HLA-DR and -DQ Eplet Mismatches and Transplant Glomerulopathy: A Nested Case–Control Study

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We conducted a nested case–control study from a cohort of adult kidney transplant recipients to assess the risk of transplant glomerulopathy (TG) as a function of donor and recipient HLA-DR and -DQ incompatibility at the eplet level. Cases (n = 52) were defined as patients diagnosed with transplant glomerulopathy based on biopsies showing glomerular basement membrane duplication without immune complex deposition. Controls (n = 104) with a similar follow-up from transplantation were randomly selected from the remaining cohort. HLAMatchmaker was used to ascertain the number of DRB1/3/4/5, DQA1 and DQB1 related eplet mismatches (eplet load). Multivariable conditional logistic regression models demonstrated an increase in the odds of TG (odds ratios [OR] of 2.84 [95% confidence interval (CI): 1.03, 7.84] and 4.62 [95% CI: 1.51, 14.14]) in the presence of 27–43 and >43 HLA-DR + DQ related eplet mismatches versus <27 eplet mismatches, respectively. When the eplet load was modeled as a continuous variable, the OR for TG was 1.25 (95% CI: 1.04, 1.50) for every 10 additional HLA-DR + DQ eplet mismatches. Our study suggests that minimization of HLA-DR + DQ eplet mismatches may decrease the incidence of transplant glomerulopathy diagnosed by indication biopsies. The role of eplet immunogenicity/antigenicity as determinants of allograft outcomes requires further study.

Abbreviations: CNI, calcineurin inhibitor; DSA, donor-specific antibody; KTR, kidney transplant recipients; TG, transplant glomerulopathy

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Introduction

Despite the improvements in kidney allograft survival observed over the past few decades, many kidney transplant recipients (KTR) experience kidney allograft failure (1). Kidney allograft failure is associated with an increased risk for mortality (2), a substantial decrease in quality-of-life (3) and a fourfold increase in health-care costs (3).

Transplant glomerulopathy (TG) is an important contributor to kidney allograft failure (4–7). TG is a morphologic pattern of chronic kidney allograft injury, which is characterized by duplication/multilamination of capillary basement membranes in the absence of immune-complex deposits (8). TG is present in 20% of kidney allograft surveillance biopsies by 5 years following transplantation (9,10). Its prevalence in indication biopsies varies from 1.6% to 7% (5,6,11–13). Currently, no treatment is available to reverse, or even mitigate, the effects of TG (14–17). Considering the absence of effective therapy, the implications of allograft failure and the scarcity of organs available for transplantation, it is important to prevent the development of TG in KTR.

TG develops as a consequence of overt and covert episodes of antibody-mediated rejection (5,18–24), typically, due to HLA class II anti-HLA antibodies (5,10,25–30). Antibodies against HLA develop when antibody-accessible polymorphic residues on HLA antigens are recognized as “nonself.” Such residues include structural and functional epitopes (31). Structural epitopes constitute the epitope’s binding face with alloantibodies, while functional epitopes determine the strength and specificity with which the epitope will bind.
with an antibody. Each donor’s functional epitope may have one or more residues that may be identified as nonself by the recipient. These residues have been termed “eplets” (32–35). HLAMatchmaker (http://www.hlamatchmaker.net) is a computer algorithm that identifies structural HLA eplet mismatches between donors and recipients that may give rise to anti-HLA antibodies (31,33,36–40).

Recent evidence suggests that the number of locus-specific eplet mismatches is a predictor for the development of anti-HLA-DR and anti-HLA-DQ antibodies and that this variable outperforms traditional low- and high-resolution antigen-based matching in predicting the development of donor-specific antibodies (DSA) (41). Whether the number of HLA-DR + DQ eplet mismatches, or the HLA-DR + DQ “eplet load,” is associated with chronic antibody-mediated injury in kidney allografts, or TG, is unknown.

In the face of this uncertainty, we conducted a nested case-control study to address the following questions: (i) Is the HLA-DR + DQ eplet load an independent risk factor for TG? (ii) Does a dose-response relationship exist between HLA-DR + DQ eplet load and the risk of TG? (iii) Are certain eplet mismatches more strongly associated with an increased risk for TG compared to other eplet mismatches? (iv) Is the risk for TG due to eplet mismatches dependent on the choice of induction or maintenance immunosuppression?

Materials and Methods

Study population and case ascertainment

We conducted a nested case-control study from a cohort of KTR transplanted at the University Health Network between January 1, 2000 and December 31, 2012 (n = 1753). Data were obtained from our in-center Comprehensive Renal Transplant Research Information System (CoReT-RIS) (42). CoReTRIS includes information on recipient and donor demographics, comorbidities, pre/posttransplant histocompatibility testing and comprehensive data on clinical outcomes, treatment and laboratory investigations following kidney transplantation. The data quality validation procedures implemented in CoReTRIS, as well as the accuracy and reproducibility of the included variables were described previously (43).

Cases were defined as adult KTR with TG, diagnosed by light microscopy and defined as >10% duplication or multilamination of the glomerular basement membrane. The diagnosis of TG was made upon exclusion of glomerular immune deposits (by electron microscopic and/or immunofluorescence examination) and thrombotic microangiopathy (TMA), since these findings on kidney allograft biopsies may also present with duplication of the glomerular basement membrane (8,44). TMA was excluded by ruling out the presence of thrombi and intimal edema as well as systemic features of TMA. All TG cases were ascertained by a single renal pathologist (RJ) without knowledge of eplet status. Two controls were randomly selected for each TG case from the remaining cohort, matching for follow-up time and the year of transplantation. This procedure is known as incidence density or risk set sampling and ensures that the odds ratio derived from the conditional logistic regression model is a direct estimate of the incidence rate ratio that would have been ascertained from the equivalent cohort study (45).

HLA typing and eplet mismatch identification

DRB1, DRB3/4/5, DQA1 and DQB1 HLA typing was performed using sequence-specific oligonucleotide probes (LABType HLA typing kit; One Lambda, Canoga Park, CA) (46,47). Respective four-digit types were assigned using the catalogue of common and well-documented (CWD) human leukocyte antigen alleles (46,48–51). The plausibility of four-digit HLA-DR and HLA-DQ types was further corroborated against the allele frequency net database, an online resource containing information on the frequencies of immune genes, their corresponding alleles and haplotypes in different populations (52). When rare HLA-DR or HLA-DQ alleles were possible, typing by sequence-specific primer polymerase chain reaction (46,47) was conducted in an effort to minimize allele misclassification. HLAMatchmaker software was used to define potential eplet mismatches between donors and recipients (31). The HLA class II version of HLAMatchmaker (HLAMatchmaker DRD2 Matching for 500 donor-recipient pairs—version 2.0) considers polymorphisms in the HLA-D regions that lead to alloantibody responses.

Exposure

HLA-DR and HLA-DQ demonstrate strong linkage disequilibrium (S3–57). Allele mismatches in HLA-DR are likely to represent an extra eplet load related to both HLA-DR and HLA-DQ and vice versa. For this reason, the main exposure in this study, HLA-DR + DQ eplet load, was modeled as the sum of HLA-DR and HLA-DQ eplet mismatches (determined by HLAMatchmaker from the amino acid chains coded by the HLA-DRB1 and HLA-DRB3/4/5 and HLA-DQA1/HLA-DQB1 alleles, respectively). To identify dose-response and threshold effects, HLA-DR + DQ eplet load was modeled as a categorical variable. The primary categorization of HLA-DR + DQ eplet load divided eplet load categories by tertiles (i.e. each category included an equal number of participants). A secondary categorization of HLA-DR + DQ eplet load was carried out by creating a binary variable using the median number of HLA-DR + DQ eplet mismatches in the study sample as the threshold. HLA-DR + DQ eplet load was also modeled as a continuous variable. Eplet loads of HLA-DR and HLA-DQ were also considered separately and modeled as categorical (three categories based on tertiles of HLA-DR or HLA-DQ eplet loads and two categories based on the median number of HLA-DR or HLA-DQ eplet mismatches, respectively) and continuous variables.

Model covariates

Potential recipient, donor and transplant covariates were evaluated at transplantation and were considered for inclusion in the conditional logistic regression model. Recipient factors included age, sex, race, peak panel reactive antibody (PRA: 0–100%) and presence of hepatitis C antibodies. Donor factors included age, sex and donor type (deceased vs. living). Transplant factors included the number of HLA-A and HLA-B antigen mismatches (0–2 each), number of transplants, transplantation era, type of induction therapy and the type of calcineurin inhibitor used for maintenance immunosuppression.

Statistical analysis

The distributions of recipient, donor and transplant characteristics at the time of transplantation were evaluated across cases and controls using the t-test or Kruskal–Wallis test for continuous variables and chi-square or Fisher’s exact test for categorical variables. Predictive values of TG were calculated for various HLA-DR and HLA-DQ eplet and antigen (serological equivalents) mismatch thresholds (58). We fit multivariable conditional logistic regression models to assess the independent association between HLA-DR + DQ, HLA-DR and HLA-DQ eplet load and TG in the setting of matched data. Both univariable (Model 1) and multivariable analyses were conducted. Each multivariable model sequentially incorporated a larger set of clinically relevant covariates while including the main exposure variable.
These covariates included recipient age, sex, race and peak PRA (Model 2).
Donor type and induction therapy were also included in Model 3 and Model 4, respectively. Missing covariate data were handled by multiple imputations using Stata’s ‘ice’ command (StataCorp, College Station, TX, www.stata.com).

**Sensitivity analyses**

Several sensitivity analyses were undertaken to assess the robustness of the main results. First, to evaluate the impact of model selection, obtain a parsimonious model and optimize model fit, we applied a process of backward, stepwise, covariate selection to Model 4 using a threshold for entry/exit of p < 0.15. Second, to account for the choice of maintenance immunosuppression, the type of calcineurin inhibitor (tacrolimus vs. cyclosporine) was also added as a covariate to the original set of covariates included in Model 4. Finally, to assess the risk for TG as a function of previously identified thresholds of eplet load, analyses using 10 HLA-DR and 17 HLA-DQ eplet mismatches as thresholds were conducted (41).

We also conducted post hoc sensitivity analyses to evaluate the independent association of HLA-DRB1 and HLA-DRB3/4/5 eplet mismatches and TG. To address the structural basis of HLA-DR immunogenicity, we compared the distribution of eplet mismatches shared by HLA-DRB1 and HLA-DRB3/4/5 among cases and controls. Finally, we report the most frequent eplet mismatches among cases versus controls (i.e. observed in >20% of TG cases and their frequency among controls ≥10% lower than cases) and evaluate those against previously identified immunogenic eplets (37, 41).

All statistical analyses were performed using Stata/IC, version 12.0. A two-tailed p-value < 0.05 was considered statistically significant. The Research Ethics Boards at the University of Toronto and University Health Network approved the study.

**Results**

After implementing the exclusion criteria, the final study cohort from which cases and controls were derived consisted of 1480 patients (Figure 1). Of a total of 59 TG cases, diagnosed on indication biopsies in the cohort, one case could not be matched with controls and six sets of cases and controls were excluded because either the donors or recipients were missing DNA samples for HLA typing. The final study sample included 52 cases and 104 controls. TG was diagnosed after a mean follow-up of 3.97 ± 2.71 years in the included cases.

The study cohort was mainly Caucasian (70.5%) while African-Canadian patients constituted 9.6% of the study sample. Standard immunosuppression included a calcineurin inhibitor (tacrolimus, n = 73; cyclosporine, n = 78), mycophenolate mofetil (n = 141) and prednisone (n = 140). Induction therapy with thymoglobulin (n = 89) or basiliximab (n = 45) was used in 134/156 (85.9%) patients. Patients with TG were younger and had higher peak PRA levels at transplantation than controls (Table 1). There was no significant difference in the sex, number of HLA class I antigen mismatches, induction or maintenance immunosuppression therapy. The proportion of patients who underwent living donor transplantation was higher among cases (63.5% vs. 43.3%; p < 0.02). The incidence of biopsy proven acute rejection, in general and acute antibody-mediated rejection, in particular, was significantly higher among cases compared with controls (71.1% vs. 16.3%; p < 0.001, and 57.7% vs. 4.8%; p < 0.001, respectively).

The median number of HLA-DR, HLA-DQ and HLA-DR+DQ eplet mismatches was higher among cases than controls (median 17 [interquartile range (IQR): 12, 27] vs. 13 [IQR: 5, 21], 21 [IQR: 12, 27] vs. 15 [IQR: 7, 29] and 37 [IQR: 27, 55] vs. 34 [IQR: 15, 49], respectively), and allowed a higher resolution between cases and controls compared with antigen mismatches (see Table 1). Mean negative predictive values of TG with zero HLA-DQ, HLA-DR and HLA-DR+DQ eplet versus antigen mismatches were 95.1% versus 87.2%, 92.9% versus 93.6% and 97% versus 94.6%, respectively. Tables S1–S4 display the predictive values of TG and frequencies of HLA-DR, HLA-DQ and HLA-DQA eplet mismatches among study participants.

Table 2 displays the results of the conditional logistic regression models evaluating the association between TG and categories of HLA-DR+DQ eplet mismatches based on tertiles of the total number of HLA-DR+DQ eplet mismatches among KTR (each including a third of the study participants). The univariable analysis (Model 1) showed OR of 2.59 (95% CI: 1.09, 6.17; p = 0.032) and 2.14 (95% CI: 0.88, 5.24; p = 0.095) in the presence of 27–43 and >43 HLA-DR+DQ eplet mismatches vs. <27 eplet mismatches, respectively. Multivariable conditional logistic regression models demonstrated an increase in the odds of TG across HLA-DR+DQ eplet mismatch categories. The fully adjusted model (Model 4) estimated...
### Table 1: Baseline characteristics of transplant glomerulopathy patients and matched controls

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 52)</th>
<th>Controls (n = 104)</th>
<th>p-Value</th>
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<tbody>
<tr>
<td>Mean recipient age (years)</td>
<td>43.7 ± 13.5</td>
<td>49.7 ± 12.2</td>
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<td>Recipient sex (%)</td>
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<td>Male</td>
<td>65.4</td>
<td>63.5</td>
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<tr>
<td>Female</td>
<td>34.6</td>
<td>36.5</td>
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</tr>
<tr>
<td>Recipient race (%)</td>
<td></td>
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<tr>
<td>African Canadian</td>
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<td>12.5</td>
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<tr>
<td>Non-African Canadian</td>
<td>96.1</td>
<td>87.5</td>
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<tr>
<td>Cause of ESRD (%)</td>
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<tr>
<td>Diabetes</td>
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<tr>
<td>Non-Diabetes</td>
<td>80.8</td>
<td>84.6</td>
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<td>Median peak panel-reactive antibodies (PRA)</td>
<td>8 (0, 80)</td>
<td>0 (0, 26)</td>
<td>0.12</td>
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<td>Mean donor age (years)</td>
<td>43.38 ± 11.7</td>
<td>45.8 ± 14.3</td>
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<td>Donor sex (%)</td>
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<tr>
<td>Male</td>
<td>40.4</td>
<td>44.2</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>59.6</td>
<td>55.8</td>
<td></td>
</tr>
<tr>
<td>Donor type (%)</td>
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<td>Deceased</td>
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<td>Living</td>
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<td>43.3</td>
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<td>HLA antigen identical living-related donors (%)</td>
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<td>HLA-A antigen mismatches</td>
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<td></td>
<td>0.35</td>
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<td>0 mismatches (%)</td>
<td>11.5</td>
<td>19.2</td>
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<tr>
<td>1 mismatch (%)</td>
<td>46.2</td>
<td>43.3</td>
<td></td>
</tr>
<tr>
<td>2 mismatches (%)</td>
<td>40.4</td>
<td>37.5</td>
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<tr>
<td>Missing (%)</td>
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<td>HLA-B antigen mismatches</td>
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<td>43.3</td>
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<td>2 mismatches (%)</td>
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<td>44.2</td>
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<tr>
<td>Missing (%)</td>
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<td>HLA-DQ antigen mismatches¹</td>
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<td>23.0</td>
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<td>1 mismatch (%)</td>
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<tr>
<td>Median HLA-DQ eplet mismatches²</td>
<td>21 (12, 27)</td>
<td>15 (7, 29)</td>
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<tr>
<td>HLA-DR antigen mismatches³</td>
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<td>0.24</td>
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<tr>
<td>0 mismatches (%)</td>
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<td>42.3</td>
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<tr>
<td>2 mismatches (%)</td>
<td>50.0</td>
<td>40.4</td>
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<tr>
<td>Median HLA-DR eplet mismatches⁴</td>
<td>17 (12, 27)</td>
<td>13 (5, 21)</td>
<td>0.01</td>
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<tr>
<td>HLA-DR + DQ antigen mismatches</td>
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<td>0.22</td>
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<td>0 mismatches (%)</td>
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<td>3 mismatches (%)</td>
<td>26.9</td>
<td>17.3</td>
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<tr>
<td>4 mismatches (%)</td>
<td>19.2</td>
<td>24.0</td>
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<tr>
<td>Median HLA-DR + DQ eplet mismatches</td>
<td>37 (27, 55)</td>
<td>34 (15, 49)</td>
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<td>Transplant era (%)</td>
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<td>2000–2004</td>
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<td>2005–2008</td>
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<td>2009–2012</td>
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<td>First transplant (%)</td>
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<td>Induction therapy (%)</td>
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<td>Lymphocyte depleting</td>
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<td>Nonlymphocyte depleting</td>
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<td>Maintenance immunosuppression (%)</td>
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<td>Cyclosporine</td>
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<td>Delayed graft function (%)</td>
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<td>21.1</td>
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Table 1: Continued

<table>
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<th>Cases (n = 52)</th>
<th>Controls (n = 104)</th>
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<tr>
<td>Acute rejection (%)(^6)</td>
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<td>Yes</td>
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<td>16.3</td>
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<td>No</td>
<td>28.9</td>
<td>83.7</td>
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</tr>
<tr>
<td>Acute antibody-mediated rejection (%)(^6)</td>
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<td>Yes</td>
<td>57.7</td>
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<tr>
<td>No</td>
<td>42.3</td>
<td>95.2</td>
<td></td>
</tr>
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</table>

Continuous variables are summarized using the mean and standard deviation (SD). Nonnormally distributed variables are presented as medians and interquartile ranges (IQR). Categorical variables are presented as proportions.

1Serologic equivalents of HLA-DQB1 chains were considered to quantify HLA-DQ antigen mismatches.

2The number of HLA-DQ eplet mismatches was determined using HLAMatchmaker from amino acid chains coded by HLA-DQA1 and HLA-DQB1 alleles.

3Serologic equivalents of HLA-DRB1 chains were considered to quantify HLA-DR antigen mismatches.

4The number of HLA-DR eplet mismatches was determined using HLAMatchmaker from amino acid chains coded by HLA-DRB1 and HLADR3/4/5 alleles.

5Includes acute cellular rejection and acute antibody-mediated rejection diagnosed in accordance with the Banff classification (44,59,87,88).

6Diagnosed in accordance with the Banff classification in the presence of acute allograft dysfunction, peritubular capillary C4d staining and histopathological features of acute antibody-mediated rejection (44,59,87,88).

Table 2: Odds ratio of transplant glomerulopathy by categories of HLA-DR + DQ eplet mismatches in multivariable conditional logistic regression models

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
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<tbody>
<tr>
<td></td>
<td>OR [95% CI]</td>
<td>p-Value</td>
<td>OR [95% CI]</td>
<td>p-Value</td>
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<td>HLA-DR + DQ eplet mismatch category</td>
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<tr>
<td>&lt;27</td>
<td>Referent</td>
<td></td>
<td>Referent</td>
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<tr>
<td>27–43</td>
<td>2.59 [1.09, 6.17]</td>
<td>0.032</td>
<td>2.41 [0.94, 6.14]</td>
<td>0.064</td>
</tr>
<tr>
<td>&gt;43</td>
<td>2.14 [0.88, 5.24]</td>
<td>0.095</td>
<td>3.54 [1.28, 9.80]</td>
<td>0.015</td>
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<td>HLA-DQ eplet mismatch category</td>
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<tr>
<td>&lt;11</td>
<td>Referent</td>
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<td>Referent</td>
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<tr>
<td>11–21</td>
<td>2.90 [1.20, 6.97]</td>
<td>0.018</td>
<td>3.20 [1.23, 8.76]</td>
<td>0.018</td>
</tr>
<tr>
<td>&gt;21</td>
<td>1.19 [0.48, 3.00]</td>
<td>0.713</td>
<td>1.85 [0.67, 5.12]</td>
<td>0.242</td>
</tr>
<tr>
<td>HLA-DR eplet mismatch category</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;13</td>
<td>Referent</td>
<td></td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>13–27</td>
<td>3.33 [1.43, 7.75]</td>
<td>0.005</td>
<td>3.14 [1.25, 7.86]</td>
<td>0.015</td>
</tr>
<tr>
<td>&gt;27</td>
<td>3.44 [1.43, 8.28]</td>
<td>0.006</td>
<td>4.18 [1.61, 10.83]</td>
<td>0.003</td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, odds ratio.

Model 1: Main exposure.
Model 2: Main exposure, recipient age, recipient sex, peak PRA and recipient race.
Model 3: Main exposure, recipient age, recipient sex, peak PRA, recipient race and donor type.
Model 4: Main exposure, recipient age, recipient sex, peak PRA, recipient race, donor type and induction.
HLA-DR, HLA-DQ and HLA-DR eplet mismatches among study participants as a threshold (see Table 3).

The fully adjusted multivariable conditional logistic regression models showed an OR of 3.21 (95% CI: 1.36, 7.56; p = 0.008), 2.42 (95% CI: 1.03, 5.70; p = 0.043) and 3.64 (95% CI: 1.42, 9.37; p = 0.007) in the presence of >36, >18 and >15 HLA-DR, HLA-DQ and HLA-DR eplet mismatches vs. the referent categories (<36, <18 and <15, respectively).

When the eplet load was modeled as a continuous variable (see Table 4), OR of 1.25 (95% CI: 1.04, 1.50; p = 0.019), 1.63 (95% CI: 1.14, 2.33; p = 0.008) and 1.27 (95% CI: 0.95, 1.69; p = 0.109) were identified for every 10 unit increase in HLA-DR, HLA-DR and HLA-DQ eplet mismatches, respectively.

### Sensitivity analysis
When a process of backward, stepwise, covariate selection was applied to Model 4, a similar relationship was identified between TG and the number of HLA-DR, HLA-DQ and HLA-DR eplet mismatches. This was true whether the primary and secondary exposures were modeled as categorical or continuous variables (see Table 5, Parsimonious Models). In the sample of cases and controls treated with calcineurin inhibitors, we established that the risk for TG, as a function of HLA-DR, HLA-DQ and HLA-DR eplet load, was independent of the choice of calcineurin inhibitor for maintenance immunosuppression (see Table 5, Adjustment for CNI).

The association between TG and HLA-DR and HLA-DQ eplet load was also confirmed when applying HLA-DR and HLA-DQ eplet mismatches as continuous variables (see Table 5, Table 4).

### Table 3: Odds ratio of transplant glomerulopathy by threshold of HLA-DR+DQ eplet mismatch in multivariable conditional logistic regression models

<table>
<thead>
<tr>
<th>Main exposure</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DR+DQ eplet mismatch category</td>
<td>OR [95% CI]</td>
<td>p-Value</td>
<td>OR [95% CI]</td>
<td>p-Value</td>
</tr>
<tr>
<td>&lt;36 Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>≥36 2.01 [1.01, 4.01] 0.047</td>
<td>2.83 [1.27, 6.29] 0.010</td>
<td>3.38 [1.41, 7.95] 0.005</td>
<td>3.21 [1.36, 7.56] 0.008</td>
<td></td>
</tr>
<tr>
<td>HLA-DQ eplet mismatch category</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>&lt;18 Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>≥18 1.50 [0.75, 3.00] 0.252</td>
<td>2.14 [0.98, 4.70] 0.057</td>
<td>2.51 [1.09, 5.78] 0.030</td>
<td>2.42 [1.03, 5.70] 0.043</td>
<td></td>
</tr>
<tr>
<td>HLA-DR eplet mismatch category</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>&lt;15 Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>≥15 2.44 [1.16, 5.12] 0.018</td>
<td>2.81 [1.22, 6.47] 0.015</td>
<td>3.79 [1.48, 9.73] 0.005</td>
<td>3.64 [1.42, 9.37] 0.007</td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, odds ratio.

Model 1: Main exposure. Model 2: Main exposure, recipient age, recipient sex, peak PRA and recipient race. Model 3: Main exposure, recipient age, recipient sex, peak PRA, recipient race and donor type. Model 4: Main exposure, recipient age, recipient sex, peak PRA, recipient race, donor type and induction.

### Table 4: Odds ratio of transplant glomerulopathy per 10 HLA-DR+DQ eplet mismatches in multivariable conditional logistic regression models

<table>
<thead>
<tr>
<th>Main exposure</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DR+DQ eplet mismatches</td>
<td>OR [95% CI]</td>
<td>p-Value</td>
<td>OR [95% CI]</td>
<td>p-Value</td>
</tr>
<tr>
<td>1.11 [0.96, 1.29] 0.157</td>
<td>1.25 [1.05, 1.50] 0.012</td>
<td>1.37 [1.12, 1.68] 0.003</td>
<td>1.25 [1.04, 1.50] 0.019</td>
<td></td>
</tr>
<tr>
<td>HLA-DQ eplet mismatches</td>
<td>1.07 [0.85, 1.35] 0.575</td>
<td>1.29 [0.98, 1.71] 0.076</td>
<td>1.44 [1.05, 1.98] 0.025</td>
<td>1.27 [0.95, 1.69] 0.109</td>
</tr>
<tr>
<td>HLA-DR eplet mismatches</td>
<td>1.38 [1.02, 1.97] 0.034</td>
<td>1.65 [1.16, 2.35] 0.005</td>
<td>1.91 [1.28, 2.85] 0.002</td>
<td>1.63 [1.14, 2.33] 0.008</td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, odds ratio.

Model 1: Main exposure. Model 2: Main exposure, recipient age, recipient sex, peak PRA and recipient race. Model 3: Main exposure, recipient age, recipient sex, peak PRA, recipient race and donor type. Model 4: Main exposure, recipient age, recipient sex, peak PRA, recipient race, donor type and induction.
HLA-DQ eplet load thresholds previously identified as predictive of DSA development (41). The fully adjusted models demonstrated an OR of TG of 2.97 (95% CI: 1.23, 7.19) and 4.78 (95% CI: 1.75, 13.04) in the \( >10 \) HLA-DR and \( >17 \) HLA-DQ eplet mismatches versus the referent categories, respectively.

Post hoc sensitivity analyses showed that both HLA-DRB1 and HLA-DRB3/4/5 related eplet mismatches were independently associated with TG (see Table 6). From Tables S2 and S5, it is apparent that some of the immunogenic eplet mismatches associated with HLA-DR in our study (i.e. 67LR, 71RRA, 48YQ, 4Q, 18L, 12AKC, 14CEH, 16HLW, 26WN, 41YNL, 81YV, 96QM, 180MM, 187Q, 71DRA and 32IYN) were shared by the amino acid chains coded by HLA-DRB1 and -DRB3/4/5 alleles (i.e. 67LR, 71RRA, 4Q, 71DRA and 32IYN) and were deemed immunogenic previously (i.e. 4Q and 71DRA) (37,41). In contrast, some previously reported immunogenic HLA-DQ eplet mismatches (37,41) appeared more frequently among the controls (see Tables S3 and S4).

**Discussion**

We conducted a nested case–control study to assess the relationship between HLA-DR + DQ eplet load and development of TG. Our analysis established that HLA-DR + DQ eplet load, defined as the sum of HLA-DR and HLA-DQ eplets, is a risk factor for TG. The risk was independent of the type of induction therapy or calcineurin inhibitor used for treatment.
maintenance immunosuppression. When modeled as a continuous variable, HLA-DR eplet load appeared to confer a greater risk for TG per number of eplet mismatches compared with HLA-DQ.

Our study is the first to link donor–recipient incompatibility at the eplet level with TG (4–7,59). There has been a growing recognition that HLA antibodies are directed toward structurally defined HLA epitopes (60) rather than HLA antigens (21,25,26). Duquesnoy et al (37), for example, showed that patients with DRB1/3/4/5 antibodies were exposed to twice as many mismatched eplets as those who did not show antibodies (21.4 ± 8.0 vs. 10.6 ± 7.8, p < 0.0001). More recently, Wiebe et al (41) showed a similar association between the number of locus-specific epitope mismatches and the development of de novo anti-HLA-DR and anti-HLA-DQ antibodies. In our study, the risk for TG as a function of eplet load persisted when we applied the HLA-DR and HLA-DQ eplet load thresholds identified by Wiebe et al (41).

The optimal strategy to classify the risk of immune-mediated injury (e.g. TG) to kidney allografts associated with HLA-DR and HLA-DQ eplets is unknown. For this reason, we evaluated several strategies to classify this exposure. We confirmed the presence of a dose–response relationship between HLA-DR + DQ and HLA-DR eplet load when the exposure was modeled both as a binary variable and a three-level categorical variable based on eplet load tertiles. In contrast, while HLA-DQ eplet load conferred a significantly increased risk for TG when the eplet load was modeled as a binary variable, when it was modeled as tertiles, the OR estimate in the presence of >21 eplet mismatches (vs. the referent category of <11 eplet mismatches) was lower than the OR in the presence of 11–21 HLA-DQ eplet mismatches and did not reach statistical significance. Our inability to observe a dose–response relationship between TG and HLA-DQ eplet load categories defined by tertiles may be related to the relatively small sample size. Alternatively, the immunogenicity (the ability to induce an antibody response) and antigenicity (the reactivity with specific antibodies) of particular eplet contributing to the eplet load may play a role (37,41).

The importance of eplet immunogenicity was evaluated by Kosmolaptsis et al (61), who reported that, in addition to the number of polymorphic amino acid mismatches, physicochemical properties (hydrophobicity and electrostatic mismatch) of recipients’ HLA type were strong predictors of class II alloantigen immunogenicity. Interestingly, a higher immunogenic potential (i.e. greater likelihood of antibody response and higher mean fluorescence intensities) was found per amino acid mismatch with HLA-DR versus HLA-DQ. Studies conducted in an era preceding DQ typing and antibody detection also found that HLA-DR compatibility, at the antigen level, carried the greatest influence on allograft outcomes (21,26). In a more recent study, two mismatches in HLA-DR51, -DR52 and -DR53 alleles conferred a greater risk of subclinical antibody-mediated rejection among desensitization patients (62). Similarly, in re-transplant candidates greater DSA reactivity was observed in response to mismatches in HLA-DR53 followed by -DR51 and -DR52 and less so with HLA-DRB1 mismatches (37). We confirmed an independent association between TG and both HLA-DRB1 and HLA-DRB3/4/5 eplet mismatches. We also established that some of the most commonly observed HLA-DR related eplet mismatches among cases were shared by the HLA-DRB1 and HLA-DRB3/4/5 chains and associated with DSA development in prior studies (37,41). These findings suggest that varying immunogenicity/antigenicity across eplets and shared mismatches may contribute to antibody-mediated injury in kidney allografts.

Absence of a dose–response relationship between HLA-DQ eplet load and TG is curious considering the dominance of anti-HLA-DQ antibodies among HLA class II de novo DSA (25,56,63–65). This observation does not necessarily challenge (25,66) the role of HLA-DQ eplet mismatches in the development of TG; but rather sheds lights on the potential diversity of eplet immunogenicity and the unique interactions of HLA-DQB/-DQA chains (in contrast to the single polymorphic HLA-DRB1 chain) with antibodies. While a greater eplet load may indicate a greater risk for experiencing antibody-mediated injuries from de novo DSA, it is the presence (or absence) of highly immunogenic eplet mismatches that help refine this risk. Furthermore, eplet-based matching considers the polymorphisms in both HLA-DQB/-DQA chains (unlike standard antigen-based matching which is limited to HLA-DQB1); yet, the current paradigm, which considers each HLA-DQB/-DQA chain to independently interact with antibodies might misrepresent the true nature of certain HLA-DQ epitope-antibody interactions (67). Population level studies are required to obtain sufficient statistical power to elucidate the immunogenicity/antigenicity of individual (and combinations of) HLA-DQ eplets and their effect on TG.

With the introduction of more potent immunosuppressive drugs, the difference in survival between well-matched and poorly-matched allografts has decreased, although fully HLA-matched donor–recipient combinations still provide the best results (68). Our study demonstrated that the risk for TG as a function of eplet load persisted even when adjusting for the choice of induction therapy and calcineurin inhibitors. Since overt TG is considered irreversible (69), with even complement inhibition being shown to be ineffective (5), minimization of HLA class II incompatibility (by avoiding highly immunogenic eplet mismatches) may be considered in future organ allocation schemes as a strategy for TG prevention.

Though the risk for immune-mediated allograft injury may be recognized prior to transplantation in the form of preformed HLA class II DSA (63,70,71) and positive cross-

matches (47), anti-class II DSA may also appear post-
transplant (72–75). Knowledge of the eplet load and the
immunogenicity of particular eplets, may be used for risk
stratification and inform personalized surveillance (DSA
monitoring and surveillance biopsies) and therapeutic
regimens. Whether such a strategy will allow timely
recognition of impending immune-mediated injury and
facilitate TG prevention is yet to be determined.

To our knowledge, this is the first study to establish a link
between HLA-DR + DQ eplet load and the risk of TG. The
association was consistent across analytical methods and
exposure metrics (i.e. continuous and categorical vari-
able). Despite these advantages, some limitations should
be noted. First, a common challenge of case–control
studies relates to a difficulty ensuring an unbiased selection
of controls. In our case, the validity of control selection is
increased since we can clearly identify the source
population from which cases are derived. Second, cases
may not be representative of all disease-related cases.
Specifically, patients with a clinical presentation typical for
TG might not be referred for a diagnostic renal biopsy
because of physician or patient preferences. Since the
clinical practice of kidney transplant physicians at University
Health Network has been to refer patients for confirmatory
kidney allograft biopsies in the setting of kidney dysfunction
or new-onset proteinuria, this concern is minimized. Our
capacity to diagnose subclinical TG, however, was impaired
by the absence of a surveillance biopsy program at our
transplant center. As a result, we may have missed earlier
cases of TG. Nevertheless, the results of our study would
be applicable to cases of biopsy-confirmed TG associated
with clinical features (i.e. graft dysfunction and/or protein-
uria). Third, misclassification of the exposure may also be
possible since HLA-DP typing was not conducted. HLA-DP
antibodies, however, are considered to be less immuno-
logically relevant in first-time KTR (76–78), which reflect the
majority of our study patients. Furthermore, Wiebe et al (41)
found that DP eplet mismatches were not associated with
the development of anti-HLA-DP de novo DSA. Fourth, as
with any observational study, unmeasured confounders,
are possible. For example, we could not account for
suboptimal exposure to maintenance immunosuppression
as a consequence of nonadherence (74,79), under-dosing,
excessive metabolism, abnormal absorption (80–82) and
drug interactions (42,83–86). It is worth noting, however,
that differential exposure to immunosuppressants is
unlikely, considering neither transplant nephrologists nor
patients were aware of the eplet load. Finally, this is a single
center study and its results may not be generalizable to
other centers.

In conclusion, our study identifies HLA-DR + DQ eplet load
as an independent risk factor for TG, a prognostically
important and therapeutically challenging disease pro-
cess. Future multicenter cohort studies evaluating the
relationship between HLA-DR + DQ eplet mismatches and the
time to developing TG on surveillance biopsies
should be conducted to confirm our findings. Population
level studies are also required to elucidate clinically
relevant thresholds of eplet load and the role of
immunogenicity/antigenicity of HLA-DR + DQ eplets in
determining kidney allograft outcomes. A better under-
standing of the immunogenicity and antigenicity of eplets
is required prior to applying eplet-based matching
strategies to identify compatible donor-recipient pairs
and guide personalized therapeutic strategies. The
diagnostic characteristics and discriminative value of
eplet mismatches compared to antigen mismatches in
relation to TG also warrant further study.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1: Positive and negative predictive values of transplant glomerulopathy on indication biopsies by thresholds of HLA-DR (A), HLA-DQ (B) and HLA-DR+DQ (C) antigen and eplet mismatches.

Table S2: Distribution of HLA-DR eplet mismatches among cases and controls in our study sample versus prior publications.

Table S3: Distribution of HLA-DQB eplet mismatches among cases and controls in our study sample versus prior publications.

Table S4: Distribution of HLA-DQA eplet mismatches among cases and controls in our study sample versus prior publications.

Table S5: Distribution of eplet mismatches shared by HLA-DRB1 and HLA-DRB3/4/5 among cases and controls.