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Human leukocyte antigen eplet mismatches and long-term clinical outcomes in pediatric renal transplantation: a pragmatic, registry based study.

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Author Contributions:

Matthew P Sypek was involved in the design of the study, coordinated data acquisition, performed the analysis, drafted the manuscript and coordinated author contributions and submission for publication

Steve Hiho was involved in coordination of tissue typing data and review and approval of the final manuscript.

Linda Cantwell was involved in coordination of tissue typing data and review and approval of the final manuscript.

Phil Clayton was involved in data analysis and review and approval of the final manuscript.

Peter Hughes was involved in the design of the study, interpretation of analysis, and review, redrafting and approval of the final manuscript.

Amelia K Le Page was involved in the design of the study, interpretation of analysis, and review, redrafting and approval of the final manuscript.

Joshua Y Kausman was involved in the design of the study, interpretation of analysis, and review, redrafting and approval of the final manuscript.

The authors declare that they have no conflicts of interest relevant to this publication.

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List of Abbreviations:

ANZDATA – Australia and New Zealand Dialysis and Transplant Registry

CAKUT – congenital abnormalities of the kidney and urinary tract

CDC – complement dependent cytotoxicity

dnDSA – de novo donor specific antibody

DSA – donor specific antibody

EpMM – eplet mismatch

GN - glomerulonephritis

HLA – human leukocyte antigen

HR – hazards ratio

IAR – interquartile range

MFI – median fluorescence intensity

NGS – next generation sequencing

OR – odds ratio

SAB – single antigen bead

SSO - sequence specific oligonucleotide

SSP – sequence specific primers

VTIS – Victoria Transplantation and Immunogenetics Service

Abstract:

Background:

HLA epitope based matching offers the potential to improve immunological risk prediction and management in children receiving renal allografts, however, studies demonstrating the association between systems for defining epitope mismatches and clinical endpoints are lacking in this population.

Methods:

We conducted a pragmatic, retrospective, registry-based study of pediatric recipients of primary renal allografts in Victoria, Australia between 1990-2014 to determine the association between HLA *epit* mismatches (EpMM) and clinical outcomes including graft failure, re-transplantation and de novo donor specific antibody (dnDSA) formation.

Results:

A total of 196 patients were included in the analysis with a median age of 11 years. Median follow up period was 15 years during which time 108 (55%) primary grafts failed and 72 patients were re-transplanted. HLA class I but not class II EpMM was a significant predictor of graft failure on univariate analysis but not in adjusted models. EpMM was associated with reduced likelihood of re-transplantation in univariate but not adjusted analysis. Within the limitations of the study, class specific EpMM was a strong predictor of dnDSA formation. Associations were stronger when considering only the subset of antibody verified EpMM.

Conclusion:

Associations between HLA EpMM and clinical outcomes in pediatric renal allograft recipients seen on univariate analysis were attenuated following adjustment for confounders. These findings are

inconclusive but suggest that HLA EpMM may provide one tool for assessing long term risk in this population whilst highlighting the need for further clinical studies.

Key Words:

Renal Transplant; Human Leukocyte Antigen; Epitope; Eplet; Graft Survival; Re-Transplantation; Donor Specific Antibodies.

Background:

With increased recognition of the dominant role of humoral immunity in long term renal allograft outcomes and advances in our understanding of human leukocyte antigen (HLA) structure, many have argued that HLA matching should be considered at the epitope level(1–5). Various systems have been proposed to define HLA epitopes including based on antibody adsorption/elution studies(6) and in silico methods analysing the sequence and structure of HLA molecules(7,8). Duquesnoy's system of defining HLA *functional* epitopes using *eplet* designations has gained popularity due to its comprehensive inclusion of all theoretical epitopes, the ease of calculating eplet mismatches (EpMM) using freely available software(9) and a growing body of evidence regarding its clinical application(10,11). This system has demonstrated value in assisting with epitope specific anti-HLA antibody analysis and acceptable mismatching for sensitized patients(12,13). However, Duquesnoy himself, and others stress the importance of the need for further clinical validation of the eplet system for defining HLA epitopes and its role in predicting alloimmune risk post-transplant(14–16).

A number of studies have demonstrated the association between EpMM and primary alloimmune responses in renal transplant recipients, including; donor specific antibody formation(17–21), transplant glomerulopathy(22) and sensitisation following graft failure(23). However, studies examining hard clinical endpoints including graft survival and re-transplantation are limited and have shown conflicting findings (18,24–26). Studies in this area are challenging due to the requirement of a long follow up period to observe clinical outcomes and the limited availability of high-resolution typing on historical cohorts.

It is well recognised that transplantation is the best treatment for most children with end-stage kidney disease(27,28) and that long term patient survival is excellent(29,30). As a result, many children will require multiple transplants throughout their lives and therefore the proposed benefits

of improved epitope matching are of particular importance in this patient population. These potential benefits have already prompted us, and other pediatric transplantation units, to integrate eplet matching into clinical practice(31–33). However, additional evidence is required to support the ongoing role of eplet matching in improving long term outcomes particularly when the potential benefits of improved immunological matching must be weighed against other factors in donor choice including donor age and organ quality.

We aimed to use a pragmatic study design to conduct a retrospective, registry-based study of pediatric renal transplant recipients over the last 25 years in Victoria, Australia to explore the relationship between epitope matching, as defined by Duquesnoy's eplets, and clinical outcomes.

Methods:

Cohort:

Patients were eligible for the study if they received a primary, kidney only transplant in Victoria, Australia, between 1990-2014, and were aged less than 18 years of age at the time of transplantation. Patients were excluded from the primary analysis if they had primary non-function of their graft or failure within the first 2 weeks following transplantation. Follow up data was available to 31st December 2017.

Clinical outcome data was obtained from the Australian and New Zealand Dialysis and Transplant Registry (ANZDATA), a binational database that collects information on all patients with end-stage kidney disease receiving renal replacement therapy in Australia and New Zealand. HLA typing information and anti-HLA antibody testing was obtained from the Australian Red Cross Blood Service, Victoria Transplantation and Immunogenetics Service (VTIS), which is responsible for all transplant immunological testing in the state of Victoria.

Outcome, exposure and confounders:

The primary outcome was time to graft failure (including death with a functioning graft). Secondary outcomes included time to re-transplantation in patients who experienced graft loss during the study period and development of de novo donor specific antibodies (dnDSA) in a subset of patients with testing available. Post-transplant DSA monitoring was performed at the discretion of treating

units, and included screening, testing for clinical indications and testing prior to relisting for subsequent transplantation after primary graft failure. Recipient serum samples (pre and post-transplant) were screened for anti-HLA antibodies by either mixed bead or single antigen bead (SAB) assays (One Lambda Inc.) as per standard protocol and assessed for presence of DSA with median fluorescence intensity (MFI) cut-off >1000 being used. Surveillance biopsies were not routinely performed in this cohort.

The exposures of interest were class specific HLA EpMM, with a secondary analysis examining only antibody verified HLA EpMM. Effect was estimated per EpMM. Potential confounders included in multivariate modelling were: recipient age at transplant, gender, primary renal disease (categorised as congenital abnormalities of the kidney and urinary tract (CAKUT), glomerulonephritis and other) and sensitisation status at primary transplant (detection of any anti-HLA antibody vs non-sensitized); and donor age and source (living donor vs deceased donor). Note that prior to February 2016 sensitisation status was based on HLA class I cytotoxic antibody detection only and after this date was based on detection of class I or class II antibodies on SAB assay according to methods outlined above.

HLA typing and Calculation of Eplet Mismatches

Due to the retrospective nature of our study design, the technology used for HLA typing evolved over the years and a variety of these technologies are reflective in this cohort, however, 4 digit molecular typing is required to calculate EpMM. Serological complement dependent cytotoxicity (CDC) HLA typing only, was used in a proportion of patient and donors, with 4-digit HLA alleles assigned using local haplotype and allele frequencies gathered from over 5000 local donors typed by next generation sequencing (NGS) methods. These same assigned alleles were used for any low resolution (ie. 2-digit) molecular typing that was performed. Low resolution typing was determined by Luminex sequence specific oligonucleotide (SSO) (One Lambda Inc.) or sequence specific primers (SSP). All high resolution results reported are from either Sanger sequencing or next generation sequencing (NGS) methods. For a proportion of typing at HLA-DRB3/4/5 and HLA-DQA/DQB no typing information was available and high resolution typing was assumed based on population linkages only. Table 1 shows the proportion of patients assigned 4 digit typing using each method.

Eplet mismatches were determined using HLAMatchmaker v02, released June 2016, available from www.epitopes.net. Class I EpMM included the A and B loci only, class II EpMM considered DRB1, DRB3/4/5, DQA1 and DQB1 loci. The *eplet* repertoire is designed to include all potential HLA epitopes, however, not all of these have been confirmed to be targets of clinically observed antibodies and remain descriptors of potential epitopes. In addition to examining total EpMM for each HLA class, we also assessed the association of the subset of antibody verified eplet mismatches which are also reported by the HLAMatchmaker application.

Statistical methods

Cox proportional hazards regression models were used to determine the association between EpMM and clinical outcomes including graft survival and re-transplantation. Due to the ad hoc nature of dnDSA detection, logistic regression models were used to determine predictors of dnDSA formation at any point during the follow up period. Confounders were considered for inclusion in multivariate adjustment if statistically significant at a p value of <0.2 on univariate analysis. Linearity of continuous predictors was tested by plotting Martingale residuals and comparing the fit of alternative fractional polynomial models; linear splines or categorisation were introduced where non-linearity was detected. The Cox proportional hazards assumption was tested using scaled Schoenfeld residual and a piecewise model was created to deal with significant violations.

The final models were adjusted for the following confounders: graft survival model – recipient age (age under 12 years vs age 12 years and over, with a piece wise model estimating separate effects for 0-10 years post-transplant and >10 years post-transplant), primary renal disease, donor age (with a linear spline knotted at age 30 years), and donor type; re-transplantation model – age at primary transplant (continuous), primary renal disease and sensitization status; dnDSA model – age at primary transplant (continuous). Eight patients were missing data on pre-transplant sensitization, sensitivity analyses imputing these as either sensitized or non-sensitized did not alter results and hence these patients were excluded from the re-transplantation adjusted model (n=3).

Additional sensitivity analyses were conducted for the dnDSA models excluding zero HLA class I and class II serological mismatch transplants at traditional A/B and DR loci and including HLA class specific serological mismatches in the adjusted model.

Results were considered statistically significant at a p value of <0.05 . All analysis were conducted using STATA 15.1, Statacorp, TX, US.

Results:

Cohort description

A total of 198 patients were identified within the ANZDATA database according to the inclusion criteria. Two patients were unable to be matched in the VTIS database and were excluded.

Table 2 shows the patient characteristics of the entire cohort. Fifty five percent were male and the median age was 11 years old (IQR 5-15). The most common cause of renal failure was congenital abnormalities of the kidney and urinary tract (CAKUT) (57%). A large majority of transplants were from living donors (79%) with a median donor age of 42.5 years (IQR 37-50). The median overall follow-up time was 15 years during which time 108 (55%) primary grafts failed and 72 patients received a re-transplantation.

Table 3 summarizes the HLA matching between donors and recipients using both HLA antigens (serological equivalents) and EpMM. Overall, the cohort was reasonably well matched, particularly at class II with 27% have zero HLA DR antigen mismatches. The median total EpMM was 10 (IQR 5-14) for class I (A and B loci) and 21 (IQR 2.5-33) for class II (DRB1, DRB345, DQA1, DQB1). This compares to a median of 5 (IQR 3-8) for class I and 8 (IQR 0-13) for class II when only antibody verified EpMM are considered. Figure 1 shows the distribution of HLA Class I and Class II total EpMM and antibody verified EpMM. Figure 2 shows the total class specific EpMM by HLA antigen mismatch, demonstrating significant overlap in EpMM across difference degrees of antigen mismatch.

Association between eplet mismatches and clinical outcomes

The associations between class specific EpMM and clinical outcomes in both univariate and adjusted models are shown in table 4.

Graft Survival

Twelve patients were excluded due to primary non-function of their graft or graft failure within the first 14 days. Of the 184 patients included in the primary analysis, 96 (52%)

experienced graft failure during the follow up period, including 9 patients who died with a functioning graft.

HLA Class I EpMM was associated with a 5% increase in hazards for graft failure for each eplet mismatch on univariate analysis (HR 1.05 per MM, 95%CI 1.01-1.09, $p=0.022$) however this association was not significant after adjustment for confounders (recipient age group and primary renal disease, and donor age and source) (adjusted HR 1.03 per MM, 95%CI 0.98-1.07, $p=0.220$). The effect size was greater when considering only antibody verified class I EpMM (HR 1.08 per MM, 95%CI 1.01-1.15, $p=0.023$) however, was also not significant after adjustment for confounders. There was no significant association between class II EpMM and graft failure on either univariate or adjusted analysis, either when considering all EpMM or only the subset of antibody verified mismatches (table 4).

Re-Transplantation

Of the 99 patients who experienced graft failure and remained alive, 72 (73%) went on to have a second transplant during the study follow up period, 50% of these were from living donors.

HLA Class II, but not class I, total EpMM was associated with a reduced likelihood of re-transplantation on univariate analysis (HR 0.98 per MM, 95%CI 0.97-1.00, $p=0.012$). After adjustment for significant confounders (age and sensitization at time of first transplant and primary renal disease) neither class I nor class II EpMM with the first kidney donor was associated with re-transplantation in this cohort (table 4). When only considering antibody verified EpMM, there were significant associations between both class I and class II and re-transplantation on univariate analysis (HR 0.93 per MM, 95%CI 0.87-1.00, $p=0.036$ and HR 0.95, 95%CI 0.92-0.98, $p=0.004$, respectively), however, after adjustment for confounders these associations were no longer statistically significant.

De Novo Donor Specific Antibodies

A total of 127 patients (65%) were tested for DSA at some point following their primary transplant. Patients with DSAs tested were slightly younger than those without testing (median age 10 vs 13 years, $p = 0.010$) but other baseline characteristics were similar (table S1). Nine patients with pre-

transplant DSA were excluded from this analysis. Of these 118 patients, 16 (14%) developed isolated class I dnDSA and 19 (16%) developed isolated class II dnDSA, with 51 patients (43%) developing both class I and class II dnDSA.

There was a strong association between class specific EpMM and dnDSA formation both on univariate analysis and when adjusting for the recipient age at time of transplantation (adjusted OR 1.11 per EpMM, 95%CI 1.03-1.19, $p = 0.006$ for class I and adjusted OR 1.05 per EpMM, 95%CI 1.02-1.08, $p < 0.001$ for class II). The associations were stronger when considering only antibody verified eplets (adjusted OR 1.21 per EpMM, 95%CI 1.06-1.36, $p = 0.003$ for class I and adjusted OR 1.21 per EpMM, 95%CI 1.13-1.33, $p = 0.001$ for class II). As expected, class I EpMM was not associated with class II dnDSA formation and vice versa.

On sensitivity analysis that excluded HLA A/B or HLA DR zero antigen mismatch transplants, the above associations were no longer observed. Similarly, no associations between EpMM and dnDSA formation were seen when HLA A/B and HLA DR antigen mismatches were included in the models.

Discussion

We present the first retrospective, registry-based study of the association between EpMM and long term clinical outcomes in a pediatric renal transplant population. Within the limitations of available data, we have demonstrated an association between HLA class I, but not class II, EpMM and graft survival in this population that is attenuated after adjustment for confounders. Similarly, associations between HLA class II EpMM and class I antibody verified EpMM and reduced likelihood of re-transplantation were seen on univariate analysis but not after model adjustment. Despite the ad hoc and incomplete data available on post-transplant dnDSA, class specific EpMM was strongly associated with this surrogate endpoint, supporting previous studies.

Our study represents a pragmatic attempt to investigate if the theoretical benefits of improved epitope matching, as defined by the eplet repertoire, are supported by long term clinical outcomes. We chose a retrospective, registry-based approach as the key clinical outcomes of interest (graft survival and re-transplantation) occur on the scale of decades in this population making a prospective study infeasible. A major weakness of this study is the limited high-resolution, extended HLA typing available on this historical cohort which necessitated a number of assumptions in assigning this for the purposes of EpMM calculations. We used the available HLA typing in

combination with information on local haplotype frequencies and extended class II linkage associations to determine the most likely complete, high resolution typing. There is limited evidence that using haplotype based assumed high resolution typing to determine quantitative EpMM may be sufficiently accurate for the purpose of epidemiological studies(34,35), however, due to this major limitation, our findings should be viewed as hypothesis generating only.

While there is a growing body of evidence linking EpMM and dnDSA formation, studies examining graft survival are limited and have shown conflicting results. Haririan et al conducted a retrospective study of 101 predominantly African American renal allograft recipients with a mean follow up of 18 months(26). They found that the number of triplet (an earlier iteration of eplets) mismatches did not have a significant association with the risk of graft loss, however, in an exploratory analysis a threshold of 10 class I triplet mismatches was predictive of graft survival (no predictive class II threshold was identified) and a subgroup analysis of 76 patients with data on HLA DQ matching did show some significant associations between triplet mismatch and graft survival. In a study of 62 adult renal allograft recipients, Silva et al found no differences in 10 year graft survival between patients with more or less than 10 HLA class I EpMM(25). Both of these studies used methods that presumed high resolution typing based on serological equivalents. In a more recent analysis, Wiebe et al examined the synergistic association of class II EpMM and non-adherence with graft failure(18). They dichotomised EpMM into high and low risk categories based on a previous study exploring associations between EpMM threshold and risk of dnDSA and reported that these risk categories interacted with adherence, with high risk, non-adherent patients more likely to experience graft failure than low risk adherent patients.

Our analysis showed a significant association with HLA class I antibody verified EpMM and graft failure, but not class II. There are several reasons why this finding may differ from previously published reports on a stronger association between class II antigen mismatches and graft failure, compared to class I antigen mismatches. Firstly, our cohort of predominantly living donor transplant recipients had around a quarter of patients with zero HLA DR antigen mismatches compared to only 6% with zero HLA A and B antigen mismatches, potentially reducing the power of the study to detect class II effects. In addition, due to the lack of historical HLA DQA, DQB and DRB3/4/5 typing, more assumptions were required to assign extended high-resolution class II typing potentially obfuscating class II EpMM associations. Information about medication adherence was not available and we were therefore unable to assess any synergistic effects of EpMM and non-adherence on graft survival.

We elected to report our results per EpMM rather than per 10 EpMM or by quantiles, or explore statistically significant EpMM cutoffs, as others have chosen to do(8,22,36,37). One of the potential benefits of an epitope based approach to HLA matching is in increased granularity of risk prediction and after testing the assumption of a linear relationship between eplet mismatches and the linear predictor in our models, we feel that reporting hazard and odds ratios per eplet allows the reader to better understand incremental risk. The clinical relevance of significant associations becomes more apparent by considering the effect per 10 eplet mismatches (HR 1.56 per 10 eplets [95% CI 1.06-2.29, $p=0.02$], compared to HR 1.05 per eplet [95% CI 1.01-1.09, $p=0.02$] for the unadjusted association between class I EpMM and graft failure, for example), however, without a clinical or biological justification for defining these cut offs the magnitude of the effect size reported seems arbitrary.

Preservation of re-transplantation opportunities is of paramount importance when considering allograft selection of pediatric renal transplant recipients. Based on studies demonstrating associations between EpMM and degree of post-transplant sensitisation(19,23) we hoped to demonstrate that EpMM was a predictor of this important clinical endpoint in a real world pediatric cohort. While we saw a signal for this on univariate analysis, particularly for HLA class II EpMM, this association did not persist in our multivariate modelling. The two most significant predictors of re-transplantation in our cohort were sensitization prior to primary graft and primary renal disease. In light of these, our study may have been underpowered to detect a true association between EpMM and re-transplantation. Alternatively, this finding may suggest that children who are demonstrated to have formed anti-HLA antibody prior to their first transplant may have an increased risk of subsequent sensitization that overwhelms any risk associated with increased EpMM, although this hypothesis remains to be tested.

There are significant limitations in the data that was available to us on post-transplant dnDSA formation, and caution should be taken when extrapolating from the results reported here. Due to the changes in technology over our study period, lack of standardized protocols across units, and cost and lack of proven benefit of post-transplant dnDSA surveillance testing, this was performed on an ad hoc and inconsistent basis across our cohort. Many samples may have been tested after primary graft failure at time of relisting and the relationship to graft nephrectomies is unknown. Testing protocols were not standardized across time and detailed antibody specificity data was not available for analysis. The use of a logistic regression model here is dubious as duration of follow up varies amongst patients and this analysis does not account for censoring, however, it was not

possible to perform survival analysis with the available data. Despite these limitations, our findings in this real world study add weight to the growing body of evidence that HLA EpMM are an important predictor of dnDSA formation, which is itself strongly associated with antibody mediated rejection and poor graft outcomes(38,39). However, caution should be exercised when examining association with surrogate outcomes and further clinical evidence is required before practice change can be recommended.

To our knowledge, this is the first study to examine the associations with the subset of antibody verified EpMM and clinical outcomes. Our models demonstrated stronger and more consistent associations between antibody verified EpMM when compared to all EpMM. The eplet system for defining HLA epitopes remains a theoretical description of functional epitopes, some of which may not be biologically relevant. Our findings suggest that considering whether or not eplets have been shown to be associated with antibody formation may be an important consideration when assessing risk based on this system of defining HLA matching. This highlights the ongoing need to standardize systems of defining HLA serological epitopes, an ongoing goal of the International HLA and Immunogenetics Workshop.

While the use of registry-based data for this analysis allowed us to capture a broad population with very long term follow-up, it presents additional limitations to our analysis. Our study population was limited to available data and as such the analysis may have been underpowered to detect all significant associations. Due to incomplete collection of rejection data by the registry over our study period, we were unable to analyse this important outcome. Information relating to specific causes of graft failure was limited and could not be meaningfully reported.

Overall, the findings of this pragmatic, registry-based study are inconclusive, however, within its many limitations, our study does suggest a signal for an association between EpMM and the important long term outcomes of graft survival and re-transplantation in this population. The lack of significant associations in our key adjusted analyses may reflect that our study was underpowered to detect a true difference in this well-matched cohort. Or alternatively, this may reflect an important reality, that immunological matching is only one factor that determines long term outcomes for children receiving renal transplants and must be weighed against a number of other factors including organ quality and expediting access to transplantation when making decisions about the best transplant option for each individual child.

References:

1. Tambur AR, Claas FHJ. Toward HLA epitope matching in clinical transplantation. *Am J Transplant*. 2013;13(12):3059–60.
2. Duquesnoy RJ. Should Epitope-Based HLA Compatibility Be Used in the Kidney Allocation System? *Hum Immunol*. 2017;78(1):24–9.
3. Filippone EJ, Farber JL. Humoral immunity in renal transplantation: Epitopes, Cw and DP, and complement-activating capability - an update. *Clin Transplant*. 2015;29(4):279–87.
4. Picascia A, Grimaldi V, Napoli C. From HLA typing to anti-HLA antibody detection and beyond : The road ahead. *Transplant Rev. Elsevier Inc.*; 2016;30(4):187–94.
5. Wiebe C, Nickerson P. Strategic Use of Epitope Matching to Improve Outcomes. *Transplantation*. 2016;100(10):2048–52.
6. El-Awar NR, Akaza T, Terasaki PI, Nguyen A. Human leukocyte antigen class I epitopes: update to 103 total epitopes, including the C locus. *Transplantation [Internet]*. 2007;84(4):532–40. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17713439>
7. Duquesnoy RJ. A Structurally Based Approach to Determine HLA Compatibility at the Humoral Immune Level. *Hum Immunol*. 2006;67(11):847–62.
8. Wiebe C, Kosmoliaptsis V, Pochinco D, Taylor C, Nickerson P. A Comparison of HLA Molecular Mismatch Methods to Determine HLA Immunogenicity. *Transplantation [Internet]*. 2018;1. Available from: <http://insights.ovid.com/crossref?an=00007890-9000000000-96662>
9. Duquesnoy RJ. Antibody-reactive epitope determination with HLAMatchmaker and its clinical applications. *Tissue Antigens*. 2011;77(6):525–34.
10. Sypek M, Kausman J, Holt S, Hughes P. HLA Epitope Matching in Kidney Transplantation: An Overview for the General Nephrologist. *Am J Kidney Dis [Internet]*. Elsevier Inc; 2018;71(5):720–31. Available from: <https://doi.org/10.1053/j.ajkd.2017.09.021>
11. Duquesnoy RJ. Are We Ready for Epitope-Based HLA Matching in Clinical Organ Transplantation? *Transplantation*. 2017;101(8):1755–65.
12. Heidt S, Witvliet MD, Haasnoot GW, Claas FHJ. The 25th anniversary of the Eurotransplant Acceptable Mismatch program for highly sensitized patients. *Transpl Immunol. Elsevier B.V.*; 2015;33(2):51–7.
13. Wiebe C, Nickerson P. Acceptable mismatching at the class II epitope level: the Canadian experience. *Curr Opin Organ Transplant*. 2014;19(4):442–6.
14. Tambur AR. HLA-DQ antibodies: are they real? Are they relevant? Why so many? *Curr Opin Organ Transplant [Internet]*. 2016;21(4):441–6. Available from:

- <http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00075200-900000000-99307>
15. Duquesnoy J. Epitope-based human leukocyte antigen matching for transplantation: a personal perspective of its future. 2018 [cited 2018 Aug 14];23(4):486–92. Available from: www.co-transplantation.com
 16. Tambur AR, Campbell P, Claas FH, Feng S, Gebel HM, Jackson AM, et al. Sensitization in Transplantation: Assessment of Risk (STAR) 2017 Working Group Meeting Report. *Am J Transplant*. 2018;18(7):1604–14.
 17. Wiebe C, Pochinco D, Blydt-Hansen TD, Ho J, Birk PE, Karpinski M, et al. Class II HLA epitope matching - A strategy to minimize de novo donor-specific antibody development and improve outcomes. *Am J Transplant*. 2013;13(12):3114–22.
 18. Wiebe C, Nevins TE, Robiner WN, Thomas W, Matas AJ, Nickerson PW. The Synergistic Effect of Class II HLA Epitope-Mismatch and Nonadherence on Acute Rejection and Graft Survival. *Am J Transplant*. 2015;15(8):2197–202.
 19. Kosmoliaptsis V, Mallon DH, Chen Y, Bolton EM, Bradley AJ, Taylor CJ. Alloantibody responses after renal transplant failure can be better predicted by donor-recipient HLA amino acid sequence and physicochemical disparities than conventional HLA matching. *Am J Transplant*. 2016;16(7):2139–47.
 20. Dankers MKA, Witvliet MD, Roelen DL, De Lange P, Korfage N, Persijn GG, et al. The Number of Amino Acid Triplet Differences Between Patient and Donor Is Predictive for the Antibody Reactivity Against Mismatched Human Leukocyte Antigens¹. *Transplantation*. 2004;77(8):1236–9.
 21. Wiebe C, Rush DN, Nevins TE, Birk PE, Blydt-Hansen T, Gibson IW, et al. Class II eplet mismatch modulates tacrolimus trough levels required to prevent donor-specific antibody development. *J Am Soc Nephrol [Internet]*. Departments of Medicine, University of Manitoba, Room 331, 777 William Avenue, Winnipeg, MB, Canada: American Society of Nephrology; 2017;28(11):3353–62. Available from: <https://www.scopus.com/inward/record.uri?eid=2-s2.0-85032640112&doi=10.1681%2FASN.2017030287&partnerID=40&md5=3a8ec85b6ac4a9e457cf450ac3ba5439>
 22. Sapir-Pichhadze R, Tinckam K, Quach K, Logan AG, Laupacis A, John R, et al. HLA-DR and -DQ eplet mismatches and transplant glomerulopathy: A nested case-control study. *Am J Transplant*. 2015;15(1):137–48.
 23. Singh P, Filippone EJ, Colombe BW, Shah AP, Zhan T, Harach M, et al. Sensitization trends

- after renal allograft failure: the role of DQ eplet mismatches in becoming highly sensitized. *Clin Transplant*. 2016;30(1):71–80.
24. Laux G, Mytilineos J, Opelz G. Critical evaluation of the amino acid triplet-epitope matching concept in cadaver kidney transplantation. *Transplantation* [Internet]. 2004 [cited 2018 Aug 14];77(6):902–7. Available from: <https://insights-ovid-com.ezp.lib.unimelb.edu.au/pubmed?pmid=15077035>
 25. Silva E, Alba A, Castro A, Carrascal M, Buckel E, Aguiló J, et al. Evaluation of HLA Matchmaker Compatibility as Predictor of Graft Survival and Presence of Anti-HLA Antibodies. *Transplant Proc* [Internet]. Elsevier; 2010 Jan 1 [cited 2018 Aug 14];42(1):266–9. Available from: <https://www.sciencedirect-com.ezp.lib.unimelb.edu.au/science/article/pii/S004113450901803X?via%3Dihub>
 26. Haririan A, Fagoaga O, Daneshvar H, Morawski K, Sillix DH, El-Amm JM, et al. Predictive value of human leucocyte antigen epitope matching using HLAMatchmaker for graft outcomes in a predominantly African-American renal transplant cohort. *Clin Transplant*. 2006;20(2):226–33.
 27. Rusai K, Szabo AJ. Recent developments in kidney transplantation in children. *Curr Opin Organ Transplant* [Internet]. 2014;19(4):381–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24991976>
 28. Ingelfinger JR, Dharnidharka VR, Fiorina P, Harmon WE. Kidney Transplantation in Children. *N Engl J Med*. 2014;371:549–58.
 29. Allain-Launay E, Roussey-Kesler G, Ranchin B, Guest G, Maisin A, Novo R, et al. Mortality in pediatric renal transplantation: A study of the French pediatric kidney database. *Pediatr Transplant*. 2009;13(6):725–30.
 30. ANZDATA Annual Report: Chapter 11 Paediatrics. http://www.anzdata.org.au/v1/report_2014.html [Internet]. 2014; Available from: http://www.anzdata.org.au/v1/report_2014.html
 31. Sypek MP, Alexander SI, Cantwell L. Optimizing Outcomes in Pediatric Renal Transplantation Through the Australian Paired Kidney Exchange Program. *Am J Transplant*. 2017;17(2):534–41.
 32. Kausman JY, Walker AM, Cantwell LS, Quinlan C, Sypek MP, Ierino FL. Application of an epitope- based allocation system in pediatric kidney transplantation. *Pediatr Transplant*. 2016;20(7):931–8.
 33. Bryan CF, Chadha V, Warady BA. Donor selection in pediatric kidney transplantation using DR and DQ eplet mismatching: A new histocompatibility paradigm. *Pediatr Transplant*. 2016;20(7):926–30.

34. Fidler S, D'Orsogna L, Irish AB, Lewis JR, Wong G, Lim WH. Correlation and agreement between eplet mismatches calculated using serological, low-intermediate and high resolution molecular human leukocyte antigen typing methods. *Oncotarget*. 2018;9(17):13116–24.
35. Ferradji A, D'Souza Y, Saw CL, Oualkacha K, Richard L, Sapir-Pichhadze R. Performance of an allele-level multi-locus HLA genotype imputation tool in hematopoietic stem cell donors from Quebec. *Immun Inflamm Dis*. 2017;5(4):551–9.
36. Kosmoliaptsis V, Mallon DH, Chen Y, Bolton EM, Bradley JA, Taylor CJ. Alloantibody Responses After Renal Transplant Failure Can Be Better Predicted by Donor–Recipient HLA Amino Acid Sequence and Physicochemical Disparities Than Conventional HLA Matching. *Am J Transplant*. 2016;16(7):2139–47.
37. Walton DC, Hiho SJ, Cantwell LS, Diviney MB, Wright ST, Snell GI, et al. HLA Matching at the Eplet Level Protects Against Chronic Lung Allograft Dysfunction. *Am J Transplant*. 2016;16(9):2695–703.
38. Tait BD, Süsal C, Gebel HM, Nickerson PW, Zachary A a, Claas FHJ, et al. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. *Transplantation*. 2013;95(1):19–47.
39. Aubert O, Loupy A, Hidalgo L, Huyen JD Van, Higgins S, Viglietti D, et al. Antibody-Mediated Rejection Due to Preexisting versus De Novo Donor-Specific Antibodies in Kidney Allograft Recipients. *J Am Soc Nephrol*. 2017;28(6):1912–23.

Legends:

Table 1 shows the methods of human leukocyte antigen (HLA) typing for recipients and donors in the cohort at each loci. For non high-resolution testing, 4-digit HLA alleles assigned using local haplotype and allele frequencies gathered from over 5000 local donors typed by next generation sequencing methods

Table 2 reports the characteristics of the entire cohort. Note sensitization refers to the detection of any anti-HLA antibody pre transplant. IQR – interquartile range; CAKUT – congenital abnormalities of the kidney and urinary tract; GN – glomerulonephritis.

Table 3 shows the HLA mismatches for the entire cohort (n=196). The number of patients at each level of HLA antigen mismatches is shown as well as the median HLA eplet mismatches for the entire cohort. HLA – human leukocyte antigen; IQR – interquartile range; EpMM – eplet mismatch.

Table 4 shows the associations between eplet mismatches and post-transplant outcomes. Hazard ratios and odds ratios are reported per eplet mismatch. Model are adjusted for the following confounders: model 1 - recipient age (age under 12 years vs age 12 years and over, with a piecewise model estimating separate effects for 0-10 years post-transplant and >10 years post-transplant), primary renal disease, donor age (with a linear spline knotted at age 30 years), and donor type; model 2 - age at primary transplant (continuous), primary renal disease and sensitization status; models 3 and 4 - age at primary transplant (continuous). CI – confidence interval; EpMM – eplet mismatch; Ab – antibody; dnDSA – de novo donor specific antibody.

Table S1: Demographics and baseline characteristics of patients who had post-transplant donor specific antibodies (DSAs) tested and those who did not. IQR – interquartile range; CAKUT – congenital anomalies of the kidney and urinary tract; GN – glomerulonephritis.

Figure 1 shows the distribution of eplet mismatches in the entire cohort (n=196) for both total eplet mismatches and mismatches in the subset of antibody verified eplets. HLA – human leukocyte antigen.

Figure 2 shows box plots of total class specific eplet mismatches by HLA antigen (serological equivalent) mismatches at the three loci traditionally considered in renal transplant HLA matching. Not the considerable overlap of eplet mismatches across levels of antigen mismatch. HLA – human leukocyte antigen.

Table 1:

Recipient Typing	Donor Typing
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	Serological Typing Only	Low/Intermediate Resolution Molecular Typing	High Resolution Molecular Typing	Assumed based on population based linkages	Total	Serological Typing Only	Low/Intermediate Resolution Molecular Typing	High Resolution Molecular Typing	Assumed based on population based linkages	Total
HLA-A/B	62 (31%)	49 (25%)	87 (44%)	-	198 (100%)	99 (50%)	-	99 (50%)	-	198 (100%)
HLA-DRB1	27 (14%)	55 (28%)	116 (59%)	-	198 (100%)	52 (26%)	61 (31%)	85 (43%)	-	198 (100%)
HLA-DRB3/4/5	24 (12%)	-	61 (31%)	113 (57%)	198 (100%)	43 (22%)	-	43 (22%)	112 (57%)	198 (100%)
HLA-DQB1/A1	52 (26%)	-	124 (63%)	22 (11%)	198 (100%)	81 (41%)	-	97 (49%)	20 (10%)	198 (100%)

Table 2:

Factor	Level	n (%)
		196 (100%)
Gender	Male	108 (55.1%)
	Female	88 (44.9%)
Age at transplant, median (IQR)		11 (5, 15)
Primary Renal Disease	CAKUT	111 (56.9%)
	GN	43 (22.1%)
	Other	41 (21.0%)
Sensitization	Non-sensitized	120 (63.8%)
	Sensitized	68 (36.2%)
Donor Type	Living Donor	155 (79.1%)
	Deceased Donor	41 (20.9%)
Donor age, median (IQR)		42.5 (37, 50)
Transplant Failure	No	88 (44.9%)
	Yes	108 (55.1%)
Re-transplantation Outcome	Functioning Primary Graft	88 (44.9%)

Death with Functioning Graft	9 (4.6%)
Re-transplanted	72 (36.7%)
Not re-transplanted	27 (13.8%)

Table 3:

HLA Class I		
HLA-A/B Serological Equivalent Mismatches, n (%)	0	14 (7.1%)
	1	59 (30.1%)
	2	87 (44.4%)
	3	27 (13.8%)
	4	9 (4.6%)
Class I EpMM - Total, median (IQR)		10 (5, 14)
Class I EpMM - Ab Verified, median (IQR)		5 (3, 8)
HLA Class II		
HLA-DR Serological Equivalent Mismatches, n (%)	0	53 (27.0%)
	1	112 (57.1%)
	2	31 (15.8%)
Class II EpMM - Total, median (IQR)		21 (2.5, 33)
Class II EpMM - Ab Verified, median (IQR)		8 (0, 13)

Table 4

	Univariate Models		Adjusted Models	
	Model 1: Graft Survival			
	n=184		n=184	
	Hazard Ratio	95% CI	Adjusted Hazard Ratio	95% CI
Class I EpMM - Total	1.05*	1.01,1.09	1.03	0.98,1.07
Class II EpMM - Total	1.01	1.00,1.02	1.00	0.99,1.02
Class I EpMM – Ab Verified	1.08*	1.01,1.15	1.05	0.98,1.12
Class II EpMM – Ab Verified	1.02	0.99,1.05	1.00	0.96,1.03
	Model 2: Re-transplantation			

	n=99		n=94	
	Hazard Ratio	95% CI	Adjusted Hazard Ratio	95% CI
Class I EpMM - Total	0.96	0.93,1.00	0.98	0.94,1.02
Class II EpMM - Total	0.98*	0.97,1.00	0.99	0.97,1.00
Class I EpMM – Ab Verified	0.93*	0.87,1.00	0.94	0.88,1.01
Class II EpMM – Ab Verified	0.95**	0.92,0.98	0.96	0.93,1.00
Model 3: Class I dnDSA				
	n=118		n=118	
	Odds Ratio	95% CI	Adjusted Odds Ratio	95% CI
Class I EpMM - Total	1.09*	1.02,1.17	1.11**	1.03,1.19
Class II EpMM - Total	1.00	0.98,1.02	0.98	0.96,1.01
Class I EpMM – Ab Verified	1.18**	1.05,1.32	1.22**	1.08,1.38
Class II EpMM – Ab Verified	0.98	0.93,1.02	0.94*	0.90,1.00
Model 4: Class II dnDSA				
	n=118		n=118	
	Odds Ratio	95% CI	Adjusted Odds Ratio	95% CI
Class I EpMM - Total	1.05	0.99,1.12	1.02	0.95,1.09
Class II EpMM - Total	1.05***	1.03,1.08	1.05***	1.02,1.08
Class I EpMM – Ab Verified	1.09	0.98,1.22	1.04	0.92,1.17
Class II EpMM – Ab Verified	1.12***	1.05,1.18	1.11**	1.04,1.18

Distribution of HLA Eplet Mismatches

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HLA Eplet Mismatches by HLA Antigen Mismatch

All Eplets

petr_13705_f2.png

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