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Identification of amino acids in antigen-binding site of class II HLA proteins independently associated with hepatitis B vaccine response

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ABSTRACT

Background & aims: Genetic factors in *class II human leukocyte antigen* (*HLA*) have been reported to be associated with inter-individual variation in hepatitis B virus (HBV) vaccine response. However, the mechanism underlying the associations remains elusive. In particular, the broad linkage disequilibrium in HLA region complicates the localization of the independent effects of genetic variants. Thus, the present study aimed to identify the most probable causal variations in class II HLA loci involved in the immune response to HBV vaccine.

Methods: We performed a case-control study to assess whether *HLA-DRB1*, *-DQB1*, and *-DPB1* 4-digit alleles were associated with the response to primary HBV vaccination in 574 healthy Japanese students. To identify causative variants, we next assessed independently associated amino acid variants in these loci using conditional logistic regression analysis. Furthermore, to clarify the functional effects of these variants on HLA proteins, we performed computational structural studies.

Results: *HLA-DRB1*01:01*, *HLA-DRB1*08:03*, *HLA-DQB1*05:01*, and *HLA-DPB1*04:02* were significantly associated with sufficient response, whereas *HLA-DPB1*05:01* was associated with poor response. We then identified amino acids independently associated with sufficient response, namely, leucine at position 26 of HLA-DRβ1 and glycine-glycine-proline-methionine at positions 84–87 of HLA-DPβ1. These amino acids were located in antigen-binding pocket 4 of HLA-DR and pocket 1 of HLA-DP, respectively, which are important structures for selective binding of antigenic peptides. In addition, the detected variations in HLA-DP protein were responsible for the differences in the electrostatic potentials of the pocket, which can explain in part the sufficient/poor vaccine responses.

Conclusion: HLA-DRβ1 position 26 and HLA-DPβ1 positions 84–87 are independently associated with anti-HBs production against HBV vaccine. Our results suggest that HBsAg presentation through these HLA pocket structures plays an important role in the inter-individual variability of HBV vaccination.

1. Introduction

Hepatitis B virus (HBV) is a major global health issue due to its high prevalence and related mortality. It is estimated that 2 billion people, approximately 30% of the global population, show serological evidence of current or past HBV infection, and 240 million people worldwide are chronically infected with HBV [1–4].

HBV vaccination is the mainstay of HBV prevention. However, vaccine failure occurs in 5–10% of recipients: individuals who cannot produce a protective level of antibodies against the hepatitis B surface antigen (anti-HBs) after a standard vaccine course [5]. Although the mechanisms that determine the different vaccine responses are not fully understood, the complex interplay between hepatitis B surface antigen (HBsAg) and host factors influences the different immune response among individuals.

Abbreviations: HBV, hepatitis B virus; anti-HBs, antibody to hepatitis B surface antigen; HBsAg, hepatitis B surface antigen; LD, linkage disequilibrium; PDB, Protein Data Bank; Leu26, leucine at position 26; GGPM, glycine-glycine-proline-methionine; DEAV, aspartic acid-glutamic acid-alanine-valine.

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The heritability of the response to HBV vaccination has been reported to be 77% [6]. *Human leukocyte antigen* (*HLA*) loci have been reported as host genetic factors for the HBV vaccine response [7–12]. The function of HLA is to present antigenic peptides to CD4+ or CD8+ T lymphocytes and to initiate subsequent immune responses. Recently, two genome-wide association studies (GWASs) have demonstrated strong associations of single nucleotide polymorphisms (SNPs) in the regions of *HLA-DR* and *-DP* with the HBV vaccine response [13,14]. Thus, we hypothesize that class II HLA is the leading candidate for genetic determinants of HBV vaccine response [15,16]. However, the mechanism of class II HLA molecules in the immune response to HBV vaccination is not completely understood. In addition, extensive linkage disequilibrium (LD) in *HLA* regions [17] complicates the localization of the independent effects of each variant [18,19].

Therefore, in the present study, we aimed to address whether independently contributing amino acid variants exist in class II HLA proteins and also to determine the potential molecular mechanisms underlying the associations. One possible hypothesis is that the vaccine response is regulated by specific amino acids located at key HLA structures, i.e. antigen-binding sites, which may alter the immune response to HBsAg. To this end, we elucidated the associations between *HLA-DRB1*, *-DQB1*, and *-DPB1* alleles and the response to primary HBV vaccination. Based on the genotyping data, we searched for independently associated amino acid variants in the antigen-binding site of class II HLA using conditional logistic regression analysis and explored the effects of these variants on the protein structure.

2. Participants and methods

2.1. Participants

Participants were recruited from healthy students studying at the University of Tsukuba and Iwate Medical University, Japan. All participants received their first HBV vaccine during this study. Students who were seropositive for HBsAg and/or antibodies against hepatitis B core antigen, or who had anti-HBs prior to enrollment were excluded. In total, 578 students were enrolled from June 2013 to August of 2014, and statistical analysis was performed for 574 participants whose *HLA-DRB1*, *HLA-DQB1*, and *HLA-DPB1* alleles were successfully genotyped. The characteristics of the participants are described in Table 1.

2.2. Vaccination and serological testing

All participants received a subcutaneous dose of 10 μg of recombinant HBV vaccine (Bimmugen, Kaketsuken, Kumamoto, Japan) three times: the initial dose and then at 1 and 6–12 months after the initial dose. The serum anti-HBs titer was measured at 30–60 days after the final dose using a chemiluminescent immunoassay (Architect, Abbott Japan Co., Ltd., Tokyo, Japan). We classified the participants according to their anti-HBs titers: less than 10 mIU/mL (non-responders), 10–100 mIU/mL (low-responders), and ⩾100 mIU/mL (responders). For statistical analysis, participants with poor responses, i.e., non-responders and low-responders, were categorized as "cases", and those with sufficient responses, i.e., responders were categorized as "controls".

2.3. Genotyping

DNA was extracted from whole blood and purified using a Quick Gene DNA whole blood kit (Kurabo Industries Ltd., Osaka, Japan). Four-digit *HLA-DRB1, HLA-DQB1,* and *HLA-DPB1* alleles were genotyped based on exon 2 of each locus using the polymerase chain reaction (PCR)-sequence-specific oligonucleotide probe method with WAK Flow genotyping kits (Wakunaga, Hiroshima, Japan).

2.4. Evaluation of linkage disequilibrium between the detected alleles

Haplotype frequencies were calculated using Arlequin v3.5 software [20]. To evaluate the LD between each detected allele, the commonly used LD indices D' and r^2 were calculated from the frequencies of the related alleles and haplotypes.

2.5. Amino acid analysis

The amino acid polymorphisms of exon 2 of all alleles in three *class II HLA* loci were obtained from the IPD-IMGT/HLA Database release 3.20.0 (April 2015; https://www.ebi.ac.uk/ipd/imgt/hla/) corresponding to 4-digit alleles of each locus. There were a total of 271 amino acid positions, including 32 polymorphic positions from 91 positions in HLA-DRβ1, 30 polymorphic positions from 91 positions in HLA-DQβ1, and 22 polymorphic positions from 89 positions in HLA-DPβ1. To assess the responsible amino acids, we first examined the amino acid positions that showed independent associations with the hepatitis B vaccine response using a logistic regression model, as previously described [17]. Next, we evaluated the contribution of the prevalence of each amino acid variant at the detected positions using Fisher's exact test.

2.6. Structural studies of HLA-DR and -DP molecules

To compare the differences between responder-associated and poor responder-associated HLA protein structures, the detected amino acid positions in three-dimensional structures and the electrostatic potentials were assessed using the Molecular Operating Environment program (MOE; Chemical Computing Group; http://www.chemcomp.com/). The protein structural data were obtained from Protein Data Bank Japan (PDBj: http://pdbj.org/) entries, as follows: 1AQD, 3WEX, and 3LQZ for HLA-DR1 (*HLA-DRB1*01:01*),

Table 1
Characteristics of participants.

	Case (n = 156)		Control (n = 418)
	Non-responder <10 mIU/mL	Low-responder 10 to <100 mIU/mL	Responder 100 ⩽ mIU/mL
Number of participants	27 (4.7%)	129 (22.5%)	418 (72.8%)
Age at first dose (years)			
Mean (range)	23.4 (19–33)	22.4 (19–39)	20.6 (19–33)
Gender			
Male	19	82	166
Female	8	47	252
GMT (mIU/mL)	4.7	42.7	746.1

GMT: geometrical mean titer of anti-hepatitis B surface antigen antibody.

HLA-DP5 (including *HLA-DPB1*05:01*), and HLA-DP2 (*HLA-DPB1*02:01*), respectively. Although protein structural data were not available for the responder-associated HLA-DP4, the targeted amino acid residues, such as residues 84–87 of HLA-DPβ1 in 3LQZ, were exactly the same as those of *HLA-DPB1*04:01* and -*DPB1*04:02*. Therefore, we used 3LQZ to compare the effects of the detected amino acid variants. Using these data, protonation and minimization were performed, and the Poisson-Boltzmann electrostatic potentials of the HLA-DR and -DP protein surfaces were calculated.

2.7. Statistical analysis

Associations between *HLA-DRB1*, *-DQB1*, *-DPB1* alleles, and the response to HBV vaccination were assessed using Fisher's exact test from two-by-two allele frequency tables. Factors having a P lower than the threshold with Bonferroni correction were considered statistically significant; the thresholds were set at $\alpha = 0.05/60$ (8.33×10^{-4}) for *HLA-DRB1*, *HLA-DQB1*, and *HLA-DPB1* alleles, and $\alpha = 0.05/271$ (1.85×10^{-4}) for amino acids on the basis of the number of detected alleles and amino acid positions. Alleles showing significant associations with "cases" (with an odds ratio [OR] > 1.0) were defined as "risk alleles", whereas those showing significant associations with controls (with an OR < 1.0) were defined as "protective alleles". For statistical analyses, R ver3.1.1 (https://www.r-project.org/) and SPSS software version 22.0 (IBM Corp. Armonk, NY) were used.

2.8. Ethical statement

All participants were recruited after they had provided written informed consent. The study protocol was in accordance with the Declaration of Helsinki and the Ethical Guidelines for Human Genome/Gene Analysis Research of the Ministry of Health, Labor, and Welfare of Japan and was approved by the Research Ethics Committees of the University of Tsukuba and Iwate Medical University.

3. Results

3.1. Associations between HLA-DRB1, -DQB1, and -DPB1 alleles and the response to primary HBV vaccination

In the present study, 4.7% (27/574) of the participants were non-responders (anti-HBs <10 mIU/mL), 22.5% (129/574) were low-responders (10–100 mIU/mL), and 72.8% (418/574) were responders (⩾100 mIU/mL; Table 1). The number of men was significantly higher than the number of women in the case group ($P < 0.0001$, OR = 2.79, 95% confidence interval [CI] = 1.90–4.09). In total, 31 *HLA-DRB1*, 14 *HLA-DQB1*, and 15 *HLA-DPB1* alleles were identified. The frequencies of the identified alleles at each locus were concordant with the frequencies determined by the HLA Laboratory (Kyoto, Japan) for the Japanese population [21] and with those determined for healthy Japanese controls in previous reports (Supplementary Table S1 for *HLA-DRB1* [22], Supplementary Table 2 for *HLA-DQB1* [23], and Supplementary Table 3 for *HLA-DPB1* [24,25]).

In our case-control study, we assessed the association between HLA alleles with anti-HBs production by HBV vaccination and revealed 11 alleles with $P < 0.05$ (listed in Table 2). Among them, 1 risk allele in *HLA-DPB1*, 2 protective alleles in *HLA-DRB1*, and 1 protective allele in *HLA-DQB1* and *-DPB1* reached statistical significance after considering multiple testing. *HLA-DPB1*05:01* was the most prevalent allele, identified in 39.4% of the participants, and was significantly associated with a poor response to the primary HBV vaccination ($P < 0.0001$, OR = 1.86, 95% CI = 1.43–2.42), whereas *HLA-DRB1*01:01*, *HLA-DRB1*08:03*, *HLA-DQB1*05:01*, and

Table 2
Association of *HLA-DRB1*, *-DQB1*, and *-DPB1* alleles with the response to primary HBV vaccination.

Allele	Frequency (%) (2n = 1,148)	Case Count (%) (2n = 312)	Control Count (%) (2n = 836)	*P* value	OR (95% CI)
DRB1					
DRB1*01:01	5.2	4 (1.3)	56 (6.7)	**0.0001**	**0.18 (0.07–0.50)**
DRB1*04:06	3.6	17 (5.4)	24 (2.9)	0.0478	1.95 (1.03–3.68)
DRB1*08:03	7.2	9 (2.9)	74 (8.9)	**0.0003**	**0.31 (0.15–0.62)**
DRB1*09:01	14.5	56 (17.9)	110 (13.2)	0.0473	1.44 (1.02–2.05)
DRB1*12:01	3.4	5 (1.6)	34 (4.1)	0.0434	0.38 (0.15–0.99)
DRB1*14:06	1.8	10 (3.2)	11 (1.3)	0.0456	2.48 (1.04–5.91)
DQB1					
DQB1*05:01	5.7	4 (1.3)	61 (7.3)	**<0.0001**	**0.16 (0.06–0.46)**
DQB1*06:04	5.5	9 (2.9)	54 (6.5)	0.0190	0.43 (0.21–0.88)
DPB1					
DPB1*04:01	4.3	5 (1.6)	44 (5.3)	0.0049	0.29 (0.12–0.75)
DPB1*04:02	9.9	14 (4.5)	100 (12.0)	**0.0001**	**0.35 (0.19–0.61)**
DPB1*05:01	39.4	157 (50.3)	295 (35.3)	**<0.0001**	**1.86 (1.43–2.42)**

Fisher's exact *P* values and ORs were calculated using the allelic model.
Alleles with $P < 0.05$ are listed. (See Supplementary Tables S1–S3 for all examined alleles).
Shaded columns indicate $P < \alpha$ ($\alpha = 0.05/60$).

*HLA-DPB1*04:02* were significantly associated with a sufficient response ($P = 0.0001$, OR = 0.18, 95% CI = 0.07–0.50; $P = 0.0003$, OR = 0.31, 95% CI = 0.15–0.62, $P < 0.0001$, OR = 0.16, 95% CI = 0.06–0.46; $P = 0.0001$, OR = 0.35, 95% CI = 0.19–0.61; respectively; Table 2).

The LD of the detected alleles is shown in Supplementary Table 4. Strong LD was observed between *HLA-DRB1*01:01* and *HLA-DQB1*05:01* ($D' = 1.00$, $r^2 = 0.92$). The *DRB1*08:03-DQB1*05:01* haplotype was not observed in our dataset, indicating that associations of these alleles were not explained by the linkage. The *D*' of the other detected alleles varied from 0.17 to 0.79.

3.2. Amino acid variants associated with the HBV vaccine response

Next, we examined all the amino acid variants in exon 2 of *HLA-DRB1*, *-DQB1*, and *-DPB1* loci. Four polymorphic amino acid positions in HLA-DRβ1 (positions 11, 16, 26, and 30), one position in HLA-DQβ1 (position 57), and six positions in HLA-DPβ1 (positions 35, 55, and 84–87) showed significant associations ($P_{omnibus} < 1.85 \times 10^{-4}$) with the response to primary HBV vaccination. To detect the independent contribution of the variants while considering the effects of the strong LD, we then used a conditional logistic regression analysis, as previously described [17]. The most significant amino acid position in three major *class II HLA* loci was position 26 of HLA-DRβ1 ($P_{omnibus} = 2.61 \times 10^{-7}$). After controlling for position 26 of HLA-DRβ1, the significance of positions 11, 16, 30 in HLA-DRβ1; 57 in HLA-DQβ1; 35 in HLA-DPβ1 disappeared, but positions 55 and 84–87 of HLA-DPβ1 remained significant and positions 84–87 of HLA-DPβ1 had the lowest *P* value ($P_{omnibus} = 2.97 \times 10^{-5}$), indicating their independent associations. These four sequential amino acids (positions 84–87) in HLA-DPβ1

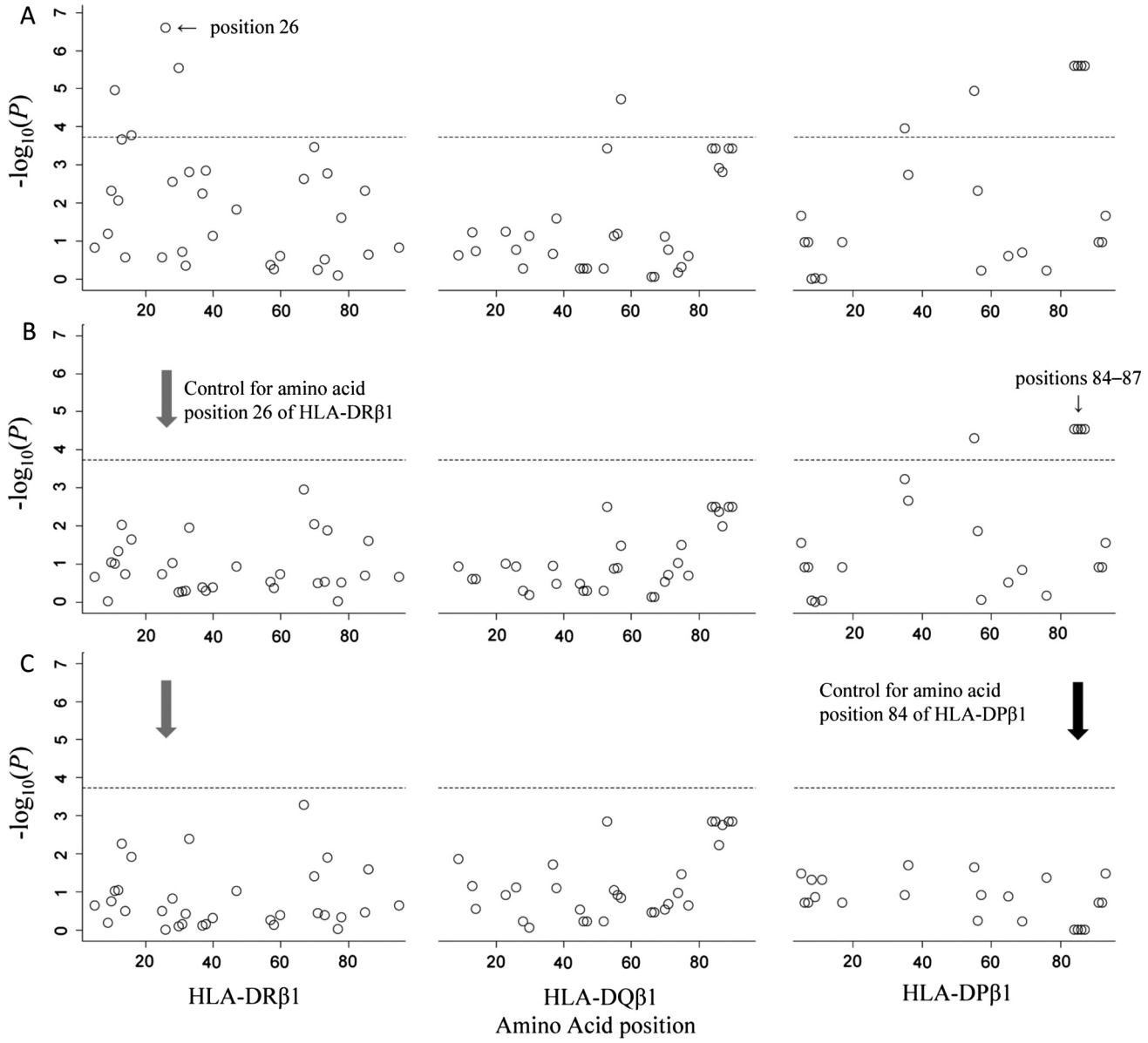

Fig. 1. Amino acid variants associated with the HBV vaccine response. The associated amino acid positions were identified using conditional logistic regression analysis. The small open circles represent $-\log_{10}$ (*P* values) for the tested amino acid positions. The horizontal dashed black lines indicate the significance threshold of $P_{omnibus} = 1.85 \times 10^{-4}$. (A) Amino acid position 26 of HLA-DRβ1 showed the strongest association with the HBV vaccine response among all of the polymorphic amino acid positions encoded by exon 2 of *HLA-DRB1*, *-DQB1*, and *-DPB1*. (B) Controlling for position 26 of HLA-DRβ1 revealed an independent association for positions 84–87 of HLA-DPβ1. (C) After controlling for position 26 of HLA-DRβ1 and position 84 of HLA-DPβ1, no significantly associated position was observed. Detailed association results are available in Supplementary Table 5.

Table 3
Amino acid residues associated with the HBV vaccine response.

Amino acid positions	Amino acid residues	Frequency		*P* value	OR	95% CI	Explainable 4-digit alleles
		Case (2n = 312) (%)	Control (2n = 836) (%)				
position 26 of DR 1	Y	57 (18.3)	112 (13.4)	0.0399	1.44	1.02–2.05	*DRB1*09:01*
	F	244 (78.2)	609 (72.8)	0.686	1.34	0.98–1.82	*DRB1*04:06* *DRB1*14:06*
	L	11 (3.5)	115 (13.8)	**<0.0001**	**0.23**	**0.12–0.43**	*DRB1*01:01* *DRB1*12:01*
positions 84–87 of DP 1	**GGPM**	88 (28.2)	365 (43.7)	**<0.0001**	**0.51**	**0.38–0.67**	*DPB1*04:01* *DPB1*04:02*
	DEAV	224 (71.8)	471 (56.3)	**<0.0001**	**1.97**	**1.49–2.62**	*DPB1*05:01*

Fisher's exact *P* values and ORs were calculated for the presence versus absence of each amino acid variant. *P* values statistically significant after correction of the significance level ($\alpha = 0.05/271$) are indicated as shaded columns.
Y: Tyrosine, F: Phenylalanine, L: leucine, GGPM: glycine-glycine-proline-methionine, DEAV: aspartic acid-glutamic acid-alanine-valine.

showed absolute LD; therefore, we treated them as a group, as reported previously [26]. After controlling for both position 26 of HLA-DRβ1 and position 84 of HLA-DPβ1, the significance of position 55 in HLA-DPβ1 disappeared and no significant association was observed. (Fig. 1 and Supplementary Table 5).

Assessment of all the variants at independently associated amino acid positions clarified that leucine at position 26 (Leu26) of HLA-DRβ1 and glycine-glycine-proline-methionine (GGPM) at positions 84–87 of HLA-DPβ1 were significantly associated with the sufficient vaccine response (responder-associated amino acids; $P < 0.0001$, OR = 0.23, 95% CI = 0.12–0.43; $P < 0.0001$, OR = 0.51, 95% CI = 0.38–0.67, respectively), whereas the sequence of aspartic acid-glutamic acid-alanine-valine (DEAV) at positions 84–87 of HLA-DPβ1 was significantly more frequent in poor responders ($P < 0.0001$, OR = 1.97, 95% CI = 1.49–2.62; Table 3). Notably, the detected amino acid variants completely explained the associations of the above-mentioned *HLA-DP* alleles; the poor responder-associated amino acids (i.e., DEAV) at positions 84–87 of HLA-DPβ1 tagged the risk allele such as *HLA-DPB1*05:01*, whereas the responder-associated amino acids (i.e., GGPM) at the same position were commonly found in the protective alleles, such as *HLA-DPB1*04:02* and *-DPB1*04:01*.

3.3. Structural studies of the HLA-DR and -DP molecules

Amino acid variations in class II HLA proteins are the basis for differential binding of antigenic peptides. In particular, the polymorphic "pockets" of the antigen-binding groove in class II HLA proteins play a critical role in peptide selection [27]. To explore the effects of the detected amino acid residues on the HLA protein

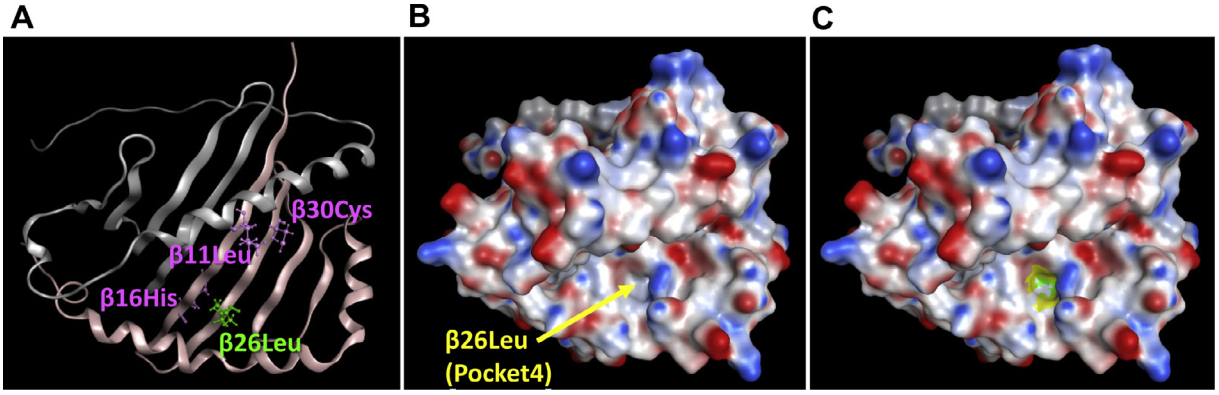

Fig. 2. Structural studies of the HLA-DR1 molecule. (A) A three-dimensional ribbon model of the HLA-DR1 (PDB:1AQD) molecule carrying responder-associated Leu26 is shown. HLA-DRα1 (4Glu-96Pro) is colored silver, and HLA-DRβ1 (4Arg-96Glu) is colored pink. The variant with the lowest *P* value (Leu26) in the β chain is indicated in green, and the other associated variants (β11,16,30) are indicated by a pink ball-stick model. (B) The electrostatic potential of HLA-DR1. Potentials less than −80 kcal/mol are colored red, those greater than 80 kcal/mol blue, and neutral potentials are colored white. The yellow arrow indicates Leu26 of DRβ1, which is in pocket 4 of the HLA-DR molecule. (C) The green area shows the surface close to Leu26 (within 4.5 Å) in (B).

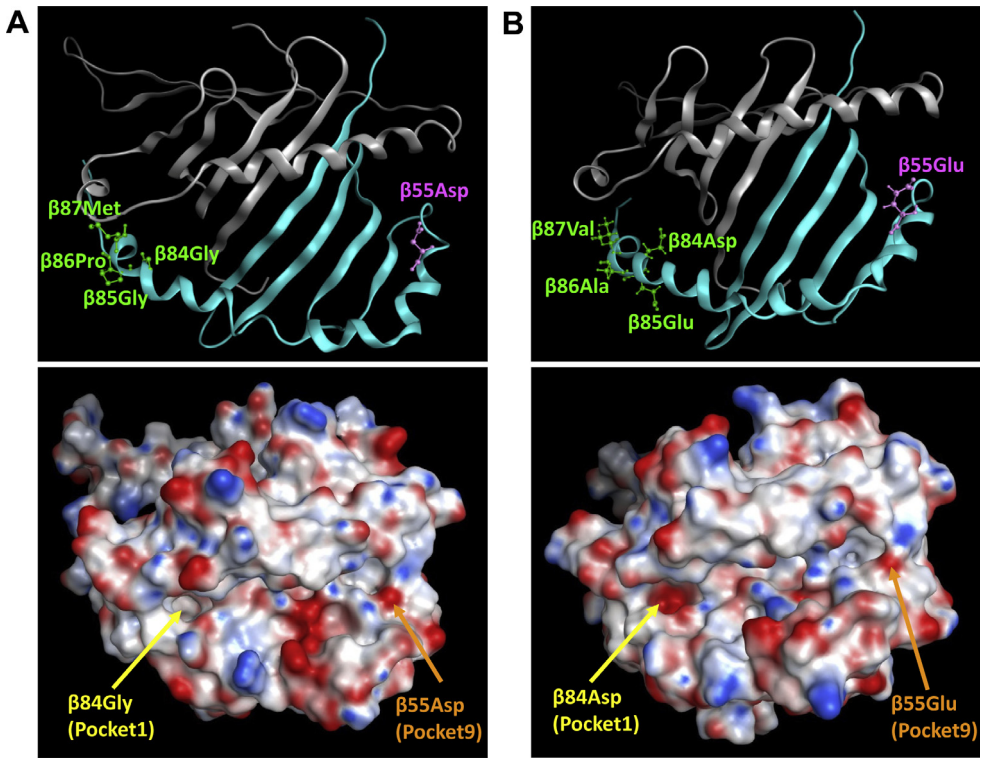

Fig. 3. Structural studies of the HLA-DP molecules. The ribbon model and the electrostatic properties of HLA-DP molecules carrying responder-associated/poor responder-associated amino acid residues are compared in A and B. The variants with the lowest *P* values (β84–87) are indicated in green, and the other associated variant (β55) is indicated by the pink ball-stick model. Potentials less than −80 kcal/mol are colored red, those greater than 80 kcal/mol are colored blue, and neutral potentials are colored white. (A) HLA-DP2 (PDB:3LQZ) molecule carrying the responder-associated amino acid variants (Gly-Gly-Pro-Met) at positions 84–87. HLA-DPα1 (1Ile-119Val) is colored silver, and HLA-DPβ1 (3Ser-96Arg) is colored cyan. The bottom of pocket 1 consists of 84Gly (yellow arrow), whereas pocket 9 consists of 55Asp (orange arrow). (B) HLA-DP5 (PDB:3WEX) molecule carrying the poor responder-associated amino acid variants (Asp-Glu-Ala-Val) at positions 84–87. HLA-DPα1 (1Ile-115Pro) is colored silver, and HLA-DPβ1 (4Pro-95Pro) is colored cyan. The bottom of pocket 1 consists of 84Asp (yellow arrow), whereas pocket 9 consists of 55Glu (orange arrow).

structure, we performed computational structural studies. The genetically significant amino acid positions in the crystal structure of the representative HLA-DR and HLA-DP molecules are shown in Figs. 2 and 3. Importantly, residue 26 of HLA-DRβ1 was located at the bottom of the pocket 4 (Fig. 2C), which interacted directly with the antigen peptides and contributed substantially to the selective recognition of the immunogenic epitope [28]. Additionally, Leu26 was found to be hydrophobic (Fig. 2B).

Residue 84 of HLA-DPβ1 chain also constituted the bottom of pocket 1 of HLA-DP molecules (Fig. 3). In our cohort, all participants had either the GGPM or DEAV motif at positions 84–87, and the electrostatic properties differed between the two motifs; the responder-associated amino acid variants (i.e., GGPM) caused pocket 1 to become hydrophobic (Fig. 3A), whereas the poor responder-associated amino acid variants (i.e., DEAV), particularly aspartic acid at position 84, induced a negative charge at the surface of the pocket (Fig. 3B), implying that these differences would affect the antigen-binding affinity of the protein.

Meanwhile, it was difficult to determine whether the associated residues 11 and 30 of HLA-DRβ1 and residue 55 of HLA-DPβ1, which formed pocket 6 of HLA-DR and pocket 9 of HLA-DP, also influenced the properties of the antigen-binding pockets, or whether their associations only resulted from the strong LD of the top two positions mentioned above.

4. Discussion

In the present study, we conducted 4-digit genotyping of *HLA-DRB1*, *HLA-DQB1*, and *HLA-DPB1* loci in Japanese students receiving a primary HBV vaccination and performed an association study evaluating the humoral immune response to HBsAg. Our key findings are as follows; (1) significantly associated protective alleles were found at all loci of *HLA-DRB1*, *HLA-DQB1*, and *HLA-DPB1*; (2) There were independently associated amino acid variants in HLA-DRβ1 and -DPβ1; (3) these amino acids were located on the surfaces of the antigen-binding pockets of HLA-DR and HLA-DP molecules.

A few reports have described 4-digit genotyping of all of three major loci (*HLA-DR*, *-DQ*, and *-DP*) simultaneously in a single cohort; thus, we assessed these loci to clarify the associations with the HBV vaccine response. The alleles detected in the present study were similar to those in previous vaccine studies in several other populations. *HLA-DRB1*01:01* and *HLA-DRB1*08:03* were found to be significantly more frequent in responders in Chinese, Caucasian, and Belgian cohorts [9,29,30] and in the Korean population [7], respectively. The identification of *HLA-DQB1*05:01* in the present study was concordant with reports from Chinese and Belgian populations [9,30]. The association between *HLA-DPB1*05:01* and a poor response to HBV vaccination has been reported in a Taiwanese cohort [8], and *HLA-DPB1*04:01* and *HLA-DPB1*04:02* have been reported as protective alleles in Taiwanese and Belgian populations [8,30]. In contrast, although *HLA-DRB1*07* has been reported as a risk allele in many populations [7,30,31], the allele frequency of *HLA-DRB1*07 (*07:01)* in the present study was only 0.26% (Supplementary Table 1); therefore, it was difficult to assess the association.

Class II HLA proteins bind and present antigenic epitopes to CD4-positive helper T cells and subsequently initiate the immune

response. In the present study, the protective alleles were detected at all three loci, suggesting that multiple class II HLA proteins contribute to the presentation of HBsAg epitopes. However, the identification of causal variants is difficult because of the strong LD among alleles in *HLA* region.

We next examined the amino acid polymorphisms in exon 2 of each locus, which encodes the antigen-binding sites. Our results demonstrated the presence of independently associated amino acid positions, all of which are located in the antigen-binding pockets of HLA-DR and HLA-DP proteins. The importance of the amino acid variations in class II HLA antigen-binding pockets, typically pockets 1, 4, 6, and 9, has been reported in various infectious and autoimmune diseases [27,32–34].

To the best of our knowledge, this is the first report examining the association between amino acid residues in HLA-DRβ1 and the HBV vaccine response. The small aliphatic residue Leu26 of HLA-DRβ1 was commonly carried in the protective alleles, such as *HLA-DRB1*01:01* and **12:01*, whereas the risk direction alleles, i.e., *HLA-DRB1*09:01*, **04:06*, and **14:06*, had large aromatic amino acids, such as phenylalanine (Phe) or tyrosine (Tyr), at this position (Table 3). Only the protective association of *HLA-DRB1*08:03* was not explainable by Leu26. Structural studies revealed that position 26 of HLA-DRβ1 was located at the surface of the pocket 4, and all of the three amino acid variants at this position had hydrophobic properties. Thus, we expect that differences in the hydrophobic interactions or shapes of the pockets, mediated by the amino acid variants at position 26 (Leu, Phe, and Tyr), may affect the antigen-binding affinity [33]. Furthermore, a cellular experimental study has shown that changing only residue 26 of HLA-DRβ1 has profound effects on T-cell recognition [35]. When the complex of the antigenic epitopes and class II HLA molecules is recognized by the T-cell receptor, variants at position 26 may induce significant functional differences in the T lymphocyte-mediated immune response.

In addition to HLA-DRβ1, conditional logistic regression analysis showed that positions 84–87 of HLA-DPβ1 were also independently associated with the HBV vaccine response. A previous study in Taiwanese individuals demonstrated an association between the response to the booster HBV vaccination and amino acid variants at these positions [8]. In the present study, our structural analysis demonstrated that the amino acid residue at position 84 constituted the bottom of the pocket 1 of HLA-DP protein. Additionally, assessment of the surface electrostatic properties clarified the obvious major difference between GGPM and DEAV, suggesting that these variants are key determinants of the affinity for HBsAg epitopes, which regulates the vaccine response. The importance of the amino acids at positions 84–87 and pocket 1 of HLA-DP molecule as the functional unit for the antigen-binding specificity was also described in other antigens [26,36].

In summary, our results indicated that the independently associated amino acid variants caused significant changes in the structural and electrostatic properties of class II HLA antigen-binding pockets, representing the major host genetic determinants of the HBV vaccine response. These amino acid changes contribute to the different anti-HBs production, likely by influencing the binding affinity of HBsAg epitopes and the presentation to CD4-positive T lymphocytes. Further studies on class II HLA proteins with regard to HBsAg at the molecular level, particularly functional analysis and trans-ethnic examinations, will facilitate the development of more potent HBV vaccines.

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6. Conflicts of interest statement

The authors report no potential conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.08.068.

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