

On Path to Informing Hierarchy of Eplet Mismatches as Determinants of Kidney Transplant Loss



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Introduction: To mitigate risks related to human leukocyte antigen (HLA) incompatibility, we assessed whether certain structurally defined HLA targets present in donors but absent from recipients, known as eplet mismatches (EMM), are associated with death-censored graft failure (DCGF).

Methods: We studied a cohort of 118,313 American 0% panel reactive antibodies (PRA) first kidney transplant recipients (2000 to 2015) from the Scientific Registry of Transplant Recipients. Imputed allele-level donor and recipient HLA-A, -B, -C, -DRB1, and -DQB1 genotypes were converted to the repertoire of EMM. We fit survival models for each EMM with significance thresholds corrected for false discovery rate and validated those in an independent PRA > 0% cohort. We conducted network-based analyses to model relationships among EMM and developed models to select the subset of EMM most predictive of DCGF.

Results: Of 412 EMM observed, 119 class I and 118 class II EMM were associated with DCGF. Network analysis showed that although 210 eplets formed profiles of 2 to 12 simultaneously occurring EMMs, 202 were singleton EMMs that were not involved in any profile. A variable selection procedure identified 55 single HLA class I and II EMMs in 70% of the dataset; of those, 15 EMMs (9 singleton and 6 involved in profiles) were predictive of DCGF in the remaining dataset.

Conclusion: Our analysis distinguished increasingly smaller subsets of EMMs associated with increased risk of DCGF. Validation of these EMMs as important predictors of transplant outcomes (in contrast to acceptable EMMs) in datasets with measured allele-level genotypes will support their role as immunodominant EMMs worthy of consideration in organ allocation schemes.

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KEYWORDS: epitope; eplet; graft failure; human leukocyte antigens; immunogenicity; kidney transplantation

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Rejection is the leading cause for premature graft loss.¹ Rejection occurs because of immune recognition of foreign targets on the donor kidney. Potent immunosuppression agents have contributed to

decreased incidence of rejection. Yet, nonadherence or dose reduction of immunosuppression because of infections or cancer make patients more vulnerable to experience immune-mediated injuries.^{2,3} The HLA gene complex has been established as a key component of the immune response to foreign antigens.⁴ Thus, allocation schemes that optimize HLA compatibility have been promoted as a strategy to improve transplant outcomes.

HLA genes are highly polymorphic, with more than 27,000 alleles identified to date.⁵ The diversity of HLA alleles enables the fine-tuning of an adaptive immune

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response; unfortunately this same diversity makes donor-recipient matching at the level of HLA alleles very challenging. While matching at the HLA allele level may not be feasible, matching at the level of HLA epitopes may be more feasible and clinically justifiable. Several algorithms have been developed to represent B-cell and T-cell epitopes on HLA. The most popular algorithm for B-cell epitopes is HLAMatchmaker.⁶ HLA-Matchmaker identifies polymorphic amino acid structurally defined HLA targets—eplets—located at accessible sites on HLA molecules that are recognizable by antibodies.⁷ Higher EMM loads, defined as eplets included within the donor's repertoire but absent from that of the recipient, are associated with increased risk of donor-specific antibodies, rejection, and graft loss.^{8–12}

We reported that antibody-verified (AbVer) eplet mismatch loads (i.e., eplets experimentally verified as targets for donor-specific antibodies) are associated with transplant glomerulopathy and graft loss.¹³ At the time this article was conceived, only 83 of the HLA class II eplets were verified,⁶ and we hypothesized that some HLA class II and class I eplets not yet verified by antibody reactivity may be independent predictors of graft failure. Further, we hypothesized that there may be a hierarchy across EMM in the tendency to induce immune-mediated injury and graft loss.

Studying individual EMMs as independent predictors of transplant outcomes requires careful consideration of many EMMs and the complex relatedness between them (resulting in high dimensionality). HLA genes are in linkage disequilibrium and certain eplets are shared by alleles within and across HLA loci.¹⁴ Consequently, donor-recipient pairs may demonstrate a selection of potentially immunodominant EMMs (from hundreds of potential eplets) appearing at the population level either as singletons or as part of profiles consisting of several simultaneously occurring EMMs. To handle this high dimensionality and differentiate between specific EMMs as determinants of transplant outcomes, there is a need for large datasets with complete outcome data, allele-level donor and recipient genotyping, as well as longitudinal capture of pertinent confounders, and effect measure modifiers. In the absence of large-scale datasets with allele-level HLA genotypes, we outline a sequence of data-driven analyses to assess risk of DCGF related to singleton (single EMM) and profiles of EMM in this retrospective cohort study of the Scientific Registry of Transplant Recipients (SRTR). Such a large registry dataset, albeit necessarily reliant on imputed allele-level genotypes nonetheless provides long-term follow-up and rigorous collection of hard clinical endpoints with enough power to evaluate risk associated with hundreds of potential EMM.

METHODS

Study Design and Population

We conducted a retrospective cohort study of kidney transplant recipients (KTRs) without evidence of preformed anti-HLA antibodies (0% peak PRAs) who received primary deceased and living kidney allografts in the United States between January 1, 2000, and January 1, 2015. Multiorgan transplant recipients and KTRs with primary graft nonfunction were excluded. Frequencies of eplets were estimated in consecutive living and deceased kidney donors ($n = 169,416$) as well recipients ($n = 176,316$) included in the SRTR dataset during the same period and for whom allele-level genotypes could be imputed and eplet repertoires assigned. The McGill University Health Centre research ethics board approved this study.

Data Source

The SRTR includes data on all donors, wait-listed candidates, and transplant recipients in the United States, submitted by the members of the Organ Procurement and Transplantation Network. The Health Resources and Services Administration, U.S. Department of Health and Human Services, provides oversight to the activities of the Organ Procurement and Transplantation Network and SRTR contractors.

Allele-level HLA type imputation and EMM estimation

Allele-level donor-recipient HLA-A, -B, -C, -DRB1, and -DQB1 types were imputed from serologic HLA-A, -B, and -DRB1 types using an algorithm provided by the National Marrow Donor Program,^{15,16} allele-level HLA haplotypes were imputed by maximum likelihood estimation independent of self-reported race. A Python program identified eplets included among the repertoire of donor eplets but missing from the recipient's repertoire of "self" eplets as mismatches. The entire genotype was considered when verifying eplet compatibility such that eplets shared by donor-recipient alleles of the same locus (e.g., HLA-A) or across loci (e.g., HLA-A, -B, -C) were excluded from the mismatch count. A total of 449 potential eplets (223 Class I: 72 AbVer, 151 non-AbVer; 226 Class II: 72 AbVer, 154 Non-AbVer) considered as per the HLA Epitope Registry (www.epregistry.com.br) accessed in September 2018.

Outcome Definition and Potential Confounding Variables

The primary endpoint was time to DCGF, defined as return to dialysis or re-transplantation. Baseline recipient, donor, and transplant characteristics were considered for inclusion in multivariable models.

Table 1. Baseline characteristics of 0% PRA first-time kidney transplant recipients from the U.S. Scientific Registry of Transplant Recipients

Variable		n	%
Recipient characteristics			
Age at transplantation (y)	0–14	4177	3.5
	15–24	6490	5.5
	25–44 (Ref)	30,478	25.8
	45–64	55,397	46.8
	≥65	21,771	18.4
Sex	Female	40,153	33.9
Self-reported race	Caucasian (Ref)	83,827	70.9
	African American	26,162	22.1
	Other	8324	7.0
Time on dialysis (mo)	Mean, SD	36.9	32.6
	Missing	23,288	19.7
Insurance type	None	269	0.2
	Private	49,439	41.8
	Public (Ref)	68,598	58.0
	Missing	7	0.0
Donor characteristics			
Age	36–45	27,419	23.2
	46–55	27,805	23.5
	55+	16,670	14.1
	<=35 (Ref)	46,419	39.2
Sex	Female	57,048	48.2
Self-reported race	Caucasian (Ref)	100,122	84.6
	African American	13,677	11.6
	Other	4514	3.8
Donor type	Deceased (SCD)	56,921	48.1
	Deceased (ECD)	12,750	10.8
	Living (Ref)	48,642	41.1
Transplant characteristics			
Donor-recipient weight ratio	DRWRc <0.9	45,651	38.6
	DRWRc >1	50,745	42.9
	DRWRc 0.9–1.0	14,380	12.1
	Missing	7537	6.4
Induction agent	Campath	9338	7.9
	IL2 Receptor Blocker	35,190	29.7
	Other	2435	2.1
	Thymoglobulin	41,350	35.0
Calcineurin inhibitor	Cyclosporine	22,242	18.8
	Tacrolimus	87,924	74.3
	No CNi	8147	6.9
Steroid	Yes	110,346	93.3
Transplant era	2000–2004 (Ref)	46,061	38.9
	2005–2009	42,585	36.0
	2010–2014	29,667	25.1
Cold ischemia time (h)	Mean, SD	12.6	11.1
	Missing	20,232	17.1%

DRWRc, donor-recipient weight ratio; ECD, expanded criteria donor; PRA, panel reactive antibodies; Ref, references; SCD, standard criteria donor.

Recipient characteristics included age, sex, race, dialysis vintage, and insurance coverage. Donor characteristics included age, sex, race, and type. Transplant characteristics included era, induction agent, calcineurin inhibitor type, steroids (yes vs. no), and donor-recipient weight ratio (Table 1).

Statistical Analysis

Patients were followed until graft failure, death, or administratively censored on May 31, 2015. We fit Cox proportional hazards models to determine independent associations between single EMM and DCGF. Models were adjusted for recipient, donor, and transplant characteristics. To avoid bias related to exclusion of donor-recipient pairs with missing data, multiple imputation was performed using the Fully Conditional Specification method to impute missing covariate values.

Given the proportionality assumption was violated for some of the EMM models, we also fit Accelerated Failure Time (AFT) models. We compared residual plots of different distributions, calculated the Akaike Information Criterion, used log-likelihood ratios as estimators, and found that the Weibull distribution offered the best-fit to our dataset¹⁷ in reference to 2 adjusted models: a model with covariates only (EMM excluded), and a second model including single EMM adjusted for the same covariates (Supplementary Material S1). When conducting multiple comparisons and estimating hazard ratio (HR) for DCGF from Cox and AFT models for hundreds of single EMM, we applied the Benjamini-Hochberg procedure¹⁸ to control for false discovery rate. To ensure associations of particular EMM with risk of DCGF are not related to type 1 error, we pursued a permutation procedure,¹⁹ showing that the *P* value estimates for the EMM associated with DCGF were, at minimum, smaller than the 99th percentile of a random distribution of *P* values estimated under the null hypothesis that the EMM have absolutely no effect on DCGF. To inform the role of eplet frequency on the observed associations with DCGF, we measured eplet distributions in donor and recipient populations.

To model the complex relatedness between HLA EMM, we applied weighted correlation network analysis.²⁰ We then evaluated profiles of EMM as risk factors for DCGF by fitting AFT models. A profile was deemed present only if all associated EMMs were observed in the donor-recipient pairs.

To identify a subset of EMM significantly associated with DCGF, we also applied Lasso penalized Cox regression^{21–23} onto training (70%) and test (30%) datasets. This method enabled feature selection by shrinkage of the number of EMMs among several and potentially correlated EMMs. HRs of DCGF of selected EMMs were then estimated by multivariable Cox regression models while accounting for false discovery rate. A similar selection of EMMs associated with DCGF was identified when including cold ischemia time and donor type (living donor as well as standard criteria and expanded criteria deceased donor).

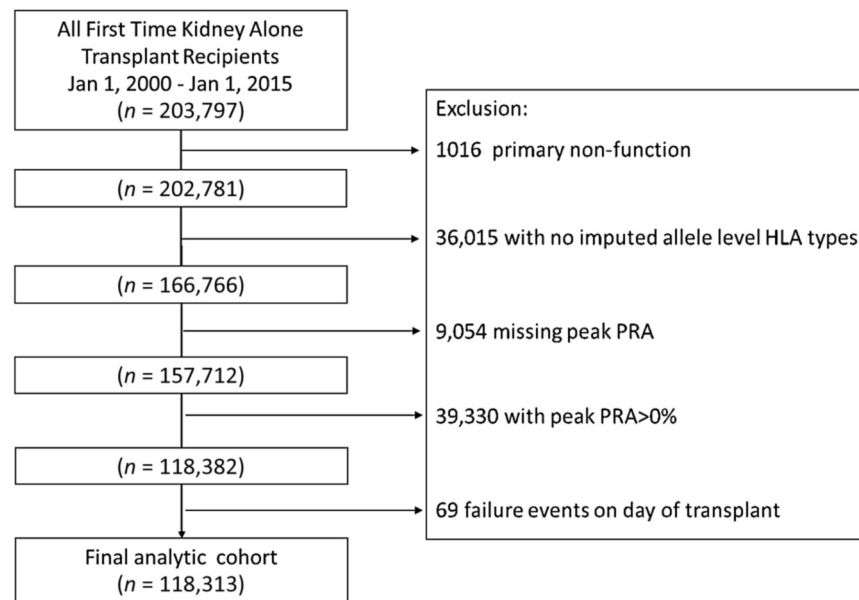


Figure 1. Study flow diagram.

Finally, we conducted sensitivity analysis to confirm the consistency of risk associated with singleton and profiles of EMM in an independent dataset of 48,384 pairs of PRA > 0% transplant recipients and their donors. In addition, given concerns that the genotype imputation may be less accurate in non-Caucasian populations,^{24–26} we repeated our main analysis in a subgroup of self-reported Caucasian donor-recipient pairs. Statistical analyses were performed using the free statistical computing R software (<https://www.r-project.org>).

RESULTS

Following application of the exclusion criteria (Figure 1, Study Flow Diagram), a total of 118,313 first-time KTR- (January 1, 2000, and January 1, 2015) from the U.S. SRTR with peak PRA 0% were included in the cohort. Baseline characteristics of the cohort and missing covariate data are presented in Table 1. A total of 19,946 KTR experienced graft failure over a median follow-up of 6.39 (interquartile range 3.12–10.01) years.

DCGF Risk Associated With Single EMM

To evaluate whether AbVer and non-AbVer EMM was associated with DCGF we fit survival models. A total of 449 potential eplets for HLA-A, B, C, DRB1, and DQB1 appeared on the HLA Epitope Registry when accessed in September 2018. Of those, 412 EMMs were observed in the study cohort with 243 EMMs (121 class I: 46 AbVer and 75 non-AbVer, and 122 class II: 48 AbVer and 74 non-AbVer) statistically significantly associated with DCGF in Cox proportional hazards models that considered a single EMM at a time, adjusted for

pertinent donor, recipient, and transplant characteristics, and controlled for false discovery rate. Given that the proportionality of hazards assumption was violated in many of the fitted Cox models for single EMM, we also fit AFT models (possible distributions of survival times can be found in [Supplementary Material S1](#)). Of the 412 EMMs observed in the study cohort, when adjusting for the same variables as the Cox models, the AFT model found 237 (119 class I [44 AbVer and 75 non-AbVer] and 118 class II [46 AbVer and 72 non-AbVer]) single EMMs that were associated with DCGF (Figure 2a and Figure 3a and b). All 237 EMMs identified by the AFT models were included within the 243 EMMs identified by the Cox model. Taken together, these survival analyses demonstrated that only half of the observed EMMs were associated with an increased risk for DCGF ([Supplementary Material S2](#)).

Frequencies of Eplets in Donor and Recipient Populations and Risk of DCGF

To assess whether DCGF risk is informed by a higher frequency of particular eplets among donors versus recipients, we assessed their frequencies in these populations. Intraclass Correlation Coefficients of 0.998 or higher were observed across eplet frequencies in the donor and recipient populations, suggesting the distribution of EMM associated with DCGF, as well as those that were not, did not segregate differently among donors and recipients (Figure 2a and Figure 3a and b).

Profiles of EMM and Risk for DCGF

Although AFT models showed that approximately half of the observed EMMs are associated with DCGF, it is

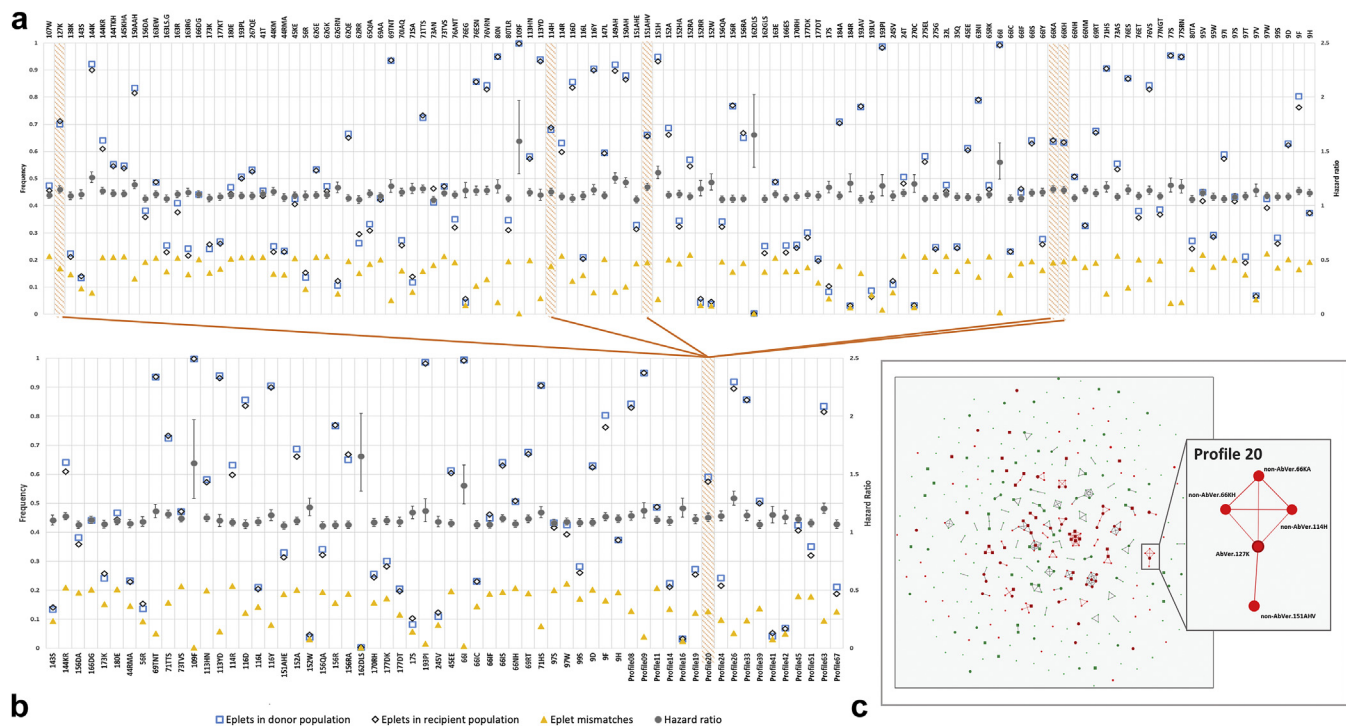


Figure 2. Human leukocyte antigen (HLA) Class I singleton and profiles of eplet mismatches associated with death-censored graft failure and their distribution in donor and recipient population. (a) Hazard ratios and 95% confidence intervals of HLA class I eplet mismatches associated with death-censored graft failure and their distribution in donor and recipient population. (b) HLA class I profiles and singleton eplet mismatches associated with death-censored graft failure and their distributions in donor and recipient populations. (c) Profile 20 associated with death-censored graft failure includes 5 antibody-verified (AbVer) and non-AbVer eplet mismatches that are also associated with graft loss. In the figure, *nodes* represent single eplet mismatches and *edges* pair together nodes of eplet mismatches that are co-represented in the studied population (Figure 5 provides detailed descriptions of profile visualization). * Models were adjusted for recipient characteristics: age, sex, time on dialysis, insurance, and cause of end-stage renal disease; donor characteristics: age, sex, and donor type; transplant characteristics: donor-recipient weight ratio, transplant era, induction agent, calcineurin inhibitor type, and steroids for maintenance immunosuppression.

possible that only a subset of these EMMs are in fact causally related to this outcome. Association of the noncausally related EMMs with DCGF may be explained by their simultaneous occurrence alongside causally related EMMs. To investigate the presence of profiles of highly correlated EMMs, we conducted weighted correlation network analysis and observed that although 202 eplets appeared as singleton mismatches, 210 appeared within a total of 67 profiles. Examples of EMM profiles are presented in Figure 2c and Figure 3d, respectively.

The observed profiles, each including 2 to 12 EMMs, segregated by HLA class such that only EMMs from the same class (I or II) formed a profile. Although some profiles included eplets from the same locus, other profiles included EMMs originating from different loci. Figure 4 shows 2 representative EMM profiles and a selection of the HLA alleles associated with them. Interestingly, only 33 of the 67 EMM profiles identified in the study cohort associated with DCGF in multivariable models adjusted for the same covariates as the models for single EMM (Supplementary Material S3). Profiles not associated with DCGF were often composed

of non-AbVer EMMs that were also individually not associated with DCGF. The complete network of singleton and profiles of EMM identified across donor-recipient pairs in the SRTR cohort are presented in Figure 5.

Addressing Interrelatedness of EMMs as Determinants of DCGF

To assess whether there is a hierarchy of EMM predictive of DCGF, we applied a variable selection procedure capable of handling highly correlated variables. Using 70% of the 0% PRA cohort, the least absolute shrinkage and selection operator (Lasso) penalized Cox regression model identified a subset of 55 single EMMs, which were validated in the remaining dataset. Of those, 15 were also statistically significantly associated with DCGF in the remaining dataset when using Cox regression models adjusting for recipient, donor, and transplant characteristics and controlling for false discovery rate. These mismatches can be mapped to both HLA class I and II loci and include AbVer and previously non-AbVer EMMs (Table 2).

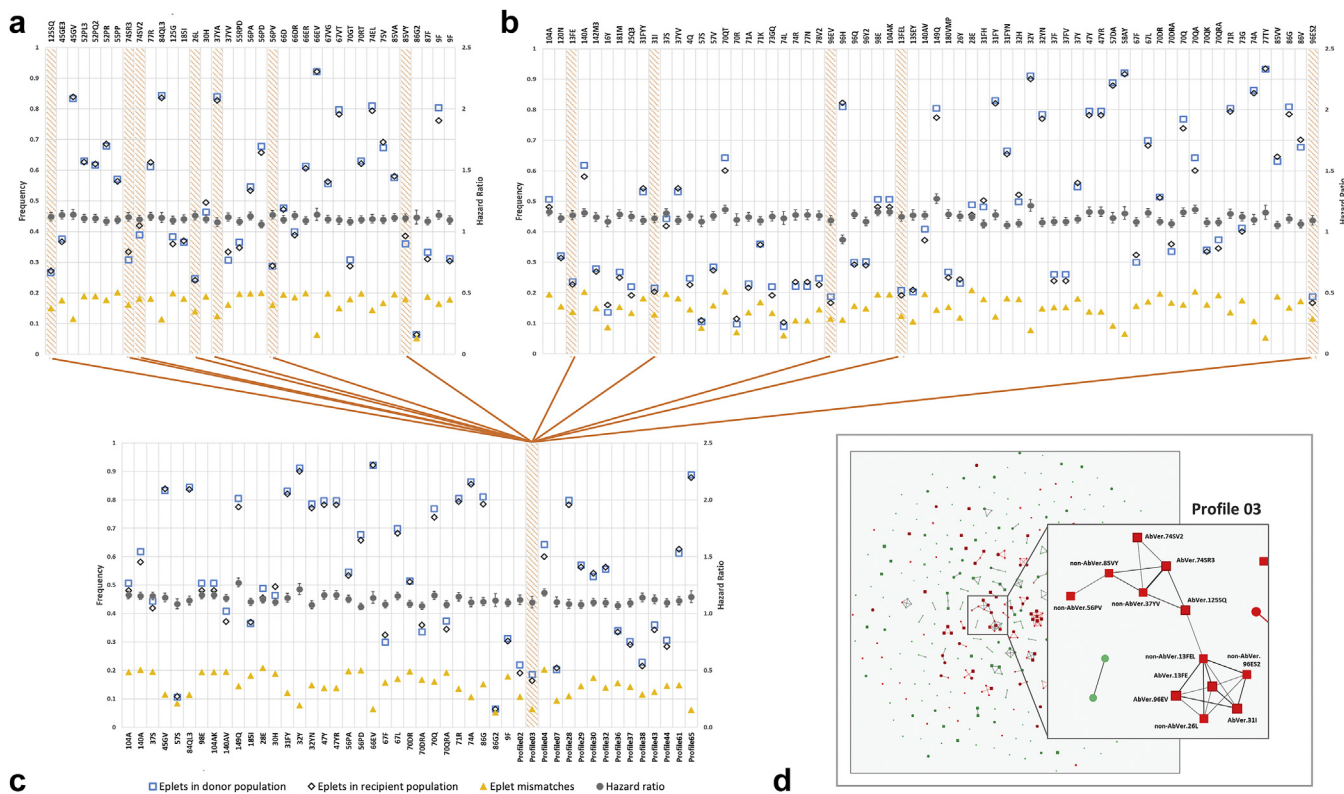


Figure 3. Selected human leukocyte antigen (HLA)-DQB1 and HLA-DRB1 singleton and profiles of eplet mismatches associated with death-censored graft failure (DCGF) and their distribution in donor and recipient populations. Hazard ratios and 95% confidence intervals of selected (a) HLA-DQB1 and (b) HLA-DRB1 eplet mismatches associated with DCGF and their distribution in donor and recipient populations. (c) HLA-DRB1/DQB1 profiles and singleton eplet mismatches associated with DCGF and their distribution in donor and recipient populations. Edges of eplet mismatch profiles also associated with DCGF are presented in red. (d) Profile 03, associated with DCGF, involves 12 antibody-verified (AbVer) and non-AbVer eplet mismatches that are also individually associated with death-censored graft failure. In the figure, *nodes* represent single eplet mismatches and *edges* pair together nodes of eplet mismatches that are significantly co-represented in the studied population (Figure 5 provides detailed descriptions of profile visualization).

* Models were adjusted for recipient characteristics: age, sex, time on dialysis, insurance, and cause of end-stage renal disease; donor characteristics: age, sex, and donor type; transplant characteristics: donor-recipient weight ratio, transplant era, induction agent, calcineurin inhibitor type, and steroids for maintenance immunosuppression.

Sensitivity Analyses

To verify the robustness of our observations, we repeated the analysis in an independent cohort of KTR with PRA >0%. We found that 90% (144 of 161) of EMMs originally observed in the 0% PRA cohort were also predictive of DCGF in the PRA >0% cohort (Supplementary Material S4A). Similarly, profiles of EMM observed in the 0% PRA cohort were also observed in the PRA >0% cohort.

To address concerns of inaccurate HLA genotype assignment (and, consequently, EMM identity) when relying on imputation in multiethnic populations, we repeated the analysis in a subcohort of self-reported Caucasian KTR and donors in whom imputation is expected to predict allele-level types more accurately. Of 188 EMM associated with DCGF in the Caucasian subgroup, 165 (88%) were also associated with DCGF in the 0% PRA cohort independent of self-reported race and ethnicity (Supplementary Material S4B).

DISCUSSION

Our analysis of the SRTR data distinguished between single HLA class I and II EMM associated with an increased risk for DCGF and those that were not. Among these EMMs, in addition to the AbVer eplets, was a subset of EMMs that were not previously verified by antibodies. Frequency of EMMs associated with DCGF was not higher in donors versus recipients. Although a sizable proportion of EMMs conferred risk for DCGF as singleton mismatches, some EMMs appeared within profiles including several simultaneously occurring EMMs. Only half of these profiles were associated with an increased risk of DCGF and they were typically composed of EMMs that were also individually associated with DCGF in AFT models. Variable selection procedures informed ≥ 8 -fold reduction in the number of EMMs (from a total of 412 EMMs observed in the study cohort to 55 EMMs identified by penalized Lasso regression of whom 49

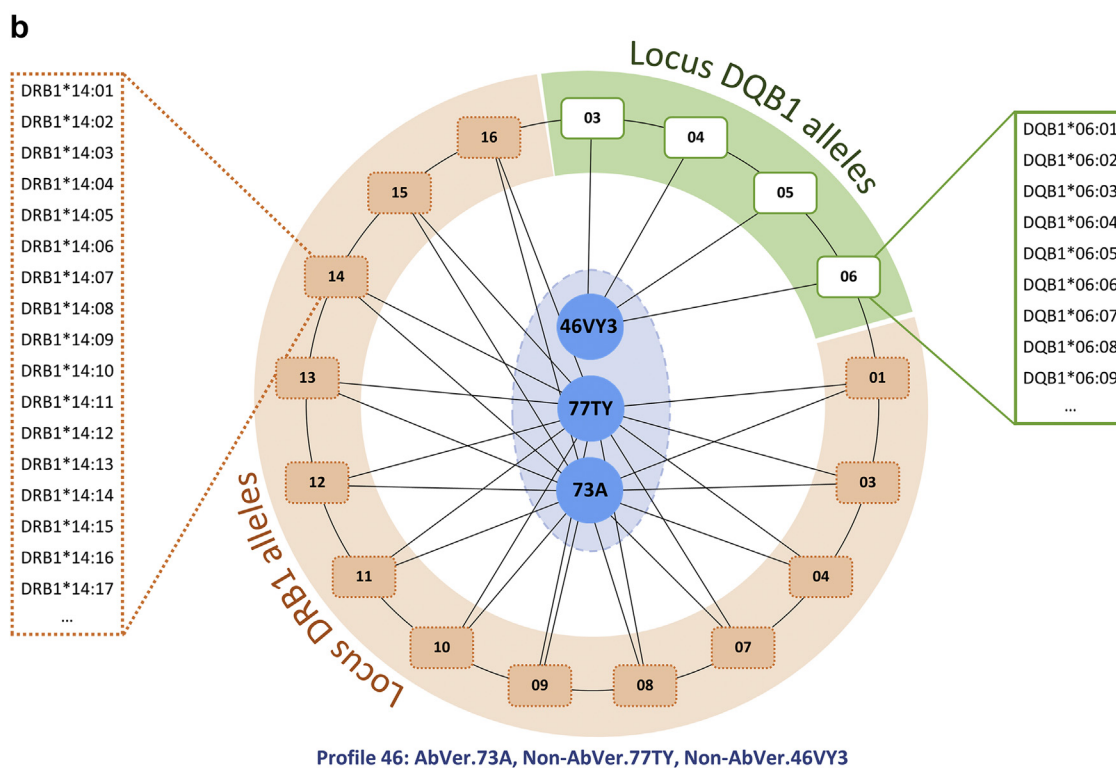
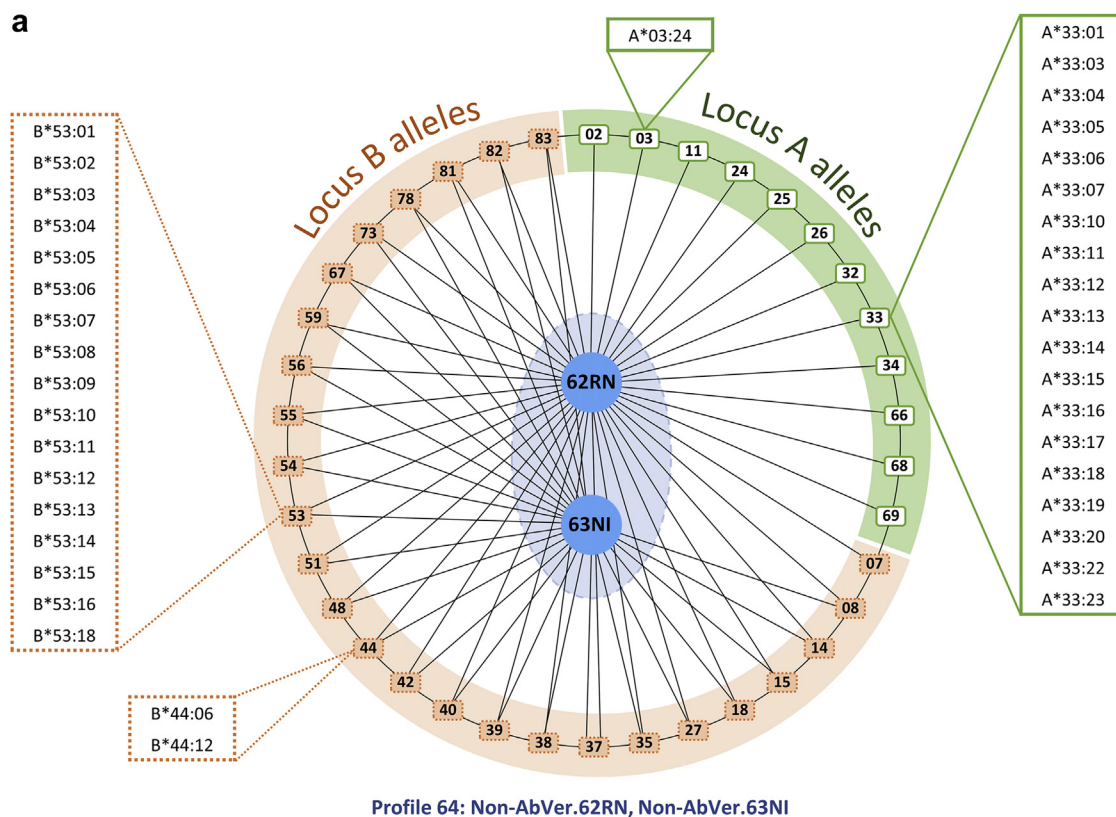


Figure 4. Examples of eplet mismatch profiles. Eplet mismatch profiles include simultaneously occurring eplet mismatches. These eplet mismatch profiles were segregated by class such that any given profile includes only eplets from human leukocyte antigen (HLA) class I or class II loci. (a) Profile 64 includes eplet mismatches non-AbVer.62RN and non-AbVer.63NI that are shared by HLA-A and HLA-B alleles. (b) Profile 46 includes the eplet mismatches AbVer.73A and non-AbVer.77TY that are shared by HLA-DRB1 alleles and AbVer.46VY3 that is shared by HLA-DQB1 alleles. AbVer, antibody verified.

* Given the large number of donor alleles that could code for each of the eplets represented in the profile, most HLA types found in association with the eplet on the HLA Epitope Registry are presented at the allele-group (first-field) level, with only a few examples of allele-level types represented in the green and orange boxes.

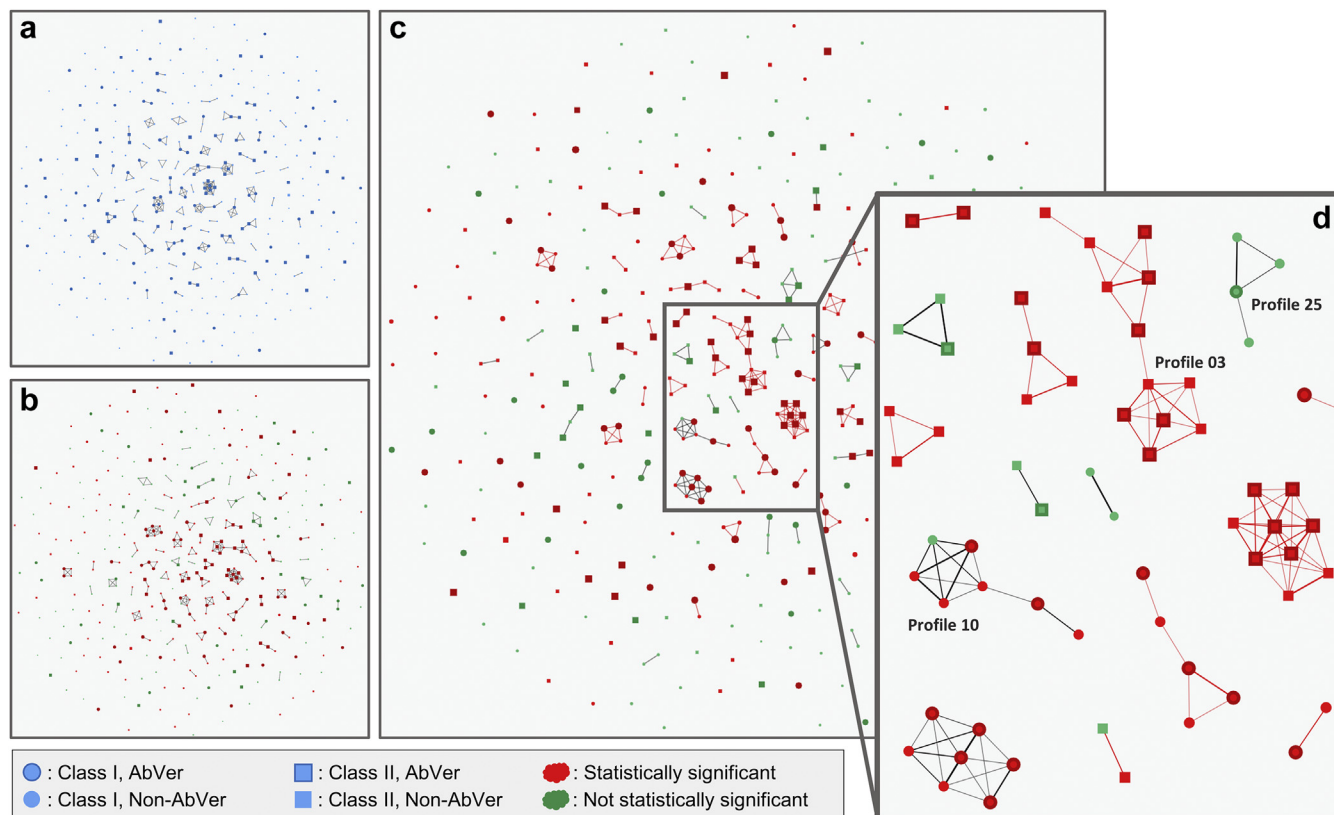


Figure 5. Profiles and singleton eplet mismatches. (a) Nodes represent single eplet mismatches. Class I and II eplet mismatches are represented by *circles* and *squares*, respectively. The *circumference* of circles and squares representing antibody-verified eplet mismatches is bolded. *Edges* pair together nodes (of eplet mismatches) that are significantly co-represented in the studied population. (b) Eplet mismatches statistically significantly associated with death-censored graft failure are represented in red. (c) Eplet mismatch profiles statistically significantly associated with death-censored graft failure have red edges connecting between nodes of eplet mismatches. (d) Eplet mismatch profiles statistically significantly associated with death-censored graft failure with eplet mismatches that have been antibody-verified are represented by bolded circumference.

were also associated with DCGF in AFT models); of those, 15 EMMs were also associated with DCGF in multivariable models corrected for false discovery rate in the remaining dataset. Our findings are expected to enable more targeted validation of high-risk EMMs as determinants of transplant outcomes. Identification of a smaller subset of EMMs that are more powerful predictors of DCGF can be studied using smaller datasets with allele-level genotypes; permit interrogation of physiochemical properties that could render them more immunogenic; and, thereafter, offer clinical justification as well as enhance feasibility of donor-recipient matching on HLA eplets.

Evidence to date has linked cumulative EMM loads with transplant outcomes.^{8–13,27–32} It has been proposed that various thresholds of acceptable cumulative EMM loads could be deemed acceptable when making decisions on organ allocation. Opponents of this approach suggest that the composition of eplets giving rise to similar mismatch loads may include single EMMs of varying immunogenicity and antigenicity, rendering the risk profile insufficiently consistent to apply in clinical

care.^{33–37} Establishing the hierarchy of EMMs as determinants of transplant outcomes has been limited by insufficient power to study single EMMs in cohorts with allele-level HLA genotypes. This is a direct consequence of the high dimensionality and interrelatedness of EMMs. The high dimensionality results from a large number of potential EMMs. Interrelatedness of EMM stems from linkage disequilibrium between HLA loci and sharing of certain eplets by HLA of the same locus and/or across loci (e.g., HLA-A, -B, and -C). Our analysis of the SRTR data identified a subset of single AbVer and new non-AbVer EMMs associated with an increased risk of DCGF. This risk cannot be attributed to differential segregation of eplets in the donor versus recipient populations or the frequency of EMM in the analytical cohort (Figures 2 and 3) but is likely a consequence of the properties of the EMMs themselves. Although risk of DCGF is not informed by the frequency of eplets among donors versus transplant candidates, it is important to note the frequency of eplets (and profiles) when seeking to secure eplet compatibility at the time of organ allocation. Organs with higher-risk eplets that are

Table 2. Eplet mismatches identified by Lasso penalized Cox regression model, their association with death-censored graft failure and appearance as singletons or within profiles

		EMM	Selected EMM by Lasso			P value	Selected EMM predictive of DCGF		Singleton EMM	Profiles of EMM	
			Hazard Ratio				*	*			
Class I	AbVer	138K	1.016	1.061	1.108	6.92E-03			Profile 14 ^o	Abv. 138K, Abv. 177KT, oth. 275G, oth. 35Q	
		144KR	1.030	1.072	1.116	7.21E-04	*	*			
		163R	0.981	1.021	1.064	3.04E-01			Profile 24 ^o	Abv. 163R, Abv. 163RG, Abv. 44KM, oth. 152HA, oth. 66NM	
		166DG	0.978	1.020	1.063	3.59E-01			*		
		62GRN	1.048	1.114	1.184	5.71E-04	*	*	Profile 42 ^o	Abv. 62GRN, Abv. 71SA, oth. 97V	
		69TNT	0.984	1.053	1.128	1.38E-01			*		
		71TTS	1.034	1.078	1.123	3.60E-04	*	*			
		90D	0.984	1.029	1.077	2.15E-01			*		
		109F	1.000	1.293	1.672	5.01E-02			*		
		113HN	1.011	1.050	1.091	1.11E-02			*		
		114Q	0.998	1.038	1.081	6.55E-02				Profile 59	oth. 114Q, oth. 245AS
		151AHV	1.033	1.079	1.127	6.38E-04	*	*	Profile 20 ^o	Abv. 127K, oth. 114H, oth. 151AHV, oth. 66KA, oth. 66KH	
		151H	1.047	1.114	1.186	6.42E-04	*	*	Profile 26 ^o	Abv. 144K, oth. 149AH, oth. 151H	
		152W	1.092	1.179	1.272	2.25E-05	*	*			
		162DLS	1.279	1.626	2.069	7.36E-05	*	*			
	170RH	0.968	1.011	1.055	6.25E-01			*			
	184R	1.020	1.112	1.212	1.58E-02				Profile 16 ^o	oth. 184R, oth. 270C	
	186R	0.976	1.086	1.210	1.32E-01			*			
	193LV	0.987	1.044	1.105	1.31E-01			*			
	193PI	0.949	1.050	1.162	3.44E-01			*			
	245V	1.006	1.060	1.116	2.97E-02			*			
	66I	1.000	1.166	1.360	5.03E-02			*			
	66NM	1.007	1.058	1.113	2.64E-02				Profile 24 ^o	Abv. 163R, Abv. 163RG, Abv. 44KM, oth. 152HA, oth. 66NM	
	71HS	1.040	1.096	1.155	6.39E-04	*	*				
	76ET	1.006	1.045	1.085	2.23E-02				Profile 10	Abv. 163LS/G, Abv. 80TLR, oth. 162GLS, oth. 166ES, oth. 199V, oth. 76ET, oth. 80TA	
	76VS	1.036	1.079	1.124	2.36E-04	*	*		Profile 08 ^o	Abv. 76VRN, oth. 76VS	
	95V	1.002	1.045	1.090	3.85E-02				Profile 13	Abv. 107W, Abv. 144TKH, Abv. 145KHA, Abv. 62GE, Abv. 62GK, oth. 65RK, oth. 95V	
	97T	0.992	1.037	1.085	1.11E-01				Profile 67 ^o	oth. 95W, oth. 97T	
	97W	0.979	1.015	1.052	4.23E-01			*			
	9F (HLA-Class I)	0.998	1.039	1.082	6.15E-02			*			
9H	1.017	1.054	1.093	3.98E-03			*				
Class II	AbVer	104A ^b	0.982	1.028	1.075	2.39E-01			*		
		40YD2	0.966	1.080	1.208	1.76E-01			*		
		45GE3	0.966	1.067	1.177	2.01E-01				Profile 43 ^o	Abv. 45GE3, oth. 66D, oth. 66DR, oth. 73G
		52PL3	0.990	1.030	1.071	1.42E-01				Profile 29 ^o	Abv. 52PL3, Abv. 55PP, oth. 66ER, oth. 70RT
		57DE ^b	0.000	559.200	2.45E+84	9.47E-01			*		
		57V ^b	0.985	1.033	1.083	1.85E-01			*		
		70QT ^b	0.997	1.055	1.117	6.31E-02			*		
		70R ^b	0.984	1.047	1.113	1.45E-01				Profile 57	Abv. 70R ^b , Abv. 70RE
		74L ^b	1.064	1.128	1.195	4.66E-05	*	*		Profile 66	Abv. 16Y, Abv. 74L, oth. 189S
		130R	1.039	1.377	1.825	2.60E-02			*		
	149Q ^b	1.097	1.151	1.208	1.15E-08	*	*				
	26L ^b (HLA-DRB1)	1.027	1.073	1.121	1.68E-03	*	*		Profile 03	Abv. 125SQ, Abv. 13FE, Abv. 31I, Abv. 74SR3, Abv. 74SV2, Abv. 96EV, oth. 13FEL, oth. 26L ^b (HLA-DRB1), oth. 37YV (HLA-DQB1), oth. 56PV, oth. 85VY, oth. 96ES2	
	28DHF	1.166	1.279	1.403	1.84E-07	*	*				
	28DY ^b	0.971	1.024	1.079	3.78E-01				Profile 48	oth. 28D ^b , oth. 28DY ^b	
	56PD	1.004	1.043	1.084	3.14E-02			*			
58AY ^b	0.989	1.052	1.119	1.10E-01				Profile 65 ^o	oth. 57DA, oth. 58AY ^b		
58EEDP ^b	0.000	0.002	8.36E+78	9.48E-01			*				
66DR	0.943	1.037	1.140	4.53E-01				Profile 43 ^o	Abv. 45GE3, oth. 66D, oth. 66DR, oth. 73G ^b		
66EV	0.985	1.045	1.109	1.41E-01			*				

(Continued on following page)

Table 2. (Continued)

EMM	Selected EMM by Lasso			Selected EMM predictive of DCGF	Singleton EMM	Profiles of EMM
	Hazard Ratio	P value				
<i>67L^b</i>	1.011	1.057	1.106	1.54E-02	*	
<i>70DK</i>	1.066	1.169	1.281	8.56E-04	*	*
<i>70Q^b</i>	0.931	0.992	1.056	7.91E-01		*
<i>71R^b</i>	1.013	1.057	1.103	1.05E-02		*
<i>86G2</i>	1.076	1.143	1.215	1.62E-05	*	*

Abv/AbVer, antibody-verified eplet mismatches; DCGF, death-censored graft failure; EMM, eplet mismatch(es); Oth, Non-antibody-verified.

^aEplet mismatch profile associated with death-censored graft failure in Accelerated Failure Time models.

^bEplet also associated with HLA-DRB3/4/5.

Models were adjusted for recipient characteristics: age, sex, time on dialysis, insurance, and cause of end-stage renal disease; donor characteristics: age, sex, and donor type; transplant characteristics: donor-recipient weight ratio, transplant era, induction agent, calcineurin inhibitor type, and steroids for maintenance immunosuppression.

Eplet mismatches associated with death-censored graft failure in Accelerated Failure Time models accounting for false discovery rate are emphasized using *italics*.

HLA-DQB1 EMMs found to be reactive by Schawelder *et al.*⁴³ are **bolded**.

An asterisk (*) in the Selected EMM predictive of DCGF column highlights eplet mismatches predictive of DCGF by the least absolute shrinkage and selection operator (Lasso) penalized Cox regression model.

An asterisk (*) in the Singleton EMM column highlights eplet mismatches not involved in profiles of eplet mismatches.

frequently observed in the population can be allocated regionally, whereas organs with higher-risk eplets that are less frequently observed in the population may benefit from coordinated national organ allocation efforts.

Only half of the EMMs observed in the analytical cohort conferred an increased risk for DCGF. These EMMs included both AbVer and previously non-AbVer EMMs. Recently, we found small effect sizes of DCGF risk by residual non-AbVer EMM loads, with most HR estimates not reaching statistical significance across the studied HLA loci excluding HLA-DRB1.¹³ Indeed, our current findings suggest that more than 40 previously non-AbVer EMMs related to HLA-DRB1 locus are associated with DCGF in AFT models. Future research is needed to establish how they may contribute in detrimental humoral and/or cellular immune responses. Interestingly, some of the already AbVer EMMs did not appear to confer an increased risk for DCGF in our cohort. These findings may be explained by the level of experimental verification for each EMM recorded in the HLA Epitope Registry, which varied from verification by human monoclonal antibodies, elution/absorption, mouse monoclonal antibodies, to sera from multiparous women.^{38,39} Alternatively, this discrepancy between antibody verification of EMM and association with DCGF may be related to interventions applied upon detection of donor-specific antibodies and rejection to prevent graft failure; none of which are longitudinally captured in the SRTR. Last, consistent with the hypothesis at the base of this analysis, these findings may also support the notion that not all eplets are created equal and this relates to their tendency to induce donor-specific antibodies, result in tissue injury, and/or respond to therapeutic interventions.

Importantly, association between any EMM and DCGF may be vulnerable to confounding related to

other simultaneously occurring EMMs. To represent the interrelatedness between EMMs, we conducted a network-based analysis. This analysis revealed that half of the EMMs appeared as singletons, whereas half were included within profiles (Figures 2 to 5). Like EMM loads, profiles of EMM segregated by HLA class. However, distinct from traditional EMM loads, eplet profiles did not include all possible donor-recipient EMMs related to the HLA locus or class but were composed of only a subset of simultaneously occurring EMMs (informed by donor-recipient pairs in our cohort). Also, some profiles identified by the weighted correlation network analysis were composed of EMMs, all of which were individually associated with DCGF (Figure 2), but other profiles also included EMMs that were not individually associated with DCGF.

To establish which of the EMMs are most predictive of DCGF, we applied a variable selection procedure. Singleton EMMs selected by the Lasso likely represent higher-risk eplets. Consistently, most of the singleton EMMs selected by Lasso penalized regression, were also associated with DCGF in AFT models. These findings support the notion that immunodominant single EMMs may inform the risk of DCGF. In immunology, antigenic competition is observed to occur between epitopes that are either shared by the same molecule or appear on 2 different molecules. When considering T-cell responses, for example, evidence suggests that recipients recognize the dominant epitope of only one of the (mismatched) HLA-DR antigens of the donor at the time of a primary rejection episode. Competition between antigens has been observed, depending on the number of precursors available in the T-cell repertoire, the affinity of the T-cell receptor for the “immunodominant” epitope and the efficiency with which epitopes are processed and presented by host antigen-presenting cells. Immunodominance of B-cell epitopes has been observed primarily with immunity secondary

to antibody response to pathogens. Antibodies and the complexes they form with the antigen may have potent regulatory effects in quantitative and qualitative terms, with rejection subsequently resulting in a response that could spread to the other mismatched antigens.^{40,41} Recognition of immunodominant epitopes, which could give rise to a primary host response, and avoidance of transplantation in this context, is expected to improve transplant outcomes.

Another important contribution of this work is the consideration of interrelatedness of EMMs. Although it is possible that EMMs selected by Lasso that are also included within profiles are themselves associated with DCGF, we cannot rule out that another eplet in the profile, or the complete EMM profile, inform risk for DCGF. Although further research is required to elucidate this point, pragmatically, our findings should allow more targeted validation of the identified subset of EMMs as important predictors of transplant outcomes using smaller cohorts of transplant recipients genotyped at the allele level. This work will also enable more detailed interrogation of the characteristics (e.g., physiochemical properties of the amino acid substitutions⁴²) that may make certain EMMs more immunogenic than others. In addition, this work may prompt additional investigation into the interplay between EMMs and recipient HLA as well as the accompanying helper T-cell epitopes as determinants of immunogenicity.

Collectively, our analyses are the first to demonstrate that a subset of EMMs determines an increased risk of graft failure. Despite this novelty, it is important to acknowledge that our study relies on imputed allele-level HLA genotypes^{32–34} and excludes HLA-DRB3/4/5, -DQA1, -DPA1, or -DPB1 loci.¹³ Importantly, genotype assignment was done using the National Marrow Donor Program algorithm, which relies on haplotype frequencies from 21 U.S. populations.^{15,16} Moreover, there is significant overlap and consistency between single EMMs associated with DCGF in self-reported Caucasian donor-recipient pairs, as well as in an independent PRA > 0% cohort, lending support to the association between the observed subset of AbVer and non-AbVer EMMs with graft failure risk. Nevertheless, missing HLA types (e.g., HLA-DRB3/4/5) may result in under- or overestimation of EMMs. For example, should a subset of HLA-DRB3/4/5 donor eplets be missing from the repertoire of the recipient HLA-DRB1/3/4/5 eplets, the resultant EMMs may be underestimated. On the other hand, if by virtue of eplet sharing across HLA-DRB1/3/4/5 loci, recipient HLA-DRB3/4/5 may include some of the donor HLA-DRB1-associated eplets, resulting in overestimation of EMMs in our dataset. Finally, the repertoire of eplets, and particularly those of HLA-DQ,

is in flux. Yet, several EMM identified by Lasso or AFT models were also found to be immunogenic in a cohort of 221 pregnancies with HLA-DQB1 listed EMMs. These EMMs (e.g., 55PP, 52PR, and 52PQ2 [52PQ+85VG]) are highlighted in [Table 2](#) and [Supplementary Material S2](#).⁴³ In addition, like our observation, some previously AbVer EMMs were found to be nonreactive (e.g., 28T, 46VY, and 52P), whereas other previously non-AbVer were found to be reactive (e.g., 85VA, 56PV). Validation of our observations in additional datasets from diverse populations with measured allele-level genotypes is required to support prioritizing donor-recipient matching on the higher-risk eplets identified by this study.

In conclusion, although evidence to date has linked cumulative EMM loads with transplant outcomes, eplet matching at the time of organ allocation requires a more refined appreciation of risk associated with specific EMMs. Our analysis of the SRTR is the first of this scale to distinguish between single EMMs associated with an increased risk of DCGF. Delineating the properties of single EMMs identified by these analyses that may render them more likely to result in graft failure could provide a breakthrough in efforts of the transplantation community to improve transplant outcomes by optimizing HLA compatibility. Simulations of how eplet matching could be implemented in organ allocation schemes while maximizing organ utilization and incorporating safeguards to avoid disparity in access to transplantation are needed.

DISCLOSURES

All the authors declared no competing interests.

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DISCLAIMER

The data reported here have been supplied by the Minneapolis Medical Research Foundation as the contractor for the Scientific Registry of Transplant Recipients (SRTR).

The interpretation and reporting of these data are the responsibility of the author(s) and in no way should be seen as an official policy of or interpretation by the SRTR or the U.S. government.

AUTHOR CONTRIBUTIONS

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF).

Material S1. Accelerated failure time models.

Material S2. Hazard ratios of death-censored graft failure by antibody-verified and non-antibody-verified eplet mismatches in the 0% PRA, PRA>0%, and Caucasian subcohorts by accelerated failure time models and connectivity scores of correlated eplet mismatches in profiles (A) and eplet mismatches appearing as singletons (B).

Material S3. Hazard ratios and confidence intervals of death-censored graft failure by observed eplet mismatch profiles.

Material S4. Relation between eplet mismatches associated with death-censored graft failure in the main analytical cohort and in sensitivity analyses.

Material S5. Members of Genome Canada Transplant Consortium.

STROBE Statement.

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