HLA-DR/DQ eplet mismatch predicts de novo donor-specific antibody development in multi-ethnic Southeast Asian kidney transplant recipients on different immunosuppression regimens

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Background:
Eplet mismatch has been recognised as a more precise strategy for determining HLA compatibility by enhancing the quantification of donor-recipient HLA differences at the molecular level. Predicting post-transplant alloimmunity using single-molecule eplet mismatch categories has not been validated in Asian cohorts.

Methods:
We examined a cohort of multi-ethnic Southeast Asian kidney transplant recipients at the National University Centre for Organ Transplantation (NUCOT), Singapore, between January 2013 and December 2022 to evaluate HLA-DR/DQ eplet mismatch as a predictor of de novo donor-specific antibody (dnDSA) development. HLA-DR/DQ single-molecule eplet mismatch was quantified using HLAMatchmaker, and we utilized previously published HLA-DR/DQ eplet mismatch thresholds to categorize recipients into alloimmune risk groups and evaluate their association with dnDSA development. Recipients with DSA pre-transplant were excluded. Four-digit HLA alleles were imputed using HaploStats when necessary. Recognizing that the predominance of cyclosporine use (71%) may alter published eplet mismatch thresholds derived from a largely tacrolimus-based (87%) cohort, we evaluated cohort-specific thresholds for HLA-DR/DQ single-molecule eplet mismatch categories.

Results:
Recipient ethnicities included Chinese (65%), Malays (17%), and Indians (14%)(Table 1). HLA-DR/DQ dnDSA developed in 29/234 (12%) recipients after a median follow-up of 5.4 years, including against isolated HLA-DR (n=7), isolated HLA-DQ (n=11), or both (n=11). HLA-DR/DQ single-molecule eplet mismatch categories correlated with HLA-DR/DQ dnDSA-free survival (p=0.001) with low-risk recipients having a dnDSA prevalence of 1% over five years (Figure 1).

Table 1. Baseline recipient demographics (n=234)

Male Donor	41%
Donor Age (years)	49±14
Deceased Donor	44%
Previous Transplant	5%
Pre-emptive Transplant	11%
Male Recipient	54%
Recipient Age (years)	47±12
Recipient Ethnicity	
Chinese	65%
Malay	17%
Indian	14%
Time from Dialysis to Transplant (years)	4.1 (0.8, 8.2)
Delayed Graft Function	15%
Induction Immunosuppression	
Basiliximab	81%
Thymoglobulin	19%
Calcineurin Inhibitor	
Cyclosporine	71%
Tacrolimus	29%
Anti-metabolite	
Mycophenolate	88%
Azathioprine	11%
None	1%
Single molecule eplet mismatch alloimmune category (Wiebe et al.)	
Low (HLA-DR <7 and HLA-DQ <9)	30%
Intermediate (Not meeting low-risk definition and HLA-DQ <15)	35%
High (HLA-DQ ≥15)	35%

Figure 1. HLA-DR/DQ dnDSA-free survival by NUCOT alloimmune risk categories

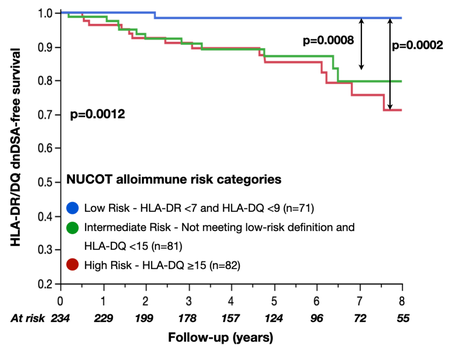
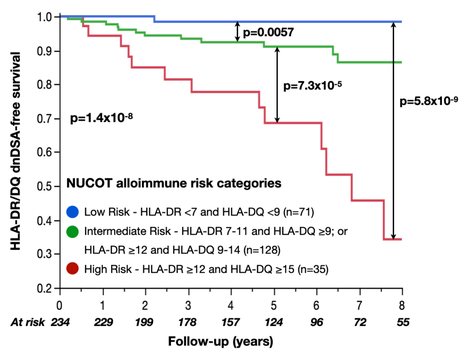


Figure 2. HLA-DR/DQ dnDSA-free survival by NUCOT alloimmune risk categories



In evaluating cohort-specific thresholds, we retained maximum HLA-DR$\beta_{1/3/4/5}$ eplet mismatch <7 and HLA-DQ$\alpha_1\beta_1$ <9 thresholds for NUCOT Low-risk due to these recipients having a low prevalence of dnDSA development. We re-examined the correlation between dnDSA development and eplet mismatch by ROC analysis after excluding these Low-risk recipients. An HLA-DR$\beta_{1/3/4/5}$ single-molecule eplet mismatch threshold of 12 and HLA-DQ$\alpha_1\beta_1$ single-molecule eplet mismatch threshold of 15 was identified. We thus defined the NUCOT High-risk category as HLA-DR ≥12 and HLA-DQ ≥15. Recipients not meeting the Low- or High-risk criteria were categorized as Intermediate-risk.

The cohort-specific alloimmune risk categories improved correlation with HLA-DR/DQ dnDSA-free survival (p<0.0001, Figure 2) and remained significant (Intermediate vs. Low HR 8.8, 95% CI 1.1-67.0, p=0.04, High vs. Intermediate HR 4.1, 95% CI 1.9-8.8, p<0.001, and High vs. Low HR 36.2, 95% CI 4.7-282, p<0.001) after adjusting for calcineurin inhibitor (cyclosporine vs. tacrolimus HR 1.7, 95% CI 0.6-4.8, p=0.4) and anti-metabolite immunosuppression (mycophenolate vs. azathioprine HR 1.6, 95% CI 0.2-14.5, p=0.7).

Conclusions:
We validated the performance of single-molecule eplet mismatch categories as a prognostic biomarker in stratifying recipients into Low-, Intermediate-, and High-risk for dnDSA development in a multi-ethnic cohort of Southeast Asian kidney transplant recipients. HLA-DR/DQ eplet thresholds for categorizing recipients as low-risk appear reproducible despite geographic, ethnic, and immunosuppression differences, identifying a group that may benefit from reduced dnDSA surveillance.

Funding Acknowledgements:
- Ministry of Health and National University Hospital, Singapore
- Canadian Institutes for Health Research
- Flynn Family Chair in Renal Transplantation
- Paul I Terasaki Research Fund