Human Leukocyte Antigen Mismatching and Graft Survival in Pediatric Heart Transplant Recipients in the United States, 1987-2012

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Abstract

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Background: The association between donor-recipient human leukocyte antigen (HLA) mismatches and graft loss is not well understood in pediatric heart recipients.

Objectives: We aimed to examine the independent association between overall and class-specific donor-recipient allelic and structural HLA mismatching and long-term graft loss in pediatric heart transplant recipients.

Methods: In this retrospective national cohort study of 4,851 heart transplant recipients 18 years of age or younger from 1987-2012, we used the Kaplan-Meier method and multivariate Cox proportional hazards regression to compare probabilities of death or re-transplantation (graft loss) by total and class-specific donor-recipient HLA-A, -B, and -DR allele mismatches. We used the HLA Matchmaker algorithm to compare probabilities of graft loss by level of class-specific HLA structural differences at the molecular level.

Results: Recipients with 4-6 mismatches had an increased independent long-term risk of graft loss compared to those with 0-3 mismatches (adjusted HR: 1.21 [95% CI: 1.05-1.40]). Median times to graft loss were 10.3 (95% CI: 9.9-11.1) and 14.3 (95% CI: 11.3-17.1) years in the groups with 4-6 vs. 0-3 mismatches, respectively. Mismatches at class I loci (HLA-A and -B) were associated with progressively higher probabilities of graft loss while mismatches at the class II locus (HLA-DR) were not. Having 10 or more class I structural eplet mismatches was associated with higher probability of graft loss (HR: 1.24 [95% CI: 1.07-1.44]) while the corresponding number of class II eplet mismatches was not. On stratification by both allele and structural eplet mismatching, only those with both 4-6 allele mismatches and 10+ class I eplet mismatches had an increased probability of graft loss.

Conclusions: Genotypic and structural-level HLA mismatching might identify recipients at increased risk of long-term graft loss who could benefit from intensified post-transplant surveillance and management. Further studies should elucidate the mechanisms by which HLA mismatches may impact graft survival.

Introduction

Despite advancements in post-transplant management, pediatric heart transplant recipients face a 5- and 15-year survival of 75% and 50%, respectively; and for recipients who survive beyond one year, there has been little, if any, improvement in long-term survival over the past 30 years (1). Identifying risk factors for long-term graft loss in pediatric patients may help guide therapeutic and surveillance strategies.

Mismatching between donor and recipient human leukocyte antigen (HLA) alleles is a potential risk factor for worsened post-transplant outcomes. HLA mismatches may be associated with a higher risk of graft rejection (2, 3), cardiac allograft vasculopathy (CAV) (4), and mortality (3, 5, 6) in adult heart transplant recipients. Pediatric transplant recipients are different from adult recipients, however. Longevity expectations and the likelihood of outliving an original graft are greater among children, and even seemingly subtle risk factors can impact long-term survival. Additionally, pediatric recipients are subject to physiologic maturation and have different immunogenic stimuli, cardiovascular comorbidities, and immunosuppressive regimens than adult recipients. Nevertheless, the impact of HLA mismatches on outcomes in pediatric heart transplant recipients is not well understood, and the few studies that have been done were able to enlist only small numbers of subjects (7, 8).

Our aim was to examine the independent association between the number of overall and class-specific donor-recipient HLA mismatches and long-term graft survival in a large national cohort of pediatric heart transplant recipients. To assist in this aim, we employed HLA Matchmaker (9), a computer program that determines the surface structural differences between donor and recipient HLA molecules and quantifies the amount of novel antigenic material to which a recipient is exposed by each specific allele mismatch.

Methods

Study design: We conducted a retrospective cohort analysis of data from the Scientific Registry of Transplant Recipients (SRTR). The SRTR includes data on all donors, wait-listed candidates, and transplant recipients in the US, submitted by the members of the Organ Procurement and Transplantation Network (OPTN). The Health Resources and Services

Administration of the Department of Health and Human Services provides oversight to the activities of the OPTN and SRTR contractors.

We included subjects aged 0-18 years undergoing primary heart transplantation in the U.S. between 1987 and 2012. We compared the earlier of all-cause mortality or re-transplantation (collectively referred to as "graft loss") in groups of recipients defined by the total number of HLA-A, -B, and -DR alleles mismatched with the donor. Since each locus has two alleles, there were six possible allele mismatches. We classified subjects into a reference group with 0-3 mismatches and a comparison group with 4-6 mismatches. This grouping was suggested to be predictive of mortality in univariate analysis presented in a recent registry report on pediatric heart transplantation from the International Society for Heart and Lung Transplantation (ISHLT) (1), and was also the most demonstrative of an association when we performed an exploratory analysis of various groupings. To determine if the association between number of mismatches and graft survival was dependent on HLA class, we compared outcomes among subjects by the number of class I HLA mismatches (0-1, 2, 3, or 4 mismatches at HLA-A and –B loci) and number of class II HLA mismatches (0, 1, or 2 mismatches at the HLA-DR locus).

Patients undergoing a second heart transplantation were considered to have had a graft failure event and were removed from analysis at the time of re-transplantation. The SRTR's ascertainment of deaths, believed to be reasonably complete by the database administrators, is based on OPTN reports from every US transplant program and monthly updates from the Social Security Administration Death Master File.

As the contractor for the SRTR, the Minneapolis Medical Research Foundation supplied the data reported here. The interpretation and reporting of these data is the authors' responsibility and in no way should be seen as an official policy of, or interpretation by, the SRTR or the U.S. government. Our institutional review board approved this study prior to data acquisition.

Analysis of structural differences between donor and recipient HLA molecules using HLA Matchmaker: To determine whether structural antigen differences might also aid in risk stratifying recipients, we entered each donor-recipient pair with genotyped HLA-A, -B, and –DR alleles into the HLA Matchmaker algorithm. HLA Matchmaker is a computer program

available online (www.hlamatchmaker.net) that is designed to assess the differences between donor and recipient HLA molecules at the structural level (9). The program uses the genotypes of donor and recipient HLA alleles to generate a list of eplets, which are polymorphic amino acid sequences in discontinuous positions that constitute the antigenic epitopes on the surface of HLA molecules. It then compares, both within loci and across loci of the same class, the specific eplets found on the donor's and the recipient's HLA molecules and calculates the number of eplets which are found in the donor, but not the recipient. The list of eplets uniquely present on the donor HLA molecules, but not shared by the recipient HLA molecules, represents the amount of novel antigenic material to which the recipient is being exposed by the graft. The HLA Matchmaker program has been studied and used for clinical applications in kidney transplantation (11-13), but has yet to be applied in heart transplantation outcomes research or clinical practice.

Using HLA Matchmaker, we assigned high-resolution four-digit alleles based on the low-resolution two-digit alleles provided in the SRTR database and known frequencies of high-resolution alleles by racial group. With these four-digit alleles, we generated the number of mismatching eplets within each class for each donor-recipient pair (14). Recipients were compared in an exploratory fashion using class-specific eplet number as a continuous variable and as a categorical variable with several different grouping schemes to identify any linear or non-linear association with graft survival. Additionally, we compared groups by number of mismatching eplets stratified by level of HLA mismatching at the allele level.

Statistical analysis and covariates: We considered the following characteristics to be potential confounders or precision variables in examining the association between the number of HLA mismatches and graft loss: donor and recipient age, sex, and race (white, black or African American, Hispanic/Latino, Asian, other), era of transplant (1987-1995, 1996-2004, 2005-2012), pre-transplant diagnosis (cardiomyopathy, congenital heart disease, other), listing status, days on wait list (as a continuous variable and as a categorical variable divided into quartiles), proportion with peak panel reactive antibodies (PRA) >10%, proportion with a positive cross-match, ischemic time, donor:recipient weight ratio (as a continuous variable and as a categorical variable divided into quartiles), donor cause

of death (anoxia, CVA/stroke, head trauma, CNS tumor, other), and proportions of recipients requiring pre-transplant dialysis, extracorporeal membrane oxygenation (ECMO), or mechanical ventilation. We classified recipients with multiple cross-match results from different testing methods as having a positive cross-match result if any of the results were positive or weakly positive.

We used the Kaplan-Meier method to estimate graft survival functions and the logrank test to compare survival estimates. We estimated the hazard ratio (HR) and 95% confidence interval (CI) for the association between HLA mismatching and graft loss independent of other risk factors for graft loss by fitting a multivariate Cox proportional hazards regression model. For the multivariate model, we selected among covariates associated with graft loss at the $p \leq 0.20$ level on univariate analysis; we considered a two-sided p-value <0.05 independent of other covariates in the model as the criterion for inclusion in the final model. We did not include in the model variables missing from more than 5% of subjects in the primary analysis. Variables which were associated with the outcome, but which were missing data from more than 5% of subjects, were assessed for confounding against the final multivariate model restricted to subjects with available data for those variables. We refitted the model using the same covariates as the final model to estimate HRs and CIs for the association between graft loss and class-specific HLA mismatches and HLA eplet mismatches.

Subjects with more HLA or eplet mismatches in one class of HLA loci were more likely to have mismatches at loci in the other class (Chi-square test p-value <0.001). For this reason, comparisons by class-specific mismatches additionally incorporated adjustment for the level of mismatching within each HLA class. We used StataSE 12 for all analyses.

Results

Study Population and Baseline Characteristics:

We identified 6,578 unique subjects who received a primary heart transplant at ages 0-18 years from 1987-2012 in the SRTR. Of these recipients, 4,851 had genotyped HLA-A, -B, and -DR alleles. The distribution of subjects by number of mismatched HLA alleles was skewed toward a greater number of mismatches (Figure 1). The 676 recipients with 0-3

total mismatches and the 4,175 recipients with 4-6 mismatches represented 3,890 and 22,413 person-years of observation, respectively.

Compared to recipients with 4-6 HLA mismatches, recipients with 0-3 mismatches were more likely to be white (65.8% vs. 58.8%, p=0.001) and were more likely to receive a graft from a white donor (69.2% vs. 60.9%, p<0.001); those with 0-3 mismatches were less likely to be black or African American (15.0% vs. 21.2%, p=0.001) and were less likely to receive a graft from a black or African American donor (11.0% vs. 19.2%, p<0.001). Recipients with 0-3 mismatches were also less likely to have a positive cross-match result (10.1% vs. 13.6%, p=0.02). The two groups were generally similar with regard to recipient age, sex, era, diagnosis, listing status, time spent on the waiting list, proportion with PRA >10%, ischemic time, pre-transplant clinical status indicated by the proportions requiring dialysis, ECMO support, and mechanical ventilation, donor-to-recipient weight ratio, and donor age, sex, and cause of death (Table 1).

Association between Number of Total and Class-Specific HLA Mismatches and Graft Survival:

Graft survival (Figure 2) was worse among recipients with a higher degree of HLA mismatching (p=0.003). Median graft survival times were 10.3 years (95% CI: 9.9-11.1 years) in the group with 4-6 HLA mismatches and 14.3 years (95% CI: 11.3-17.1 years) for the group with 0-3 HLA mismatches. The unadjusted hazard ratio comparing the risk of mortality or re-transplantation between recipients with 4-6 versus 0-3 total HLA mismatches was 1.24 (95% CI: 1.08-1.43, p=0.003).

In a multivariate regression model (Table 2), having 4-6 total HLA mismatches was associated with a hazard ratio of 1.21 (95% CI: 1.05-1.40, p=0.007) after adjustment by recipient age, sex, race, era of transplantation, diagnosis, ECMO or ventilator support, and donor cause of death. Specific adjusted hazard ratios for subjects with 4, 5, and 6 total mismatches, as compared to the reference group, were 1.21 (95% CI: 1.04-1.42, p=0.02), 1.19 (95% CI: 1.03-1.39, p=0.02), and 1.24 (95% CI: 1.06-1.46, p=0.009), respectively. PRA > 10% (HR: 1.14; 95% CI: 1.02-1.27, p=0.02), positive cross-match results (HR: 1.22; 95% CI: 1.08-1.39, p=0.002), and pre-transplant dialysis (HR: 2.16; 95% CI: 1.68-2.80, p<0.001) were associated with the outcome on univariate analysis, but were excluded from the final model due to excessive missing data. We detected no evidence of confounding by these

variables when they were assessed against the final model restricted to subjects with complete PRA, cross match, and dialysis data.

Kaplan-Meier estimates of graft survival in groups defined by class-specific mismatches (Figure 3) show progressively worsening graft survival associated with increasing number of mismatches at class I loci (p=0.04), but no association with the number of class II mismatches (p=0.71). Median graft survival times by number of class I mismatches were 16.1 (95% CI: 11.5-.), 11.4 (95% CI: 9.8-13.9), 10.6 (95% CI: 9.9-11.7), and 10.4 (95% CI: 9.6-11.5) years among recipients with 0-1, 2, 3, and 4 mismatches, respectively. Compared to those with 0-1 class I HLA mismatches, those with 2, 3, and 4 class I mismatches experienced hazard ratios for death and re-transplantation of 1.32 (95% CI: 1.00-1.75, p=0.05), 1.38 (95% CI: 1.05-1.80, p=0.02), and 1.42 (95% CI: 1.08-1.86, p=0.01), respectively, after adjustment for the level of class II mismatching and other covariates identified for the multivariate model (Table 3). Subjects with 1 and 2 HLA-DR mismatches experienced adjusted hazard ratios of 0.99 (95% CI: 0.78-1.26, p=0.94) and 1.02 (95% CI: 0.80-1.29, p=0.90), respectively, when compared to those with zero HLA-DR mismatches.

Association Between Number of Mismatched Class-Specific HLA Eplets and Graft Survival:

Recipients with more HLA allele mismatches generally had a greater number of eplet mismatches; however, there was a broad range of eplet mismatch numbers within each group defined by the number of HLA allele mismatches (Figure 5). For example, the number of mismatched eplets ranged from 6 to 38 in the group with 3 HLA allele mismatches, and from 12 to 65 in the group with 6 HLA allele mismatches.

Subjects with 10 or more mismatched eplets at class I loci had worse graft survival over the follow-up period compared to subjects with fewer than 10 mismatched eplets at class I loci (p=0.005, Figure 4). Median graft survival times were 13.5 years (95% CI: 11.3-16.9 years) in the group with fewer than 10 mismatched eplets and 10.5 years (95% CI: 9.9-11.2 years) in the group with 10 or more eplet mismatches. The adjusted hazard ratio associated with having 10 or more class I eplet mismatches was 1.24 (95% CI: 1.07-1.44, p=0.005, Table 3). There was no association between the number of class II eplet mismatches and graft survival using any grouping of eplet numbers.

Stratification of groups with 0-3 and 4-6 HLA allele mismatches by number of mismatched class I eplets showed that graft survival in recipients with 4-6 allele mismatches, but fewer than 10 mismatched class I eplets, was nearly indistinguishable from graft survival in all recipients with 0-3 mismatches (Figure 5). Only those with higher degrees of both allele mismatching and eplet mismatching were at risk for poorer survival (p=0.003). These results are reflected in the adjusted hazard ratios associated with having 0-3 allele mismatches with 10 or more class I eplet mismatches (HR: 1.05, 95%CI: 0.80-1.36, p=0.74), 4-6 allele mismatches with fewer than 10 class I eplet mismatches (HR: 1.01, 95%CI: 0.76-1.32, p=0.97), and 4-6 allele mismatches with more than 10 class I eplet mismatches (HR: 1.26, 95%CI: 1.04-1.53, p=0.02), as compared to those with 0-3 allele mismatches and fewer than 10 class I eplet mismatches.

Discussion

In this nationally representative cohort of over 4,500 pediatric heart transplant recipients followed for over 20 years, subjects with four or more donor-recipient mismatches at HLA-A, -B, and -DR alleles experienced a greater long-term risk of death or re-transplantation than subjects with fewer than four mismatches, independent of other risk factors for graft loss. More specifically, the number of class I mismatches was associated with progressively worsening graft survival in a "dose-dependent" manner, although there appeared to be a threshold at two or more class I mismatches, beyond which incremental decreases in graft survival were relatively small. Mismatches at HLA-DR were not associated with graft survival.

Analysis based on the structural differences of donor-recipient HLA molecules, as determined by the HLA Matchmaker program, are consistent with these findings; having 10 or more mismatched eplets at class I loci was associated with worse long-term graft survival, while mismatched HLA-DR eplets were not associated with graft survival. When recipients were stratified by both allele mismatches and eplet mismatches, those with a higher degree of allele mismatching, but fewer than 10 class I eplet mismatches, experienced graft survival that was nearly identical to recipients with fewer HLA allele mismatches.

There is evidence in adult heart transplant recipients that HLA allele mismatches

are associated with worse outcomes, including a higher risk of graft rejection during the first year after transplantation (2) and worsened overall survival (4-6). These studies have implicated mismatches at both class I and class II loci in association with worse survival among recipients (5, 15), and have indicated that matching at the HLA-DR locus may be associated with a lower incidence of acute rejection (3, 16) and cardiac allograft vasculopathy (4).

However, there are reasons to characterize risk factors specifically in pediatric heart transplant recipients, who differ from adult recipients in their state of physiologic and immunologic maturation, indications for transplantation, sensitization stimuli, interacting comorbidities, and longevity expectations. Data regarding the impact of HLA mismatching on outcomes in pediatric recipients are limited. HLA-DR mismatching was associated with a greater risk of high-grade rejection in a cohort of 38 pediatric heart transplant recipients treated with cyclosporine (7). A later study found no association between the number of HLA-DR mismatches and risk of developing CAV in a group of 337 pediatric heart transplant recipients (8). Recent ISHLT pediatric heart transplantation registry reports note that recipients who underwent transplantation between 1991 and 2001 had improved unadjusted 10-year survival if they had 0-3 HLA mismatches as compared to 4 or more mismatches, but no further analysis was done on the subject (1). No large, nationally representative, and multivariate analyses have been published characterizing the impact of HLA mismatching in pediatric heart transplant recipients; and no studies on the outcomes of either adult or pediatric heart transplant recipients have utilized structural analysis of eplet mismatches.

There is obvious biologic plausibility linking HLA mismatches to graft failure. HLA molecules are central to the immune system's ability to distinguish self from foreign tissue. The class-specific distribution of HLA molecules in the tissue may explain the differential association we observed with long-term graft loss. Class I HLA molecules are expressed on nearly every nucleated cell type, while class II antigens are restricted to professional antigen presenting cells and activated endothelial cells. It stands to reason that molecules present in the donor myocardium, and not those restricted to donor immune cells, which presumably will be cleared over time, would influence the antigenicity of the graft. Indeed, the detection of donor-specific antibodies (DSAs) against class I HLA molecules is more

highly associated with acute rejection and graft survival in heart recipients than is the presence of class II DSAs (17, 18).

One exception, however, might be that mismatched class II molecules in the endothelium of graft coronary arteries could predispose recipients to allograft vasculopathy, an association reported in adult heart recipients (4) and supported by data suggesting that DSAs against class II HLA molecules may be associated with allograft vasculopathy (19, 20). Due to limitations in the SRTR database, we were unable to determine the cause of graft failure or compare the risks of rejection and allograft vasculopathy in our groups. It is possible that, since it seems to be less common and less aggressive in pediatric recipients (21), the contribution of cardiac allograft vasculopathy to graft failure via class II HLA mismatching may not have been apparent over the period of time most patients were observed for this analysis.

The notion that certain HLA allele mismatches may be more antigenic than others, and that some allele mismatches may be inconsequential, is supported by outcomes in renal transplant recipients (22). HLA molecules, although coded by highly polymorphic alleles, have, to varying degrees, conserved antigenic structural elements within each class that may be recognized as self, even on mismatched donor HLA molecules. For this reason, quantifying the degree of donor-recipient eplet mismatching has the potential to augment the accuracy of recipient risk stratification based on HLA mismatching. In our cohort, for example, the survival of recipients with a higher degree of allele mismatching, but fewer than ten class I eplet mismatches, was nearly identical to the survival of recipients with fewer HLA allele mismatches. On the other hand, the group of patients with both a high number of allele mismatches and a high degree of structural difference, representing the majority of the cohort, experienced substantially reduced graft survival in comparison; this group's median time to graft loss was nearly 4 years shorter than the median time to graft loss in the rest of the cohort. It is also important to point out that the increased risk associated with a higher degree of HLA mismatching in our cohort appears to persist over the long term, in contrast to many other known post-transplant risk factors that are associated primarily with early graft failures.

Prospective matching of HLA alleles in heart allocation is currently difficult given the scarcity of donors and time constraints on the procurement process. Additionally,

prospective HLA matching could result in racial disparities in graft allocation, as it did in renal transplantation (23, 24). Nevertheless, given the ongoing need to enhance long-term graft survival, our findings may have significant clinical implications in post-transplant care. Although we cite the associations between class-specific DSAs and graft outcomes above to support our findings, it is important to remember that DSAs, despite being easily detected and measured, are an imperfect proxy for immunologic activity against the donor graft. Specifically, not all graft injury is antibody-mediated, not all DSAs result in antibody-mediated rejection or graft injury, and it remains difficult to predict which DSAs will be pathological in a given patient (18, 25, 26). HLA mismatching at the allelic and structural levels may predispose recipients to graft injury via pathways that do not involve DSAs, such as T cell-mediated responses, which are not easily measured or predicted in the clinical setting. Indeed, although we were not able to analyze DSA measurements in our cohort, HLA mismatching was associated with graft loss even among those with low PRA levels and negative cross-match results. Thus, knowledge of mismatching may help identify those at risk for graft loss due antibody-mediated injury as well as other immunologic processes and guide decisions regarding post-transplant surveillance and immunosuppression.

Further study is required to identify whether the increased graft loss in recipients with HLA mismatching is accounted for by rejection, vasculopathy, or progressive graft dysfunction, and whether these entities are mediated by DSAs. The analysis of intra- and inter-locus eplet differences may help clarify the connections between HLA mismatching, the production of clinically important DSAs, and graft loss. Furthermore, high-resolution HLA genotyping and the expansion of loci that are routinely genotyped will enhance our understanding of which mismatches are clinically important. It would also be interesting to study the application of eplet matching to the virtual cross-match, where it might ultimately improve the interpretability of the virtual cross-match and perhaps increase the donor pool for highly sensitized candidates.

Study Limitations

This study has several limitations. First, our retrospective design limits us from completely controlling for differences between our comparison groups and prevents us from observing the influence of the prolonged wait times, increased ischemic times, and

racial disparities that might occur with heart allocation based on prospective HLA matching. Second, approximately 25% of potential subjects were missing HLA genotype data. These data appeared to be missing more frequently among patients transplanted in earlier eras, but those with missing data did not have different survival estimates compared to those with mismatch data after adjustment for year of transplant (HR 1.06 (95%CI: 0.98-1.16). We had to assume that subjects without an identified second allele at a given locus were homozygous for the identified allele, and we based our analysis of eplet mismatches on high-resolution allele types that were assumed based on the provided low-resolution alleles and each subject's race. Although these assumptions influenced the number of allele and eplet mismatches assigned to each subject, there is no way to test their validity. Third, advances in the HLA typing have resulted in the discovery of new and distinct HLA types over the nearly 25 years of follow-up described in this study, presumably making the identification of mismatches more accurate over time. Our analysis, however, did not detect a changing association between mismatches and survival by era of transplant. Additionally, our analysis was limited to the HLA-A, -B, and -DR loci. These loci have historically been considered the most important loci in influencing clinical outcomes, which has resulted in limited collection of data on other loci in the SRTR. The addition of HLA-C and DQ in the future may improve risk stratification based on HLA allele and eplet mismatches.

Conclusion

Improving survival beyond the first post-transplant year in pediatric heart recipients remains challenging. Here, we have demonstrated that considering both genotypic and epitope-level HLA mismatching may help identify recipients who are at increased risk, and for whom intensified post-transplant surveillance and management may be appropriate. Identification of risk factors associated with long-term graft loss can additionally generate hypotheses for mechanistic studies designed to elucidate the etiology of graft failure and, hopefully, to test interventions capable of prolonging graft survival.

References

1. Dipchand AI, Kirk R, Edwards LB, et al. The Registry of the International Society for Heart and Lung Transplantation: 16th Official Pediatric Heart Transplantation Report. J Heart Lung Transplant. 2013;32(10):979-88.
2. Jarcho J, Naftel DC, Shroyer TW, et al. Influence of HLA mismatch on rejection after heart transplantation: a multiinstitutional study. *J Heart Lung Transplant*. 1994;13(4):583-95.
3. Ansari D, Bućin D, Nilsson J. Human Leukocyte Antigen Matching in Heart Transplantation: Systematic Review and Meta-analysis. Transpl Int. 2014 Apr 11 [Epub ahead of print], http://dx.doi.org/10.1111/tri.12335
4. Taylor DO, Stehlik J, Edwards LB, et al. Registry of the International Society for Heart and Lung Transplantation: Twenty-sixth Official Adult Heart Transplant Report. J Heart Lung Transplant. 2009;28(10):1007-22.
5. Hosenpud JD, Edwards EB, Lin HM, Daily OP. Influence of HLA Matching on Thoracic Transplant Outcomes: An Analysis From the UNOS/ISHLT Thoracic Registry. *Circulation.* 1996;94(2):170-174.
6. Opelz G, Wujciak T. The influence of HLA compatibility on graft survival after heart transplantation. N Engl J Med. 1994;330(12):816-9.
7. Herzberg GZ, Rossi AF, Courtney M, Gelb BD. The effects of HLA mismatching and immunosuppressive therapy on early rejection outcome in pediatric heart transplant recipients. J Heart Lung Transplant. 1998;17(12):1195-1200.
8. Hathout E, Beeson WL, Kuhn M, et al. Cardiac allograft vasculopathy in pediatric heart transplant recipients. Transpl Int. 2006;19(3):184-9.
9. Duquesnoy R. Clinical usefulness of HLA Matchmaker in HLA epitope matching for organ transplantation. Curr Opin Immunol. 2008;20(5):594-601.
10. Leppke S, Leighton T, Zaun D, et al. Scientific Registry of Transplant Recipients: Collecting, analyzing, and reporting data on transplantation in the United States. Transplant Rev. 2013;27(2):50-56.
11. Silva E, Alba A, Castro A, et al. Evaluation of HLAMatchmaker compatibility as predictor of graft survival and presence of anti-HLA antibodies. *Transplant Proc. 2010;42(1):266-9.*

12. Dankers MK, Witvliet MD, Roelen DL, et al. The number of amino acid triplet differences between patient and donor is predicitve for the antibody reactivity against mismatched HLA antigens. *Transplantation. 2004;77(8): 1236-9.*
13. Claas FH, Witvliet MD, Duquesnoy RJ, Persijn GG, Doxiadis II. The acceptable mismatch program as a fast tool for highly sensitized patients awaiting a cadaveric kidney transplantation: short waiting time and excellent graft outcome. *Transplantation. 2004;78(2):190-93.*
14. Maiers M, Gragert L, Klitz W. High-resolution HLA alleles and haplotypes in the United States population, Hum Immunol. 2007;68(9):779-88.
15. Frist WH, Oyer PE, Baldwin JC, Stinson EB, Shumway NE. HLA Compatibility and Cardiac Transplant Recipient Survival. Ann Thorac Surg. 1987;44(3):242-246.
16. DeMattos AM, Head MA, Everett J, et al. HLA-DR mismatching correlates with early cardiac allograft rejection incidence and graft survival when high-confidence-level serologic DR typing is used. Transplantation. 1994;57(4):626-30.
17. Frank R, Molina MR, Wald JW, Goldberg LR, Kamoun M, Lal P. Correlation of circulating donor-specific anti-HLA antibodies and presence of cd4 in endomyocardial biopsy with heart allograft outcomes: a single-center, retrospective study. J Heart Lung Transplant. 2013;32(4):410-17.
18. Ho EK, Vasilescu ER, Vlad G, Marboe CC, Addonizio LJ, Suciu-Foca N. HLA antibodies in pediatric heart transplantation. Pediatr Transplant. 2011;15(5): 458–464.
19. Topilsky Y, Gandhi MJ, Hasin T, et al. Donor-specific antibodies to class II antigens are associated with accelerated cardiac allograft vasculopathy: a three-dimensional volumetric intravascular ultrasound study. Transplantation. 2013;95(2):389-96.
20. Xydas S, Yang JK, Burke EM, et al. Utility of post-transplant Anti-HLA Antibody Measurements in Pediatric Cardiac Transplant Recipients. J Heart Lung Transplant. 2005;24(9):1289–96.
21. Pahl E, Naftel DC, Kuhn MA, et al. The impact and outcome of transplant coronary artery disease in a pediatric population: a 9-year multi-institutional study. J Heart Lung Transplant. 2005;24(6):645-51.

22. Doxiadis II, Smits JM, Schreuder GM, et al. Association between specific HLA combinations and probability of kidney allograft loss: the taboo concept. Lancet. 1996;348(9031):850-53.
23. Roberts JP, Wolfe RA, Bragg-Gresham JL, et al. Effect of changing the priority for HLA matching on the rates and outcomes of kidney transplantation in minority groups. N Engl J Med. 2004; 350(6):545-51.
24. Amaral S, Patzer RE, Kutner N, McClellan W. Racial disparities in access to pediatric kidney transplantation since Share 35. J Am Soc Nephrol. 2012;23(6):1069-77.
25. Peng DM, Law YM, Kemna MS, Warner P, Nelson K, Boucek RJ. Donor-specific antibodies: Can they predict C4d deposition in pediatric heart recipients? Pediatric Transplantation. 2013;17(5):429-35.
26. Campbell P. Clinical relevance of human leukocyte antigen antibodies in liver, heart, lung, and intestine transplantation. Curr Opin Organ Transplant. 2013;18(4):463-9.

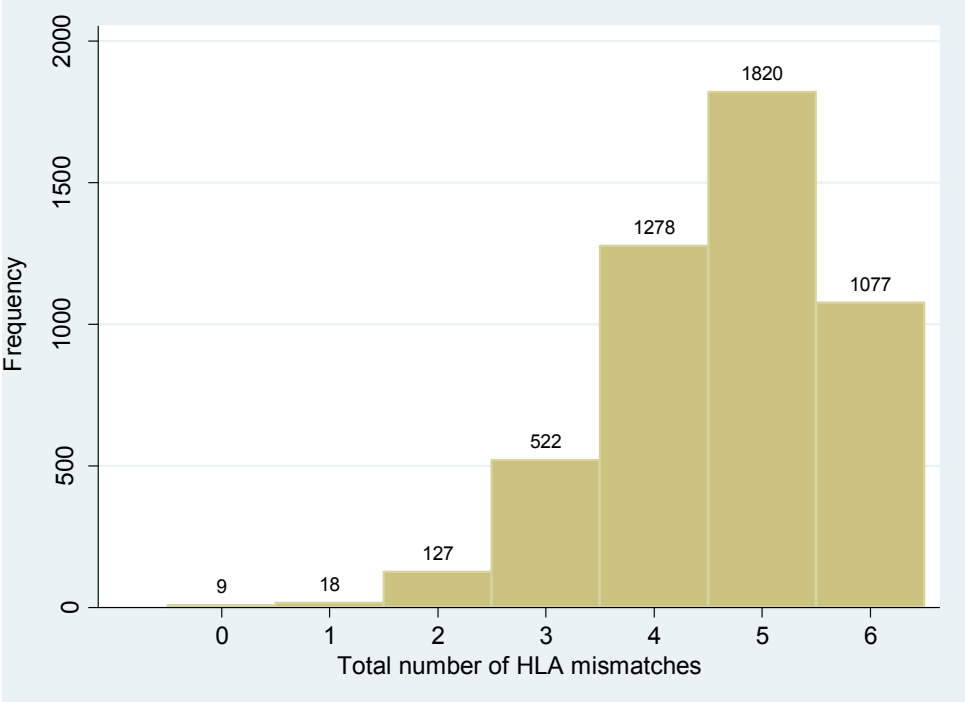


Figure 1

Frequency of HLA mismatches

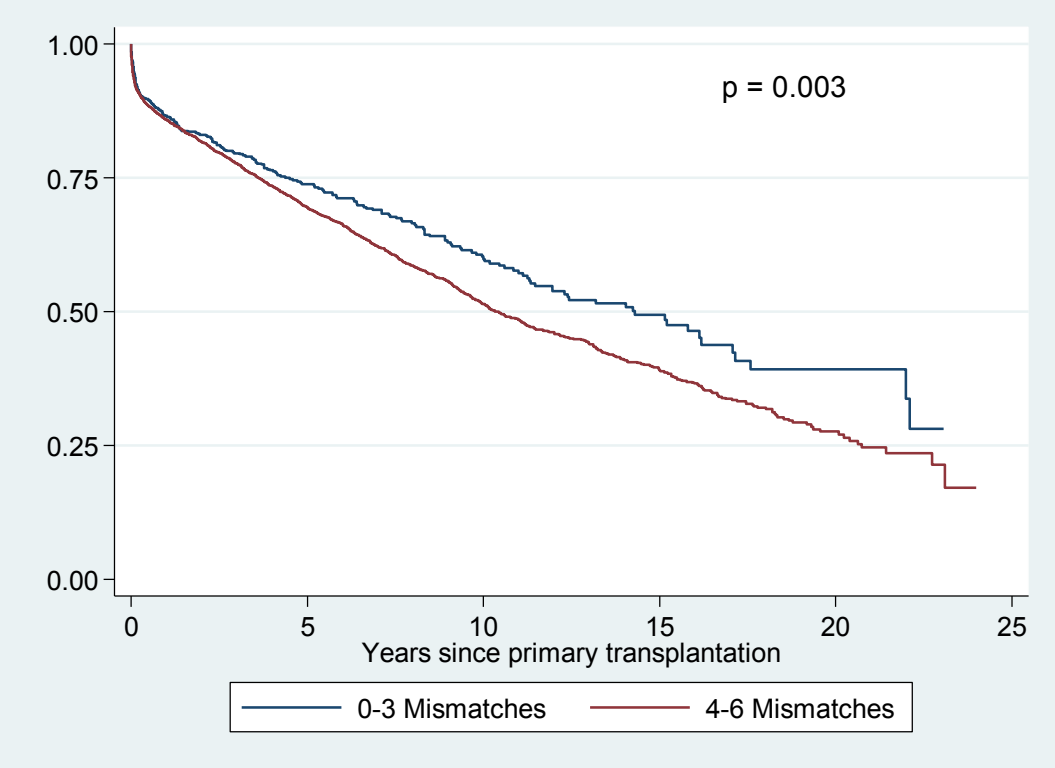
The frequencies of total mismatches at HLA-A, -B, and –DR loci among 4,851 primary heart transplant recipients ≤18 years of age in the US from 1987-2012 are skewed, consistent with highly polymorphic HLA genes.

Table 1: Recipient and donor characteristics by number of HLA-A, -B, and -DR allele mismatches for primary heart transplant recipients ≤ 18 years of age, U.S. 1987-2012.

Variable *	0-3 Mismatches †	4-6 Mismatches †	p-value ‡
n (%)	676 (13.9)	4175 (86.1)	
Recipient Characteristics			
Years of observation, med (IQR)	4.0 (1.0-9.0)	3.9 (1.0-8.3)	0.16 ||
Age at tx in years, med (IQR)	6 (0-14)	5 (0-13)	0.63 ||
Male sex, n(%)	394 (58.3)	2366 (56.7)	0.43
Race, n (%)			0.001
White	444 (65.8)	2451 (58.8)	
Black or AA	101 (15.0)	884 (21.2)	
Hispanic/Latino	103 (15.3)	612 (14.7)	
Asian	16 (2.4)	147 (3.5)	
Other	11 (1.6)	78 (1.9)	
Era of transplant, n (%)			0.72
1987-1995	159 (23.5)	959 (23.0)	
1996-2004	254 (37.6)	1523 (36.4)	
2005-2012	263 (38.9)	1693 (40.6)	
Pre-transplant diagnosis, n (%)			0.34
Cardiomyopathy	339 (50.2)	2211 (53.0)	
Congenital HD	324 (47.9)	1874 (44.9)	
Other	13 (1.9)	89 (2.1)	
Last listing status, n (%)			0.84
1A, 1B, or old status 1	535 (80.2)	3280 (79.9)	
2	132 (19.8)	827 (20.1)	
Days listed, med (IQR)	38 (14-91)	38 (13-89)	0.59 ||
PRA > 10%, n (%)	135 (21.6)	788 (20.4)	0.48
Pos. cross-match result, n (%)	60 (10.1)	504 (13.6)	0.02
Pre-tx dialysis, n (%)	14 (2.6)	77 (2.2)	0.63
Pre-tx ECMO, n (%)	31 (4.6)	163 (3.9)	0.40
Pre-tx ventilator, n (%)	95 (14.1)	621 (14.9)	0.56
Donor Characteristics			
Age in years, med (IQR)	6 (1-16)	6 (1-16)	0.96 ||
Male Sex, n(%)	391 (57.8)	2441 (58.5)	0.75
Ischemic time in mins, mn±SD	211.0±80.9	216.2±79.6	0.12 §
Don:rec weight ratio, mn ± SD	1.45± 0.77	1.41± 0.79	0.34 §
Race, n (%)			<0.001
White	467 (69.2)	2537 (60.9)	
Black or AA	74 (11.0)	802 (19.2)	
Hispanic/Latino	124 (18.4)	737 (17.7)	
Asian	5 (0.7)	61 (1.5)	
Other	5 (0.7)	31 (0.7)	
Cause of death, n (%)			0.26
Anoxia	140 (20.8)	1016 (24.4)	
CVA/Stroke	71 (10.6)	409 (9.8)	
Head Trauma	397 (59.0)	2358 (56.7)	
CNS tumor	8 (1.2)	32 (0.8)	
Other	57 (8.5)	348 (8.4)	

Table 1 (cont'd): Recipient and donor characteristics by number of HLA-A, -B, and -DR allele mismatches for primary heart transplant recipients ≤ 18 years of age, U.S. 1987-2012.

* Missing data are reported if missing for >5% of subjects in either group: PRA data are missing for 51 (7.5%) in the 0-3 mismatch cohort and 304 (7.3%) in the 4-6 mismatch cohort; cross-match results are missing for 83 (12.3%) in the 0-3 mismatch cohort and 479 (11.5%) in the 4-6 mismatch cohort; ischemic times are missing for 37 (5.5%) in the 0-3 mismatch cohort and 255 (6.1%) in the 4-6 mismatch cohort; pre-transplant dialysis data are missing for 130 (19.2%) in the 0-3 mismatch cohort and 727 (17.4%) in the 4-6 mismatch cohort; donor-to-recipient weight ratio is missing for 65 (9.6%) in the 0-3 mismatch cohort and 317 (7.6%) in the 4-6 mismatch cohort.
† Numbers for each categorical variable may not add up to total due to missing data.'
‡ Chi-square test
§ T-test of means
|| Wilcoxon rank-sum test
¶ Abbreviation: AA—African American; CNS—central nervous system; CVA—cerebrovascular accident; ECMO—extracorporeal membrane oxygenation; HD—heart disease; IQR—interquartile range; SD—standard deviation.



Years	0	5	10	15	20
Mismatches			Subjects at risk		
0-3	676	295	148	54	9
4-6	4175	1725	767	271	53

Figure 2

Freedom from graft loss by number of HLA mismatches

Kaplan-Meier estimates of graft survival in recipients ≤18 years of age show superior long-term graft survival in recipients with 0-3 HLA-A, -B, and -DR mismatches compared to those with 4-6 mismatches (p=0.003).

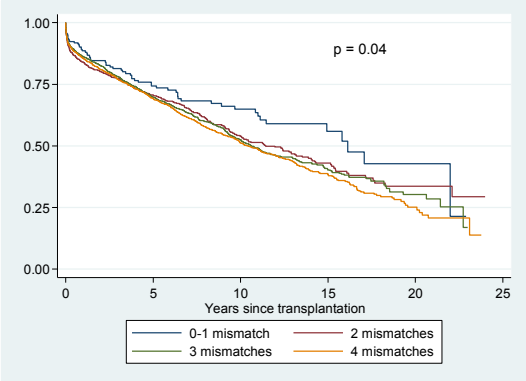
Table 2: Multivariate Cox proportional hazards regression analysis of recipient and donor characteristics associated with recipient death or re-transplantation. *

	HR	(95% CI)	p-value
4-6 vs. 0-3 HLA mismatches	1.21	(1.05-1.40)	0.007
Recipient characteristics			
1-year increase in age	1.03	(1.02-1.03)	<0.001
Male sex	0.85	(0.77-0.93)	0.001
Black/AA vs. Caucasian race	1.71	(1.53-1.91)	<0.001
Black/AA vs. Hispanic race	1.76	(1.49-2.07)	<0.001
Black/AA vs. Asian race	1.53	(1.13-2.08)	0.006
Era: 1996-2004 vs. 1987-1995	0.81	(0.72-0.90)	<0.001
Era: 2005-2012 vs. 1996-2004	0.71	(0.62-0.81)	<0.001
Congenital HD vs. cardiomyopathy	1.41	(1.27-1.55)	<0.001
Pre-transplant ECMO	1.67	(1.29-2.15)	<0.001
Pre-transplant ventilator	1.22	(1.05-1.42)	0.009
Donor characteristics			
Cause of death: CVA/stroke vs anoxia	1.23	(1.05-1.45)	0.005
Cause of death: head trauma vs. CVA/stroke	0.83	(0.72-0.96)	0.01
Cause of death: CNS tumor vs. CVA/stroke	0.47	(0.25-0.89)	0.02

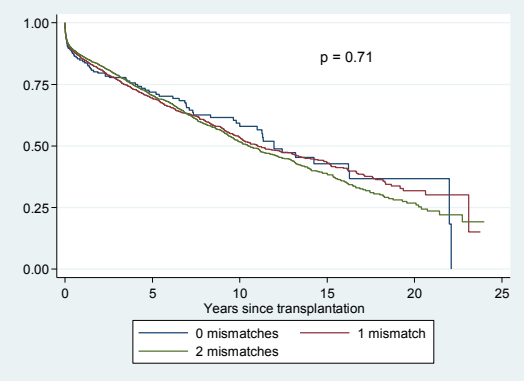
* Variables were omitted from the multivariate model despite association with graft failure due to excess missing data, including: PRA >10% (HR 1.14 [95% CI: 1.02-1.27, p=0.02]); positive cross-match result (HR 1.22 [95% CI: 1.08-1.39, p=0.002]); and pre-transplant dialysis (HR 2.16 [95% CI: 1.68-2.80, p<0.001]). Inclusion of these variables did not confound the model.

† Abbreviation: AA—African American; CNS—central nervous system; CVA—cerebrovascular accident; ECMO—extracorporeal membrane oxygenation; HD—heart disease; HR—hazard ratio; SD—standard deviation.

(a) Class I (HLA-A and -B) allele mismatches



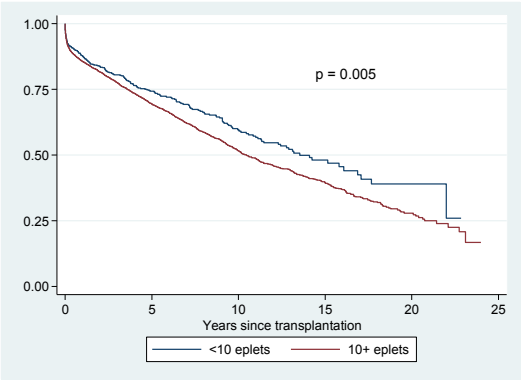
(b) Class II (HLA-DR) allele mismatches



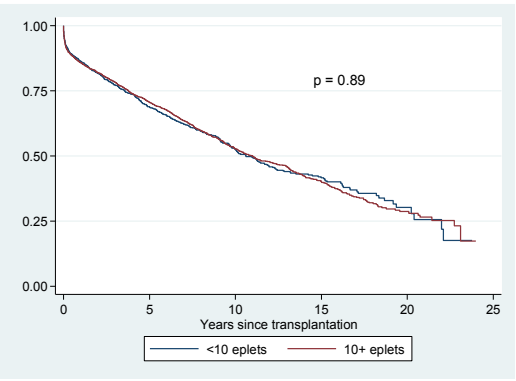
Yrs	0	5	10	15	20
Mismatches		Subjects at risk			
0-1	198	94	49	17	4
2	849	373	176	70	13
3	1994	814	363	125	22
4	1920	799	374	139	27

Yrs	0	5	10	15	20
Mismatches		Subjects at Risk			
0	205	89	47	17	3
1	1995	804	378	149	23
2	2672	1139	496	164	37

(c) Class I (HLA-A and -B) eplet mismatches



(d) Class II (HLA-DR) eplets mismatches



Yrs	0	5	10	15	20
Eplets		Subjects at risk			
<10	634	282	135	41	6
10+	4217	1738	780	284	56

Yrs	0	5	10	15	20
Eplets		Subjects at Risk			
<10	1444	584	261	103	16
10+	3407	1436	654	222	46

Figure 3

Freedom from graft loss by number of class-specific HLA allele and eplet mismatches

Kaplan-Meier estimates of graft survival by (a) class I and (b) class II allele mismatches and (c) class I eplet and (d) class II eplet mismatches. Recipients with fewer class I HLA allele (p=0.04) and eplet (p=0.005) mismatches had superior long-term graft survival. Class II HLA mismatches were not associated with differences in graft survival.

Table 3: Adjusted* hazard ratios for graft failure by number of human leukocyte antigen allele and eplet mismatches at class I and class II loci in recipients ≤ 18 years of age in the U.S., 1987-2012.

Number of HLA mismatches			
Class I (HLA-A and –B loci)			
0-1 mismatches (n=198)	Reference		
2 mismatches (n=849)	1.32	(1.00-1.75)	0.05
3 mismatches (n=1992)	1.38	(1.05-1.80)	0.02
4 mismatches (n=1920)	1.42	(1.08-1.86)	0.01
Class II (HLA-DR locus)			
0 mismatches (n=205)	Reference		
1 mismatch (n=1995)	0.99	(0.78-1.26)	0.94
2 mismatches (n=2672)	1.02	(0.80-1.29)	0.90
Number of eplet mismatches			
Class I (HLA-A and –B loci)			
<10 eplets (n=639)	Reference		
10+ eplets (n=4212)	1.24	(1.07-1.44)	0.005
Class II (HLA-DR locus)			
<10 eplets (n= 1444)	Reference		
10+ eplets (n= 3407)	1.00	(0.91-1.11)	0.96

* Class-specific mismatches and novel eplet count (<10 vs. 10+) were each included in a multivariate model adjusting for level of mismatching or novel eplet count at loci in each class as well as recipient age, sex, race, transplant era, diagnosis, pre-transplant ECMO, pre-transplant ventilator support, and donor cause of death.

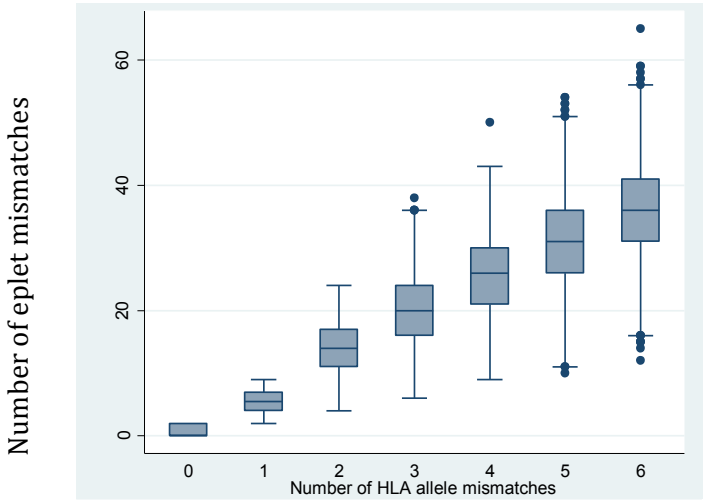
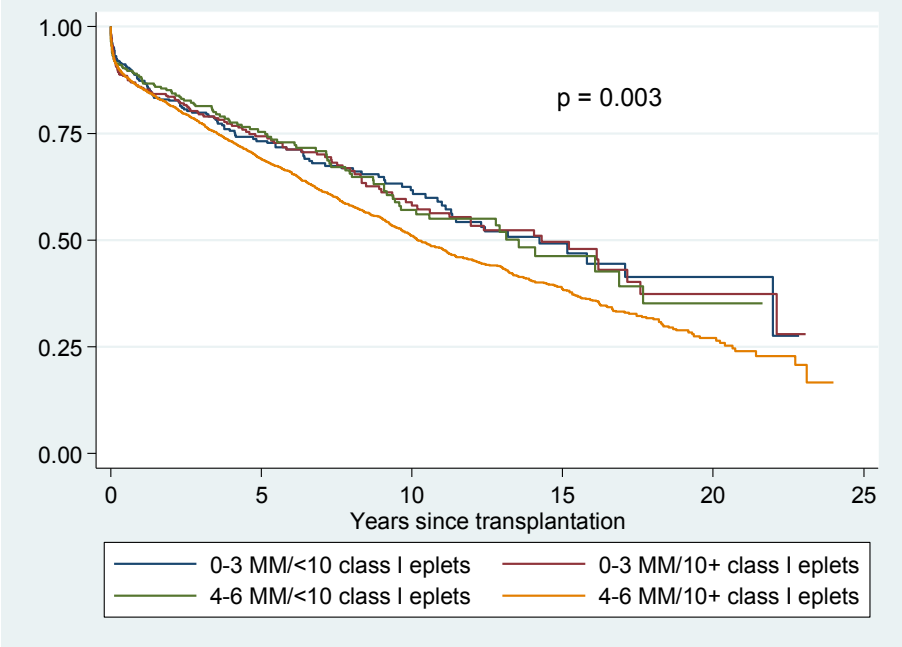


Figure 4

Number of eplet mismatches by number of HLA mismatches
Number of total eplet mismatches by total number of HLA allele mismatches show increasing number of eplet mismatches with higher number of HLA allele mismatches ($p<0.001$), although there is considerable overlap of the ranges.



Years	0	5	10	15	20
Mismatches	Subjects at risk				
0-3 MM/<10 eplets	320	151	75	23	3
0-3 MM/10+ eplets	356	144	73	31	6
4-6 MM/<10 eplets	314	131	60	18	3
4-6 MM/10+ eplets	3861	1594	707	253	50

Figure 5

Freedom from graft loss by HLA allele mismatches stratified by number of class I eplet mismatches

Kaplan-Meier estimates of graft survival by total HLA allele mismatches (0-3 vs 4-6) stratified by number of class I eplet mismatches (<10 vs 10+). Only subjects with 4-6 allele mismatches and 10+ eplet mismatches had worse graft survival compared to the remainder of the cohort (p=0.003).