posuwan N, Payungporn S, Tangkijvanich P, et al. Genetic association of human leukocyte antigens with chronicity or resolution of hepatitis B infection in thai population. *PLoS One*. 2014;9:e86007.
13. Corach D, Lao O, Bobillo C, et al. Inferring continental ancestry of argentineans from Autosomal, Y-chromosomal and mitochondrial DNA. *Ann Hum Genet*. 2010;74:65-76.
14. Flichman DM, Blejer JL, Livellara BI, et al. Prevalence and trends of markers of hepatitis B virus, hepatitis C virus and human Immunodeficiency virus in Argentine blood donors. *BMC Infect Dis*. 2014;14:218.
15. Piñeiro Y, Leone FG, Pezzano SC, et al. Hepatitis B virus genetic diversity in Argentina: dissimilar genotype distribution in two different geographical regions; description of hepatitis B surface antigen variants. *J Clin Virol*. 2008;42:381-388.
16. Remondegui C, Ceballos S, Echenique G, Roman R. Revisión epidemiológica y clínica de pacientes con hepatitis virales en Jujuy. *Acta Gastroenterológica Americana*. 2006;36(S3):S63.
17. Garay ME, Marina G, Martínez M, et al. Prevalencia intermedia de infección por virus B en la provincia de Salta, Argentina. *Acta Gastroenterológica Americana*. 2006;36(S3):S63.

18. Zuccarelli G, Alechine E, Caputo M, Bobillo C, Corach D, Sala A. Rapid screening for Native American mitochondrial and Y-chromosome haplogroups detection in routine DNA analysis. *Forensic Sci Int Genet*. 2011;5:105-108.
19. Shriver MD, Smith MW, Jin L, et al. Ethnic-affiliation estimation by use of population-specific DNA markers. *Am J Hum Genet*. 1997;60:957-964.
20. Sugiyama M, Inui A, Shin-I T, et al. Easy-to-use phylogenetic analysis system for hepatitis B virus infection. *Hepatol Res*. 2011;41:936-945.
21. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21:263-265.
22. Sala A, Corach D. Analysis of admixture and genetic structure of two Native American groups of Southern Argentinean Patagonia. *Mol Biol Rep*. 2014;41:1533-1543.
23. Liu J, Lewinger JP, Gilliland FD, Gauderman WJ, Conti DV. Confounding and heterogeneity in genetic association studies with admixed populations. *Am J Epidemiol*. 2013;177:351-360.
24. Avena S, Via M, Ziv E, et al. Heterogeneity in genetic admixture across different regions of Argentina. *PLoS One*. 2012;7:e34695.
25. Bensabath G, Hadler SC, Soares MC, et al. Prevalence and role in fulminant hepatitis in the Amazon Basin. *JAMA*. 1987;258:479-483.
26. Heyward WL, Lanier AP, Bender TR, et al. Primary hepatocellular carcinoma in Alaskan natives, 1969-1979. *Int J Cancer*. 1981;28:47-50.
27. Miranda EC, Moia Lde J, Amaral Ido S, et al. Hepatitis B and C virus infection and the hepatocellular carcinoma in the East Amazon, Brazil. *Rev Soc Bras Med Trop*. 2004;37(Suppl. 2):47-51.
28. McMahon BJ, Lanier AP, Wainwright RB. Hepatitis B and hepatocellular carcinoma in Eskimo/Inuit population. *Int J Circumpolar Health*. 1998;57(Suppl 1):414-419.
29. Ching LK, Gounder PP, Bulkow L, et al. Incidence of hepatocellular carcinoma according to hepatitis B virus genotype in Alaska Native people. *Liver Int*. 2016;36:1507-1515.
30. Hayashi S, Khan AA, Ogawa K, et al. Hepatocellular carcinoma-associated core N51H mutation with BCP PC mutations in HBV genotype F1b enhances infectivity and Up-regulates c-Myc and GAB2. *Hepatology*. 2016;64(Suppl. 1):291A-292A.
31. Imamura T, Yokosuka O, Kurihara T, et al. Distribution of hepatitis B viral genotypes and mutations in the core promoter and precore regions in acute forms of liver disease in patients from Chiba, Japan. *Gut*. 2003;52:1630-1637.
32. González López Ledesma MM, Mojsiejczuk LN, Rodrigo B, et al. Hepatitis B virus genotype distribution and genotype-specific BCP/preCore substitutions in acute and chronic infections in Argentina. *PLoS One*. 2015;10:e0121436.
33. An P, Winkler C, Guan L, et al. A common HLA-DPA1 variant is a major determinant of hepatitis B virus clearance in Han Chinese. *J Infect Dis*. 2011;203:943-947.
34. Guo X, Zhang Y, Li J, et al. Strong influence of human leukocyte antigen (HLA)-DP gene variants on development of persistent chronic hepatitis B virus carriers in the Han Chinese population. *Hepatology*. 2011;53:422-428.
35. Hu L, Zhai X, Liu J, et al. Genetic variants in human leukocyte antigen/DP-DQ influence both hepatitis B virus clearance and hepatocellular carcinoma development. *Hepatology*. 2012;55:1426-1431.
36. Wong DK, Watanabe T, Tanaka Y, et al. Role of HLA-DP polymorphisms on chronicity and disease activity of hepatitis B infection in Southern Chinese. *PLoS One*. 2013;8:e66920.
37. Thomas R, Thio CL, Apps R, et al. A novel variant marking HLA-DP expression levels predicts recovery from hepatitis B virus infection. *J Virol*. 2012;86:6979-6985.
38. Nédélec Y, Sanz J, Baharian G, et al. Genetic ancestry and natural selection drive population differences in immune responses to pathogens. *Cell*. 2016;167:657-669.
39. International HapMap 3 Consortium. Integrating common and rare genetic variation in diverse human populations. *Nature* 2010; 467:52-58.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Trinks J, Nishida N, Hulaniuk ML, et al. Role of HLA-DP and HLA-DQ on the clearance of hepatitis B virus and the risk of chronic infection in a multiethnic population. *Liver Int*. 2017;00:1-12. https://doi.org/10.1111/liv.13405