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Response to hepatitis B vaccination is co-determined by *HLA-DPA1* and *-DPB1*

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ABSTRACT

Background and aims: No report explored the combined effects of *HLA-DPA1* and *-DPB1* with long-term response to hepatitis B (HB) vaccination (HBVac). The specific aims of the study were to assess the combined effects and relative contributions of *DPA1* and *DPB1* genes.
Methods: The cases were 152 adolescents who had undetectable (<1.0 mIU/mL) post-booster anti-HBs titers and the controls were adolescents who had residual anti-HBs ≥ 10 mIU/mL at aged 16 years (n = 207) or had detectable (≥1.0 mIU/mL) anti-HBs titers after booster HBVac (n = 481). *HLA-DPA1* and *-DPB1* genotypes were determined by sequence-based typing.
Results: *HLA-DPA1*01:03:01* was correlated with lower ORs of undetectable anti-HBs titers, while *-DPA1*02:02:02* and *-DPB1*05:01:01* were correlated with higher ORs. The ORs for *HLA-DPA1*01:03:01-DPB1*05:01:01* and *DPA1*02:02:02-DPB1*protective* combinatory types were significantly less than 1.0. As compared with subjects who had no protective allele, the adjusted ORs (95% CI) were 0.545 (0.328–0.906), 0.350 (0.174–0.702), and 0.122 (0.058–0.257), for subjects who had protective alleles on *DPA1*only, *DPB1* only, and both genes, respectively. Analyses of amino acid polymorphisms showed that subjects who carried Arg81-Pro158-Val191-Pro259α + Met234β and Gln62-Arg82α + Met234β combinations had 4.3-to-4.6 folds of risks.
Conclusion: Both *DPA1* and *DPB1* genes contribute to the persistence of immunological response to primary infantile HBVac. The effects of *HLA-DP* risk alleles were dominated by the protective alleles and there were significant gene-gene interactions. Our findings provide evidences for the design of more potent HB vaccine.

1. Introduction

Hepatitis B (HB) vaccination is an effective measure to reduce the risks of HB virus (HBV) infection [1–3]. In the cohort born after the implementation of universal HB vaccination program in Taiwan, HBsAg-positive rates as well as childhood liver cancer declined significantly [4–7]. However, among individuals who had received HB vaccination during infancy but were negative for HBsAg, anti-HBs, and anti-HBc at adolescence or youth, a significant proportion of them did not have protective levels of anti-HBs titers after receiving HB booster, indicating that they might have lost immunological memory against HBsAg [8,9]. Furthermore, breakthrough and chronic HBV infections were repeatedly

Abbreviations: HBV, hepatitis B virus; HB, hepatitis B; HBVac, hepatitis B vaccination; GWAS, genome-wide association study; SNP, single nucleotide polymorphism; HLA, human leukocyte antigen; HBsAg, hepatitis B surface antigen; anti-HBs, antibodies against hepatitis B surface antigen; anti-HBc, antibodies against hepatitis B core antigen; PCR, polymerase chain reaction; OR, odds ratio; CI, confidence interval (CI); LD, linkage disequilibrium.

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reported in individuals who had received complete doses of HB vaccine [8,10–12]. To explore the determinants of long-term responses to HB vaccination will be of great help in the development of more potent HB vaccine and might further reduce the risk of HBV infection.

Over 70% of the variations in the post-vaccination antibody titers were attributable to genetic factors [13]. Genome-wide association studies on outcomes of HBV infection had identified several common genetic variants on human leukocyte antigen (*HLA*)-*DPB1* loci showing genome-wide significance [14–16]. It is reasonable to hypothesize that *HLA-DPB1* loci may also play important role in the response to HB vaccination. We identified several significant *HLA-DPB1* alleles with the response to booster HB vaccination in individuals who had competed primary HB during infancy but without protective levels of residual anti-HBs titers at adolescence [17]. Additionally, our previous genome-wide association study further confirmed that *HLA-DP* plays critical roles in the host response to booster HB vaccination. A total of 10 promising SNPs clustered within a 4.7 Kb region of *HLA-DP* and the most significant SNP rs7770370 is near the junction *of DPA1* and *DPB1* [18]. However, to our knowledge, no report explored the combinatory effects of *HLA-DPA1* and *-DPB1* with long-term response to HB vaccination.

In the present study, we tried to dissect the relative contributions of *DPA1* and *DPB1* genes on the long-term persistence of response to HB vaccination. We assayed the *DPA1* and *DPB1* genotypes in a group of cases who had undetectable anti-HBs titers after a booster dose of HB vaccination and two groups of control subjects who were anti-HBs-positive at enrollment or had detectable anti-HBs titers after booster HB vaccination.

2. Participants and methods

2.1. The study population

The study population was enrolled from our previous cohort study that explored the long-term effect of HB vaccination [8]. In brief, a total of 8813 senior high school freshmen were recruited during October 2003 to May 2008. A booster dose of 20 μg HB vaccine (Engerix-B, GlaxoSmithKline Biologicals, Rixensart, Belgium) was administered to individual who was HBsAg-negative and anti-HBs-negative at enrollment, received ≥ 3 doses HB vaccine before 18 months of age, and did not receive any booster HB vaccination during childhood and adolescence. Blood samples were drawn at the day of booster administration (pre-booster) and 1-month after booster (post-booster) and assayed for anti-HBs titer. Among the booster recipients, 83 subjects were excluded for anti-HBc-positivity, pre-booster anti-HBs titers ≥ 10 mIU/mL, or without post-booster blood samples.

We used a nested case-control study design to explore the influences of *HLA-DP* genetic variants on response to HB vaccination. There were 1 case and 2 control groups in the study. The subjects of the case group (CS; n = 152) and control group I (CN I; n = 481) were randomly selected from booster recipients whose post-booster anti-HBs titers < 1.0 and ≥ 1.0 mIU/mL, respectively. Subjects of control II (CN II; n = 207) were randomly selected from adolescents who had received primary infantile HB vaccine and were HBsAg negative and anti-HBs positive at recruitment. The study protocol conformed to the Declaration of Helsinki and was reviewed and approved by the Institutional Review Board of Mackay Memorial Hospital (IRB no: 12MMHIS191).

2.2. Serologic testing

The pre-booster blood samples were assayed for HBsAg and anti-HBc by microparticle enzyme immunoassays (MEIA) with commercial kits AxSYN HBsAg (V2) and AxSYN CORE (Abbott Diagnostics, North Chicago, IL, USA), respectively. Anti-HBs titers in the pre- and post-booster blood samples of booster recipients were determined by a quantitative method with commercial kit AxSYN AUSAB (Abbott Diagnostics). The detection limit of this quantitative method was 1.0 mIU/mL.

2.3. HLA-DPB1 and HLA-DPA1 typing

HLA-DPB1 type was determined by sequencing-based typing (SBT) method as described previously [17].

HLA-DPA1 genotype was also determined by SBT method that was proposed by Rozemuller et al. [19]. Since only exon 2 of *HLA-DPA1* was sequenced, equivocal allele allocation were assigned to the allele with the lowest definition number. In case of heterozygous individuals with genotype ambiguities that could not be resolved by group specific primer sets, allele was assigned according to the most common alleles found in Taiwanese population. The colon delimited HLA allele names with three-field format were used to assign HLA types [20].

2.4. HLA-DPA1 and –DPB1 proteins

To explore the potential influences of HLA-DP variants on the response to HB vaccination, we downloaded the amino acid sequences of the common *HLA-DPA1* and *–DPB1* alleles from the IPD-IMGT/HLA Database release 3.28.0 (March 2017; http://www.ebi.ac.uk/ipd/imgt/hla/).

2.5. Statistical analysis

We used the Pearson's chi-square test or student's *t* test to assess whether there was significant difference in the characteristics and frequency distributions of *HLA-DP* alleles between the case and control groups. Two-sided exact probabilities for the test of equal proportion of the presence of specific *DPA1* and *DPB1* alleles among the case and control groups were calculated by Binomial distribution and then adjusted for multiple comparisons by multiplying the crude exact probabilities with the numbers of common alleles. The odds ratio (OR) and its 95% confidence interval (CI) of undetectable anti-HBs titers was calculated by logistic regression analysis. Factors significantly correlated with the ORs of undetectable anti-HBs titers were further subject to multivariate logistic regression analyses with forward selection method. The Breslow-Day Test was used to test the homogeneity of the estimated ORs for the presence of specific *HLA-DP* alleles. Factor analyses were used to determine the linkage among polymorphic sites and the strength of correlation between linkage disequilibrium (LD) blocks were manifested by the Pearson's correlation coefficients. All statistical analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Characteristics of study subjects

Table 1 shows that age and intervals among the dates of birth and the 1st and the last doses of primary infantile HB vaccination were similar among groups. There was more male student in the case group. The frequency distributions of common *DPA1* and *DPB1* alleles were significantly different among three groups ($\chi^2 = 30.5$, $p = 0.00027$; $\chi^2 = 82.3$, $p = 9.2 \times 10^{-8}$), whereas were not significantly different between two control groups ($p = 0.44$ and 0.14, respectively). Accordingly, we combined the two control groups for further analyses (see Table 2).

Table 1
Characteristics of 152 cases and 688 controls.

Variable	Cases n = 152		Control I n = 481		Control II n = 207		p-value
Age of residual anti-HBs titer determination (years; mean [SD])	15.6	(0.4)	15.6	(0.4)	15.5	(0.4)	>0.10
Male sex (n, %)	107	(70.4)	288	(59.9)	91	(44.0)	<0.0001
Interval (Median [Q1-Q3])							
Birth to VD1, days	6	(3–14)	6	(3–16)	7	(4–18)	>0.10
Birth to VD4, years	1.05	(1.01–1.12)	1.04	(1.01–1.14)	1.07	(1.01–1.19)	>0.10
VD1 to VD4, years	1.02	(1.00–1.09)	1.02	(0.99–1.09)	1.02	(0.99–1.13)	>0.10
	2n	(%)	2n	(%)	2n	(%)	
HLA-DPA1							0.00027
01:03:01	35	(11.5)	242	(25.2)	101	(24.4)	
02:01:01	32	(10.5)	84	(8.7)	32	(7.7)	
02:02:01	5	(1.6)	17	(1.8)	5	(1.2)	
02:02:02	226	(74.3)	592	(61.5)	270	(65.2)	
04:01	6	(2.0)	27	(2.8)	6	(1.4)	
HLA-DPB1							<0.0001
01:01:01	10	(3.3)	39	(4.1)	12	(2.9)	
02:01:02	28	(9.2)	140	(14.6)	49	(11.8)	
02:02	2	(0.7)	43	(4.5)	26	(6.3)	
03:01:01	6	(2.0)	61	(6.3)	15	(3.6)	
04:01:01	4	(1.3)	50	(5.2)	32	(7.7)	
04:02:01	1	(0.3)	12	(1.2)	7	(1.7)	
05:01:01	208	(68.4)	474	(49.3)	216	(52.2)	
09:01	8	(2.6)	7	(0.7)	6	(1.4)	
13:01	21	(6.9)	65	(6.8)	18	(4.3)	
14:01	5	(1.6)	32	(3.3)	14	(3.4)	
17:01	2	(0.7)	12	(1.2)	2	(0.5)	
19:01	3	(1.0)	13	(1.4)	5	(1.2)	
21:01	4	(1.3)	7	(0.7)	6	(1.4)	
Others[a]	2	(0.7)	7	(0.7)	6	(1.4)	

Notes:VD1, date of first dose of primary infantile HB vaccination; VD4, date of last doses of primary infantile HB vaccination.
[a] Include *DPB1*01:02* (n = 1), *25:01* (n = 3), *31:01* (n = 1), *41:01* (n = 1), *45:01* (n = 2), *47:01* (n = 1), *48:01* (n = 4), and *75:01* (n = 1).

Table 2
Associations for common *HLA-DPA1* and *–DPB1* alleles with non-response to HB vaccination.

	Cases (n = 152)	Controls (n = 688)	Adjusted OR[a]		
	2/1/0 alleles	2/1/0 alleles	OR	(95% CI)	$P_{corr.}$[b]
DPA1					
01:03:01	5/25/122	44/255/389	0.40	(0.28–0.58)	7.6×10^{-6}
02:01:01	2/28/122	5/106/577	1.28	(0.85–1.93)	>0.15
02:02:01	0/5/147	1/20/667	1.03	(0.40–2.66)	>0.15
02:02:02	89/48/15	280/302/106	1.66	(1.27–2.18)	1.2×10^{-3}
04:01	0/6/146	0/33/655	0.82	(0.34–1.98)	>0.15
DPB1					
01:01:01	2/6/144	2/47/639	0.83	(0.42–1.63)	>0.15
02:01:02	1/26/125	16/157/515	0.67	(0.44–1.01)	>0.15
02:02:01	0/2/150	5/59/624	0.15	(0.04–0.60)	0.094
03:01:01	0/6/146	2/72/614	0.33	(0.14–0.76)	0.12
04:01:01	0/4/148	3/76/609	0.22	(0.08–0.61)	0.045
04:02:01	0/1/151	0/19/669	0.26	(0.03–1.96)	>0.15
05:01:01	75/58/19	183/324/181	2.03	(1.56–2.63)	1.6×10^{-6}
09:01	0/8/144	0/13/675	2.87	(1.15–7.11)	>0.15
13:01	1/19/132	2/79/607	1.16	(0.70–1.91)	>0.15
14:01	0/5/147	0/46/642	0.44	(0.17–1.13)	>0.15
17:01	0/2/150	0/14/674	0.67	(0.15–3.01)	>0.15
19:01	0/3/149	1/16/671	0.79	(0.24–2.63)	>0.15
21:01	0/4/148	0/13/675	1.35	(0.43–4.24)	>0.15

[a] ORs were adjusted for sex and pre-booster residual anti-HBs titers.
[b] Corrected p-values = crude two-sided exact probability × multiple comparisons (×5 and × 13 for *HLA-DPA1* and *–DPB1*, respectively).

3.2. Association analyses for common HLA-DP alleles

The ORs of undetectable anti-HBs titers were significantly correlated with *DPA1*02:02:02* and *DPB1*05:01:01* alleles while was inversely correlated with *DPA1*01:03:01* and *DPB1*04:01:01* alleles. The ORs for *DPB1*02:02, 03:01:01, 04:01:01*, *04:02:01*, and *14:01* were similar ($\chi^2_4 = 3.049$, p = 0.55), whereas their frequencies was low. To obtain more reliable estimates of association, we combined them as the 'protective' alleles. Similarly, *DPB1*05:01:01* and *09:01* were combined as the 'risk' alleles.

3.3. Effects of DPA1 and DPB1 combinatory types

Among subjects who carried *DPA1*01:03:01,* the ORs were 0.545 and 0.154 for subjects who carried *DPB1*risk* and **protective* alleles, respectively (Table 3). In subjects who carried *DPA1*02:02:02,* the ORs were 2.075 and 0.212 for subjects who carried *DPB1*risk* and ** protective* alleles, respectively.

As compared with Group 1, the adjusted ORs were significantly decreased for the other 3 groups and the lowest for Group 4 (Table 4). The ORs for Group 4 were 0.349 (95% CI, 0.132–0.922;

Table 3
Combined effects of *HLA-DPA1* and *–DPB1* alleles with non-response to HB vaccination.

		Cases		Controls				Exact p-value	Corrected p-value
DPA1	*DPB1*[a]	n	%	n	%	OR[b]	(95% CI)		
01:03:01	Risk	22	14.5	164	23.8	0.545	(0.335–0.887)	0.013	0.052
01:03:01	Protective	8	5.3	183	26.6	0.154	(0.074–0.320)	4.56×10^{-10}	1.82×10^{-9}
02:02:02	Risk	129	84.9	501	72.8	2.075	(1.288–3.343)	8.31×10^{-4}	0.0033
02:02:02	Protective	12	7.9	196	28.5	0.215	(0.116–0.397)	1.21×10^{-8}	4.84×10^{-8}

Note: CI, confidence interval; OR, odds ratio.
[a] Risk alleles include *DPB1*05:01:01* and *09:01* and protective alleles include *DPB1*02:02:01, 03:01:01, 04:01:01, 04:02:01*, and *14:01*.
[b] ORs of undetectable anti-HBs titers for the presences of specific combinatory types were adjusted for sex and pre-booster residual anti-HBs titers.

p = 0.034) and 0.210 (95% CI, 0.089–0.496; p = 0.0004) in the comparisons with Group 2 and 3, respectively. The ORs were similar for Group 2 and Group 3 (p = 0.28). As compared with subjects who had no risk allele in both *DPA1* and *DPB1* genes, the OR was not the highest for subjects who have risk alleles in both genes (Supplemental Table 1).

3.4. Linkage analyses for amino acid polymorphisms

For the 5 common *DPA1* alleles in the study, amino acid positions 42, 49, 59, 62, 81, 103 ~ 104, 114, 122, 127, 142, 158, 191, 221, and 259 are dimorphic (Supplemental Table 2). Linkage analyses showed that there are 2 LD blocks, designed as Block A1 and Block A2, of the DPA1 polymorphisms. Block A1 and Block A2 are closely linked ($r^2 = 0.88$, $p < 0.0001$). For the 13 common *DPB1* alleles, amino acid positions 37 ~ 40, 64 ~ 65, 84 ~ 86, 94, 98, 105, 113 ~ 116, 125, 199, 207, 223, and 234 are polymorphic and 2 LD blocks, designed as Block B1 and Block B2, were identified (Supplemental Table 3).

3.5. Association analyses for amino acid polymorphisms

Univariate association analyses showed that Block A1, Block A2, amino acid positions 42 and 114 of DPA1 (Supplemental Table 4) and Block B1, Block B2, and amino acid positions 94, 98, and 234 of DPB1 (Supplemental Table 5) were significantly correlated with the risks of undetectable anti-HBs titers. Multivariate analyses showed that Block A1 and Block A2 were the most significant DPA1 markers and for DPB1 was the amino acid polymorphism at position 234. Table 5 shows that subjects who were Block A1-RPVP (Arg81-Pro158-Val191-Pro259) homozygote and had ≥ 1 copies of Met234β had significantly elevated risks (adjusted OR = 4.310, 95% CI: 2.188–8.490; $p = 2.45 \times 10^{-5}$; Model I). The adjusted OR was 4.560 (95% CI, 2.313–8.987; $p = 1.17 \times 10^{-5}$) for subjects who were Block A2-QR (Gln62-Arg142) homozygote and had ≥ 1 copies Met234β (Model II).

4. Discussion

We previously showed that risks of undetectable post-booster anti-HBs titers were significantly corrected with *HLA-DBP1* alleles [17] and common genetic variants spread on the *DPB1* and *DPA1* region were the most significant genetic determinants of response to booster HB vaccination [18]. In the present study, we explored the effects of *DPA1* and *DPB1* alleles, genotypes, combinatory types, and amino acid variants on the risks of non-response to booster HB vaccination. To our knowledge, no report had assessed the combined effects of *DPA1* and *DPB1* with the response to booster HB vaccination before. In addition, study subjects had completed primary HB vaccination during infancy and received residual anti-HBs titer determinations at aged 16 years. The cases were those who had undetectable pre- and post-booster anti-HBs titers, it is reasonable to assume that these cases had very low or no immunological memory against primary infantile HB vaccination. Accordingly, our findings can be interpreted as the effects of *HLA-DP* with the long-term persistence of HB vaccination-induced immunity.

In the study, we found that without regard to *DPA1*, the *DPB1*05:01:01* is correlated with significantly higher OR. Yet, the OR was less than 1.0 for subjects carrying the *DPA1* protective-DPB1*05:01:01* combinatory type. Similarly, *DPA1*02:02:02* is correlated with significantly elevated OR when *DPB1* are left un-considered, and the adjusted ORs was less than 1.0 for subjects carrying *DPA1*02:02:02-DPB1*protective* combinatory type. Like HLA DR1 protein [21], the peptide-binding groove of DP molecules are formed by α_1 and β_1 domains [22]. Differences in the residues of either α_1 or β_1 domains may accordingly produce changes in the peptide-binding groove then accommodate different peptides. Studies on the crystal structures of DP5, which is the product of *DPA*02:02* and *DPB1*05:01*, and DP2, which is the product of *DPA1*01:03* and *DPB1*02:01*, demonstrated several distinct properties of their peptide-binding grooves [22,23]. Accordingly, our findings are of biological senses and it is necessary to type the *DPA1* and *DPB1* at the same time when exploring the effects of HLA-DP with vaccination or pathogen clearance.

Table 4
Combined effects of *HLA-DPA1* and *–DPB1* alleles with non-response to HB vaccination.

			Cases		Controls			
Group	*DPA1*01:03:01*	*DPB1*protective* alleles[a]	n	%	n	%	OR[b]	(95% CI)
1	No	No	112	73.7	310	45.1	1.000	(reference)
2	Yes	No	22	14.5	116	16.9	0.545	(0.328–0.906)
3	No	Yes	10	6.6	79	11.5	0.350	(0.174–0.702)
4	Yes	Yes	8	5.3	183	26.6	0.122	(0.058–0.257)
	p-value of trend test						3.38×10^{-9}	

Note: CI, confidence interval; OR, odds ratio.
[a] Include *DPB1*02:02, 03:01:01, 04:01:01, 04:02:01*, and *14:01*.
[b] ORs of undetectable anti-HBs titers were adjusted for sex and pre-booster residual anti-HBs titers.

Table 5
Combined effects of polymorphic amino acid positions of HLA-DPA1 and –DPB1 with non-response to HB vaccination.

		Cases		Controls				
DPA1 amino acid	DPB1 amino acid	n	%	n	%	OR[a]	(95% CI)	p-value
Model I								
Block A1[b]	Position 234							
QLFT homozygote or heterozygote	VV	10	6.6	137	19.9	1.00	(reference)	
QLFT homozygote or heterozygote	MM or MV	20	13.2	162	23.5	1.673	(0.755–3.710)	0.10
RPVP homozygote	VV	8	5.3	36	5.2	2.925	(1.071–7.990)	0.012
RPVP homozygote	MM or MV	114	75.0	353	51.3	4.310	(2.188–8.490)	2.45×10^{-5}
Model II								
Block A2[c]	Position 234							
MK homozygote or heterozygote	VV	10	6.6	143	20.8	1.000	(reference)	
MK homozygote or heterozygote	MM or MV	24	15.8	177	25.7	1.912	(0.883–4.140)	0.79
QR homozygote	VV	8	5.3	30	4.4	3.703	(1.342–10.221)	0.28
QR homozygote	MM or MV	110	72.4	338	49.1	4.560	(2.313–8.987)	1.17×10^{-5}

Note: CI, confidence interval; OR, odds ratio.
[a] ORs were adjusted for sex and pre-booster residual anti-HBs titers.
[b] Amino acid positions: 81–158-191–259.
[c] Amino acid positions: 62–142.

We found that subjects who carried protective alleles at either *DPB1* or *DPA1* had substantially decreased ORs and the ORs decreased further for subjects who carried protective alleles at both *DPB1* and *DPA1* genes. However, as compared with subjects who had no risk allele in both genes, the OR was not the highest for subjects who had risk alleles in both genes. In addition, the ORs for subjects carrying the *DPA1* 01:03:01-DPB1*05:01:01* or *DPA1*02:02:02-DPB1*protective* combinatory type were both less than 1.0. These findings indicate that the effects induced by the *HLA-DP* risk alleles are dominated by the protective alleles and are probably equally controlled by both *DPA1* and *DPB1* genes. Study on the interactions between HBsAg peptides and DPA-DPB dimers are necessary to validate our speculation.

In the study, several polymorphic amino acid positions of DPA1 and DPB1 were correlated with undetectable anti-HBs titers. It is biological sense that the Block A1 and A2 of the DP α chain and Block B1 and B2 of the DP β are all located on the peptide-binding groove of the DP2 and DP5 molecules [22,23]. Unexpectedly, multivariate analysis showed that Met234Valβ dimorphism, which locates outside the peptide-binding groove, was the most significant determinant. To date, there is only 2 reports of DP crystal structures, and none has depicted the structure outside the peptide-binding groove [22,23]. Although the structures of other HLA class II molecules had been shown by several reports, the influence of domains outside the peptide-binding groove is still unclear [24]. Even so, the influence of the Met234Valβ dimorphism should not be totally excluded. The Met234Valβ dimorphism is the product of SNP rs11551421 A/G polymorphism. The LD data of the 1000 Human Genome Project Phase 3-Southern Han Chinese shows that SNP rs11551421 is almost completely linked with rs3097649, a 3 prime UTR variant, and rs9391751, a TF binding site [25]. It seems likely that Met234β correlates with regulation of transcription of HLA-DP. The influences of SNP rs11551421, rs3097649, and rs9391751 need further exploration.

In conclusion, we identified 3 risk and 6 protective *DP* alleles that increased and decreased the ORs of undetectable anti-HBs titers. The effects of *DP*risk* alleles can be totally neutralized by the presence of *DP*protective* alleles, unfortunately, more than 50% of the study population did not carry *DP*protective* alleles. It is of great public health relevance to introduce HB vaccine that will induce more persistent immunological memory for these populations. Our findings provide information for the design of more potent HB vaccine.

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Data accessibility statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.09.001.

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