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# Highly Immunogenic DQB1 Mismatch Eplets Are Associated With Development of Chronic Active Antibody-Mediated Rejection: A First Report From Japan

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## ABSTRACT

**Background.** De novo donor-specific antibody (dnDSA), especially against class II HLA, correlates with chronic active antibody-mediated rejection (CAAMR), which eventually leads to graft loss. It would be helpful if we could identify the patients at high risk of dnDSA development in terms of histocompatibility. Structure-based matching strategy assessing mismatched epitopes/eplets by comparing polymorphic amino acid sequences can predict the risk of development of dnDSA and CAAMR. However, it has not been evaluated in Japanese patients whose diversity in HLA is limited.

**Patients and Methods.** We retrospectively studied 55 living related kidney transplant patients and ascertained donor and recipient HLA-A, -B, -DRB1, and -DQB1. The number of mismatched eplets was determined using an algorithm, HLAMatchmaker version 3. The relationship between characteristics of mismatched eplets and development of CAAMR was evaluated.

**Results.** There were 8 patients in the CAAMR group and 47 in the control group. The numbers of mismatched HLAs ( $3.6 \pm 1.2$  in CAAMR and  $3.7 \pm 2.0$  in control groups), mismatched eplets ( $32.2 \pm 10.4$  in CAAMR and  $34.4 \pm 19.8$  in control groups), mismatched DRB1 eplets ( $11.2 \pm 4.3$  in CAAMR and  $11.5 \pm 7.9$  in control groups), and mismatched DQB1 eplets ( $9.2 \pm 4.3$  in CAAMR and  $10.5 \pm 7.3$  in control groups) were not significantly different. Significantly more patients had at least one highly immunogenic mismatched eplet (62.5% in CAAMR and 25.5% in control groups;  $P = .024$  by  $\chi^2$  test).

**Conclusions.** The presence of highly immunogenic mismatched eplets is associated with development of CAAMR.

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**A**CCORDING to recent progress with immunosuppressive agents and regimens, HLA mismatch is insensitive to predict graft survival after kidney transplantation. However, chronic active antibody-mediated rejection (CAAMR) is still a leading cause of long-term allograft loss [1]. De novo donor-specific antibody (dnDSA), especially against mismatched class II HLA, correlates with development of CAAMR after kidney transplantation [2]. There are several well-known risk factors that predict the development of CAAMR, such as history of acute T cell-mediated rejection, inadequate immunosuppression, and nonadherence to immunosuppression, which are all postoperative factors [3,4]. However, to date, there are no preoperative risk factors for predicting development of CAAMR. Therefore, it

would be helpful if we could identify in advance patients at high risk of developing CAAMR, in terms of histocompatibility. It is necessary to introduce new methods to analyze histocompatibility other than traditional HLA matching.

Eplets, formerly known as triplets, are defined as short polymorphic amino acid sequences derived from structure-based matching [5,6]. An epitope, the part of an HLA that is recognized by antibodies, consists of one or multiple

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**Table 1. Patient Characteristics**

	CAAMR (n = 8)	Control (n = 47)	P value
Recipient age	26.6 ± 20.3	38.8 ± 19.1	ns*
Donor age	47.6 ± 13.5	53.2 ± 11.0	.022*
Male:female	3:5	26:21	ns†
Duration of dialysis (y)	1.7 ± 1.8	2.7 ± 4.1	ns*
Observational period (y)	8.0 ± 3.2	8.1 ± 3.2	ns*
Steroid withdrawal	3 cases (37.5%)	13 cases (27.7%)	ns†
CNI (TAC:CsA)	8:0	39:8	ns†
ABO-incompatible	2 cases (25.0%)	18 cases (38.2%)	ns*
dnDSA (class and locus)	Class I: 3 cases (A in 2 cases and B in 1 case) Class II: 7 cases (DRB1 in 2 cases and in 6 cases)	n/a	n/a
History of ATMR	4 cases (50.0%)	6 cases (12.7%)	.042†

Abbreviations: ATMR, acute T cell-mediated rejection; dnDSA, de novo donor-specific antibody; CAAMR, chronic active antibody-mediated rejection; CNI, calcineurin inhibitor; CsA, cyclosporine; n/a, not applicable; ns, not significant; TAC, tacrolimus.

\*Unpaired *t* test.

† $\chi^2$  Test.

combinations of eplets [5,6]. HLA-Matchmaker, developed by Duquesnoy, is a computer algorithm that can detect mismatched eplets by comparing amino acids sequences of donor and recipient HLA molecules [5]. There are several reports demonstrating the usefulness of HLA-Matchmaker to identify recipients who developed dnDSA leading to CAAMR and graft loss [7]. Wiebe et al also reported that higher number of mismatched eplets in class II HLA and nonadherence synergistically affects rejection-free graft survival [8]. However, no study has evaluated the usefulness of HLA-Matchmaker in the Japanese population, in which HLA diversity is limited.

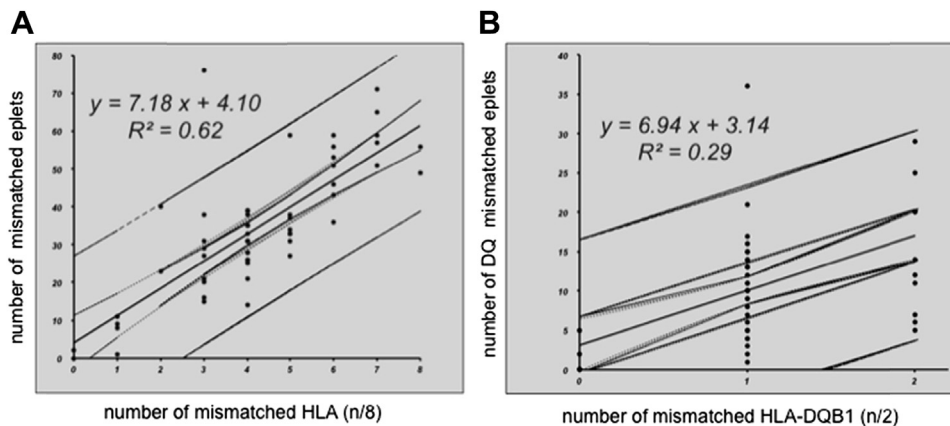
Therefore, we aimed to determine the usefulness of an eplet-based matching strategy using HLA-Matchmaker to assess the risk of CAAMR in our patients who underwent kidney transplantation.

## PATIENTS AND METHODS

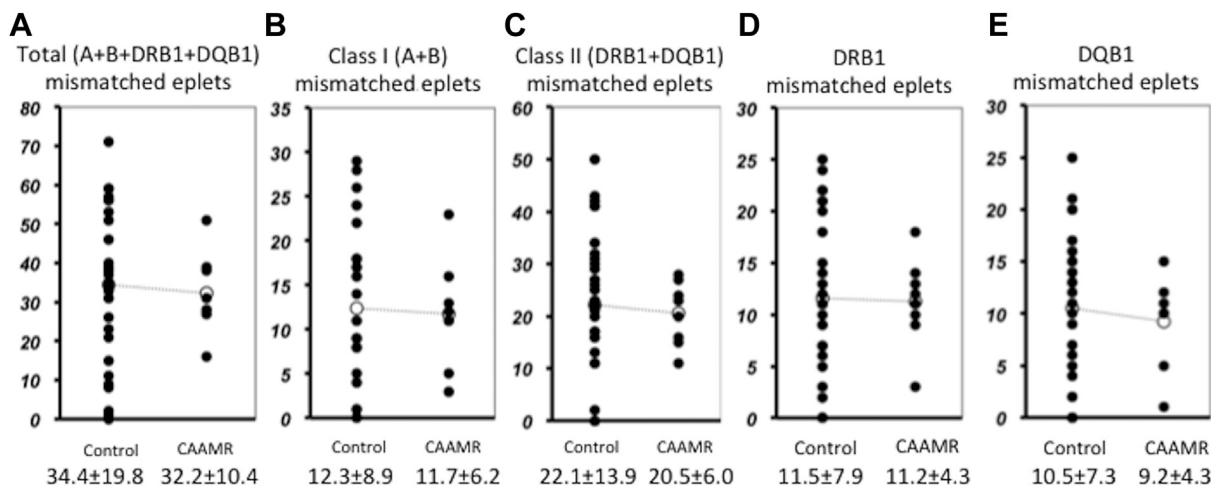
We retrospectively studied 95 living kidney transplant recipients who underwent surgery from 2006 to 2012. Thirty-seven pairs of recipients and donors whose HLA-A, -B, -DRB1, and -DQB1 loci were

not ascertained at a high resolution (4 digits) were excluded. Three recipients with preformed DSA were also excluded. Fifty-five participants were divided into the CAAMR group (n = 8), which developed CAAMR, and control group (n = 47), which did not develop CAAMR. Diagnosis of CAAMR was made according to the Banff 2013 criteria [9]. Serologic evidence of dnDSA was determined by LABScreen (One Lambda, California, CA, USA) on Luminex and/or flow cytometric crossmatching. Patients' characteristics are shown in Table 1. There was no significant difference between the CAAMR and control groups for recipients' age, gender, ABO-incompatible cases, calcineurin inhibitor (tacrolimus or cyclosporine), steroid early discontinuation regimen, and HLA mismatch. However, donors' age was significantly younger and history of acute T cell-mediated rejection prior to CAAMR was more frequent in the CAAMR group than the control group. In the CAAMR group, dnDSA determined by LABScreen belonged to HLA class I in 3 cases (2 HLA-A and 1 HLA-B) and HLA class II in 7 cases (2 HLA-DRB1 and 6 HLA-DQB1, including duplication), suggesting that DQB1 mismatch is closely associated with CAAMR.

Among these 55 patients in the CAAMR and control groups, mismatched eplets were determined by HLA-Matchmaker version 3. We evaluated the correlation between the number of mismatched HLAs and mismatched eplets. To determine the impact of the number of mismatched eplets on development of CAAMR, the



**Fig 1.** The relationship between number of mismatched HLA and number of mismatched eplets. (A) Total number of mismatched HLAs correlated with total number of mismatched eplets. (B) The number of mismatched HLA-DQB1 (0, 1, or 2) also correlated with DQB1 mismatched eplets with low coefficient *r*.



**Fig 2.** Comparison of mismatched eplets between CAAMR and control groups. Total number of mismatched eplets (A), class I (B), class II (C), or mismatched eplets belonging to DRB1 (D), or DQB1 (E) was not significantly different between the CAAMR and control groups. Abbreviation: CAAMR, chronic active antibody-mediated rejection.

number of mismatched eplets in each HLA locus or class was compared between the CAAMR and control groups. In addition, the percentage of recipients who had highly immunogenic eplets belonging to HLA-DRB1 and -DQB1, identified by Wiebe et al by analyzing kidney transplant recipients who developed dnDSA [7], was compared between the 2 groups. *P* values were calculated with the unpaired Student *t* test or  $\chi^2$  test using GraphPad Prism version 5 (GraphPad Software Inc., La Jolla, CA). Statistical significance was defined by *P* < .05. We obtained approval for the study from the Institutional Review Board of Hokkaido University Hospital.

## RESULTS

### Correlation Between Number of Mismatched HLAs and Mismatched Eplets

First, we analyzed the correlation between the number of mismatched HLAs and mismatched eplets. There was a significant correlation between total number of mismatched HLAs (HLA-A, -B, -DRB1, and -DQB1) and total number of mismatched eplets with a formula: the number of mismatched eplets =  $7.18 \times$  the number of mismatched HLA + 4.10 (*P* < .05,  $R^2 = 0.62$ ; Fig 1A). When we compared correlation between the numbers of mismatched HLA-DQB1 and mismatched DQB1 eplets, there was a significant but weak correlation with a formula: the number of mismatched eplets =  $6.94 \times$  the number of mismatched HLA + 3.14 (*P* < .05,  $R^2 = 0.29$ ; Fig 1B).

### Number of Mismatched HLAs or Eplets Does Not Affect Development of CAAMR

To determine the impact of the number of mismatched eplets on development of CAAMR, we compared the number of mismatched eplets in each HLA locus between the CAAMR and control groups. There was no significant difference in total number ( $32.2 \pm 10.4$  in CAAMR and  $34.4 \pm 19.8$  in control; Fig 2A), HLA class I ( $11.7 \pm 6.2$  in CAAMR and  $12.3 \pm 8.9$  in control groups; Fig 2B), HLA class II ( $20.5 \pm 6.0$  in CAAMR and  $22.1 \pm 13.9$  in control groups; Fig 2C), HLA-DRB1 ( $11.2 \pm 4.3$  in CAAMR and  $11.5 \pm 7.9$  in control groups; Fig 2D), and HLA-DQB1 ( $9.2 \pm 4.3$  in CAAMR and  $10.5 \pm 7.3$  in control groups; Fig 2E). Similarly, there was no significant difference in number of total (A, B, DRB1, and DQB1) mismatched HLAs ( $3.6 \pm 1.2$  in CAAMR and  $3.7 \pm 2.0$  in control groups) and DQB1 ( $0.9 \pm 0.3$  in CAAMR and  $0.8 \pm 0.7$  in control groups). These results suggest that the number of mismatched eplets or HLAs did not affect the risk of CAAMR in our cohort.

### Comparison of Frequency of Recipients Who Had Highly Immunogenic Eplets

To characterize mismatched eplets in the CAAMR group, we focused on highly immunogenic eplets that formed the epitopes that corresponded to some of Terasaki's epitopes

**Table 2. Highly Immunogenic Eplets**

	HLA Locus	CAAMR (n = 8)	Control (n = 47)	<i>P</i> Value
Highly immunogenic mismatched eplets per recipient	DRB1	0.25 ± 0.70	0.25 ± 0.60	ns*
	DQB1	1.37 ± 1.18	0.68 ± 1.20	ns*
Recipient with at least one mismatched highly immunogenic eplets	DRB1	1 case (12.5%)	8 cases (17.0%)	ns <sup>†</sup>
	DQB1	6 cases (62.5%)	12 cases (25.5%)	.024 <sup>†</sup>

Abbreviations: CAAMR, chronic active antibody-mediated rejection; ns, not significant.

\*Unpaired *t* test.

<sup>†</sup> $\chi^2$  Test.

[7]. When we analyzed the percentage of recipients who had one or more highly immunogenic mismatched eplets, a significantly higher number of recipients had at least one highly immunogenic mismatched eplet of DQB1 in the CAAMR group (62.5% in CAAMR and 25.5% in control groups;  $P = .024$  by  $\chi^2$  test; Table 2). Mean number of highly immunogenic mismatched eplets per patient was  $1.37 \pm 1.18$  in the CAAMR group and  $0.68 \pm 1.20$  in the control group. There was no significant difference in the percentage of recipients who had highly immunogenic mismatched eplets in DRB1 (12.5% in CAAMR and 17.0% in control groups by  $\chi^2$  test; Table 2). Mean number of highly immunogenic mismatched eplets per patient was  $0.25 \pm 0.70$  in the CAAMR group and  $0.25 \pm 0.60$  in the control group. These results suggest that highly immunogenic mismatched DQB1 eplets affect the development of CAAMR by inducing dnDSA after kidney transplantation.

## DISCUSSION

Our data suggest the usefulness of analyzing mismatched eplets to predict the recipients at high risk of CAAMR in the Japanese population, whose HLA diversity is limited. In accordance with other recent reports [4,10], mismatched HLA-DQB1 was associated with CAAMR in our cohort. In addition, our results indicated that the presence of highly immunogenic mismatched eplets in HLA-DQB1 determined by HLAMatchmaker, but not simply the number of mismatched eplets, is a risk factor for development of CAAMR after kidney transplantation. In contrast to previous reports [7], there was no significant difference in the number of mismatched epitopes belonging to DRB1 or DQB1. It is necessary to determine whether this discrepancy was caused by our small number of cases or if it is a Japanese population-specific finding.

How the immunogenicity of each eplet is determined has not been clarified yet. Kosmoliaptsis's group has reported that electrostatic status may affect the immunogenicity of mismatched eplets [11,12]. However, it is necessary to investigate what other factors affect immunogenicity of eplets/epitopes. In addition, the highly immunogenic eplets were a small part of the similar eplets/epitopes, and analysis is underway to identify new high- and low-immunogenic eplets. The future study may enable tailor-made immunosuppression based on immunologic risk group determined by characteristics of mismatched eplets.

One of the limitations of the current study was that, in addition to the limited number of recipients analyzed, we did not evaluate all the HLA loci. Although we evaluated the major HLA-A, -B, -DRB1, and -DQB1 loci, which are diverse and induce a major alloresponse (ie, dnDSA), we have not studied other HLAs such as HLA-C, -DR3/4/5, -DRA, -DPA1, -DPB1, and -DQA1. It is also important to

increase the number of study subjects and analyze the correlation of other traditional factors such as nonadherence and history of acute T cell-mediated rejection.

In conclusion, our study suggests that the presence of highly immunogenic mismatched eplets, but not simply the number of mismatched eplets, is associated with development of CAAMR after kidney transplantation. Our results also suggest that molecular matching is applicable to identification of patients who are at risk of CAAMR. We may be able to change the strength of immunosuppression according to the risk of CAAMR in the future.

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