# Assessing DQB1 Immunogenicity Using the Two Mismatches One DSA Approach in a Local Kidney Transp

### Introduction

De novo antibodies to mismatched donor HLA antigens (dnDSAs) are associated with rejection and inferior graft survival in solid organ transplantation. It is well recognized that not all HLA mismatches are equal in their potential to induce dnDSA formation. Understanding the immunogenicity of individual HLA mismatches is essential for immunological risk stratification to improve graft outcomes in transplant recipients. The aim of this study is to identify the relative immunogenicity of DQB1 antigen and eplet mismatches in kidney transplant recipients utilizing the two mismatches one DSA (2MM1DSA) approach conceptualized by Tambur et al.

## **Objectives**

- Decipher the relative immunogenicity of DQB1 antigen mismatches in context of recipients' DQB1 type.
- Assess the immunogenicity of DQB1 eplet mismatches.

### Methods

This study includes 57 kidney transplant recipients who have two DQB1 antigen mismatches and developed dnDSA to only one of the donor DQB1 antigens. Patients with antibody reactivities specific to only DQA1 donor antigens were excluded. Relative antigen immunogenicity is evaluated using the frequency of DSA in comparison to that of non-DSA antigens.

Eplet mismatches were assessed using HLAMatchmaker based on imputed donor and recipient high resolution DQB1 typing. Patient's antibody profiles were analyzed to identify the mismatched eplets that are the potential targets of DSAs. Immunogenicity of individual eplets was calculated as the percentage of times when the mismatched eplet is the potential DSA target.

#### Reference

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#### Results

#### Prevalence and Relative Immunogenicity of DQ Antigens

The total numbers of individual DQ antigens are comparable between recipients and donors. After classifying donor's DQ antigens into DSA and non-DSA, we identified that DQ2-DQA1\*05 (10/57, 18%), DQ5-DQA1\*01 (11/57, 19%), and DQ7-DQA1\*05 (14/57, 25%) are the most prevalent DQ DSAs in this study.

We then assessed the DSA rates for each DQ antigen by calculating the percentages of DSAs in comparison to that of non-DSAs. Excluding the antigens with 0 events observed in the DSA or non-DSA group, we found that DQ2-DQA1\*05 and DQ7-DQA1\*05 exhibited significantly higher percentages of DSA compared to non-DSA (83.3% and 82.4%, respectively); whereas DQ2-DQA1\*02, DQ6-DQA1\*01, and DQ8-DQA1\*03 showed relatively low DSA rates in this cohort.

Self		Donor	DSA	non-DSA	DSA		
	Total n (%)	Total n (%)	n	n	%	p value	
DQ2-DQA1*02	7 (6%)	7 (6%)	1	6	14.3%	0.113	
DQ2-DQA1*03	0	2 (2%)	0	2	0.0%	0.456	
DQ2-DQA1*05	9 (8%)	<b>12</b> (11%)	10	2	83.3%	0.029	
DQ4-DQA1*03	<b>3</b> (3%)	2 (2%)	1	1	50.0%	1	
DQ4-DQA1*04	9 (8%)	<b>10</b> (9%)	5	5	50.0%	1	
DQ5-DQA1*01	<b>24</b> (21%)	<b>21</b> (18%)	11	10	52.4%	1	
DQ6-DQA1*01	20 (18%)	<b>13</b> (11%)	4	9	30.8%	0.238	
DQ7-DQA1*03	7 (6%)	7 (6%)	4	3	57.1%	1	
DQ7-DQA1*05	<b>14</b> (12%)	<b>17</b> (15%)	14	3	82.4%	0.007	
DQ7-DQA1*06	<b>1</b> (1%)	1 (1%)	1	0	100.0%	1	
DQ8-DQA1*03	<b>13</b> (11%)	<b>15</b> (13%)	4	11	26.7%	0.09	
DQ9-DQA1*02	2 (2%)	4 (4%)	0	4	0.0%	0.118	
DQ9-DQA1*03	5 (4%)	<b>3</b> (3%)	2	1	66.7%	1	

#### Relative Immunogenicity of DQ Antigens in Context of **Recipients' Own Types**

As the immunogenicity of HLA mismatches is dependent on recipients' own types, we calculated the DSA rates of DQ mismatches taking patient types into account. Predictably based on similarities of amino acid sequences, we observed very low DSA rates between DQ5 recipients and DQ6 donors, and vice versa, as well as when patient and donor both carry DQB1\*03 alleles (highlighted in red boxes in the heat map). On the other hand, we detected very high DSA rates to DQ7 specifically in non-DQB1\*03 patients in this cohort. Furthermore, relatively high proportions of DQ2 DSA in DQ6 patients (88%) and DQ5 DSA in DQ7 patients (81%) were also observed.

		DSA% (n of DSA, n of non-DSA)						
		DQ2	DQ4	DQ5	DQ6	DQ7	DQ8	DQ9
Self Type	DQ2		60 (3,2)	20 (1,4)	50 (2,2)	82 (9,2)	20 (1,4)	0 (0,2)
	DQ4	0 (0,2)		50 (2,2)	25 (1,3)	78 (7,2)	50 (1,1)	33 (1,2)
	DQ5	44 (4,5)	60 (3,2)		0 (0,11)	83 (10,2)	57 (4,3)	75 (3,1)
	DQ6	88 (7,1)	14 (1,6)	0 (0,9)		91 (10,1)	50 (2,2)	0 (0,1)
	DQ7	39 (5,8)	67 (2,1)	81 (13,3)	- (2,0)		0 (0,7)	0 (0,3)
	DQ8	50 (4,4)	50 (1,1)	71 (5,2)	67 (2,1)	20 (1,4)		0 (0,1)
	DQ9	- (2,0)	- (2,0)	- (1,0)	50 (1,1)	50 (1,1)	0 (0,5)	
Note: "-" is assigned as the DSA% when 0 non-DSA cases were observed to avoid misrepresentation of the percentages								

#### **Results-cont.** Relative Immunogenicity of DQB1 Eplets

In an attempt to determine the relative immunogenicity of DQB1 eplets in this cohort, we first identified the mismatched eplets in the DSA and non-DSA alleles and excluded the eplets that are mismatched in both alleles. Next, we examined each patient's DSA antibody profile and identified eplets that are the potential antibody targets. We then calculated eplet immunogenicity as the percentage of times when the eplet is considered as the potential DSA target.

In this study, we observed the highest relative immunogenicity for eplets 52PQ and 55PP, which are present on DQ5/6 and DQ7/8/9 DSAs, respectively. On the contrary, several eplets, such as 66ER and 66DR, showed very low frequencies of DSA target, despite being highly exposed and antibody verified.

Mismatched eplet	Exposition	Confirmed	MM Total (n)	DSA target (n)	DSA target (%)	DSA Specificities (n)
52PQ	High	Yes	16	14	87.5	DQ5(10), DQ6(4)
55PP	High	Yes	23	19	82.6	DQ7(13), DQ8(4), DQ9(2)
67VG	High	Yes	15	12	80	DQ5(10), DQ6(2)
45EV	High	Yes	25	19	76	DQ7(19)
30H	Intermediate		20	13	65	DQ5(10), DQ6(3)
55PPD	High		24	15	62.5	DQ7(13), DQ9(2)
57V	High	Yes	17	9	52.9	DQ5(7), DQ6(2)
125SQ	Low	Yes	19	10	52.6	DQ5(10)
37YV	High		21	11	52.4	DQ5(11)
52LL	High	Yes	21	11	52.4	DQ2(11)
1161	High	Yes	21	11	52.4	DQ5(11)
87Y	Intermediate	Yes	23	12	52.2	DQ5(10), DQ6(2)
55R	High	Yes	16	5	31.3	DQ4(2), DQ5(1), DQ6(2)
55PPA	High		15	4	26.7	DQ8(4)
9F	Low		15	2	13.3	DQ4(2)
74S	High	Yes	25	3	12	DQ4(2), DQ5(1)
70RT	High		18	1	5.6	DQ7(1)
66ER	High	Yes	21	1	4.8	DQ7(1)
66DR	High	Yes	22	1	4.5	DQ2(1)
167H	High		25	1	4	DQ7(1)
66D	High		23	0	0	
56PA	High		18	0	0	

Note: Only the eplets observed more than 15 times were listed in the table.

## Conclusions

- DQ2-DQA1\*05 and DQ7-DQA1\*05 antigens present the highest relative immunogenicity in this 2MM1DSA cohort, particularly in recipients carrying DQ6 and non-DQB1\*03 alleles, respectively.
- U Very low DSA rates were observed between DQ5 recipients and DQ6 donors, and vice versa, and when patient and donor both carry DQB1\*03 alleles.
- □ In this preliminary study, we observed a broad spectrum of immunogenicity of individual DQB1 eplets. Further studies in a larger cohort is necessary to validate these findings.



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