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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.

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