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HLA Eplet Mismatches in Kidney Transplantation: More Than Just Adding Things Up

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Since the ground-breaking work of Paul Terasaki and colleagues[1] in the nascent years of kidney transplantation, human leukocyte antigens (HLA) have been recognized as a major target of the alloimmune response in solid organ transplantation. Our understanding of these molecules as alloantigens has evolved with advancing technologies over the past half century; from the classification under broad serological grouping (eg, A2, B27, DR11) based on cytotoxicity assays to the recognition of the major histocompatibility complex as the most polymorphic loci in the human genome with more than 29,000 HLA and related alleles now described.[2]

This evolution in understanding has left the field of transplant histocompatibility with an as yet unresolved dilemma: on one hand the traditional approach of assessing immunological risk in solid organ transplantation based on mismatches of serologically grouped antigens at the -A, -B, and -DRB1 loci is a crude simplification of alloantigen exposure, whereas the sheer number of unique HLA alleles makes considering individual allele mismatches highly impractical and fails to appreciate the shared characteristics among allele groups. This tension between oversimplification and overwhelming complexity, and the increased recognition of the humoral immune response as a major determinant of long-term transplant outcomes, has led to attempts to consider HLA alloantigens not as discrete antigen mismatches, but rather from the perspective of their surface regions to which antibodies can, or are predicted to, bind.

Several groups have developed systematic approaches to describe a comprehensive list of HLA epitopes relevant to transplantation based on the amino acid sequences and 3-dimensional structure of HLA molecules. The most widely reported of these systems is the *eplet* repertoire, developed by Rene Duquesnoy and colleagues.[3] Using *in silico* techniques, this group has described a list of all clusters of amino acids on HLA molecules that have the potential to act as a key functional unit in determining antibody specificity in the alloimmune response. Although an epitope is defined as the portion of an antigen that makes contact with a particular antibody (or T-cell receptor), an eplet is intended to represent the smallest functional unit capable of determining antibody specificity that forms a smaller portion (~3 angstroms diameter) of the larger overall epitope (~15 angstroms diameter). The eplet system offers an appealingly comprehensive method for defining all potential HLA epitopes relevant to transplantation; however, only a portion of these have been verified to be describing targets of alloantibodies, and the biological and clinical relevance of each eplet designation is yet to be defined.

Eplets have established roles in helping to predict crossmatch results in acceptable mismatch programs for highly sensitized patients and in the analysis of anti-HLA antibody profiles. A growing body of evidence supports their utility in predicting posttransplant clinical outcomes including *de novo* donor-specific antibody formation, rejection, and graft survival. Early clinical studies considered HLA eplet mismatch load as a predictor of outcome, where the sum of eplet mismatches has often been used to define a risk threshold.[4] This approach considers all eplet mismatches as having equal clinical relevance and the biological basis for the concept of risk thresholds remains unclear. More recently, Wiebe *et al.*[5] have demonstrated that single-molecule eplet mismatch was a better predictor of adverse clinical

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outcomes in kidney transplantation than total eplet mismatch, and McCaughan and colleagues[6] were able to identify a single high-risk epitope mismatch that was associated with a more than 4-fold increase in the risk of *de novo* donor-specific antibodies in a cardiothoracic transplant cohort, adding weight to the argument that not all eplet mismatches are of equal clinical importance.

In this issue of *Kidney International Reports*, Mohammadhasanzadeh and colleagues[7] use a data-driven approach in a large registry cohort to add further insights into differential clinical implications of specific eplet mismatches. The association between HLA eplet mismatch and death-censored graft survival was examined in a retrospective cohort of 118,313 unsensitized, first kidney transplant receipts from the US Scientific Registry of Transplant Recipients. Eplet mismatches were determined using imputed allele-level donor and recipient HLA typing at the -A, -B, -C, -DRB1, and -DQB1 loci. The authors' initial analysis found that approximately half of the 412 eplet mismatches observed in the cohort were associated with death-censored graft survival. Using a range of statistical techniques, including network analysis and variable selection procedures, they progressively refined this list down to 55 single HLA eplet mismatches that were most predictive of the outcome in their derivation cohort (of which, 15 were predictive in the validation cohort).

This work is an ambitious attempt to move past the concept of eplet mismatch load and address the hierarchy of risk associated with individual eplet mismatches and associated clusters of mismatched eplets. However, the unavoidable reliance on imputed high-resolution HLA typing in this registry cohort introduces potential inaccuracies in the defining of eplet mismatches that limit the clinical applicability of the article's conclusions. Two recent studies by Senev *et al*.[8] and Engen *et al*.[9] both highlight the potential for eplet misclassification based on high-resolution typing imputed using the National Marrow Donor Program algorithm, particularly for HLA class II and in nonwhite populations. Imputation of 2 field HLA typing is an unavoidable necessity when collating a dataset of sufficient size and with adequate duration of follow-up to address the question of the clinical relevance of individual eplet mismatches, yet this will inevitably impact the reliability of attributing the observed associations to specific eplets, particularly when the authors were unable to determine mismatches at -DRB3/4/5, -DQA, -DPA, and DPB. Mohammadhasanzadeh and colleagues[7] readily acknowledge this limitation of their work and conclude that although their findings require validation from diverse populations with allele-level genotypes before they can be used in prioritizing donor-recipient matching that avoid higher-risk eplet mismatches, they offer a short list of candidate eplet mismatches that could be studied to understand the properties that confer an increased risk of graft failure.

In using a broad armamentarium of statistical techniques to interrogate the relationship between individual eplet mismatches in a large cohort with a sufficient duration of follow-up to observe meaningful clinical outcomes, the authors highlight a number of challenges in the feasibility of using eplet mismatch data in organ allocation and decision making. Their initial analysis found that only approximately half of the eplet mismatches were associated with graft failure, and these included both antibody-verified and non–antibody-verified eplets, raising questions about the role of serological verification in identifying clinical risk and whether some eplet mismatches may induce an alloimmune response independent of antibody formation. In modeling the complex interrelatedness of eplet mismatches through network analysis, they highlight important limitations in simply adding the number of individual eplet mismatches in risk stratification when antibody formation may be targeted at the most immunogenic eplet of a larger eplet cluster or group. Ultimately, in demonstrating a hierarchy of risk associated with specific eplet mismatches, Mohammadhasanzadeh and colleagues[7] question the validity of quantitative eplet mismatch loads as a tool for assessing risk at the time of organ allocation, where each eplet mismatch is considered to hold equal weight.

As solid organ transplantation is moving into an era of widespread use of high-resolution molecular HLA typing and the detection of allele-specific anti-HLA antibodies through single-antigen bead assays, risk stratification based on HLA epitope mismatch is becoming both appealing and potentially feasible. Although it has been shown that overall eplet mismatch load and other systems for defining HLA molecular mismatch are valuable tools in determining immunological risk posttransplant, it is clear that not all eplet mismatches confer equal risk. If we are to aspire to use eplet mismatches in delivering personalized and precise medicine through both organ selection and the tailoring of immunosuppression, it is vital that we not only understand the clinical relevance

of specific eplets but also develop practical systems to incorporate this information into the complex processes of organ allocation and clinical decision making in kidney transplantation.

DISCLOSURE

Both authors declared no competing interests.

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