American Journal of Transplantation 2015; 15: 137–148
Wiley Periodicals Inc.

doi: 10.1111/ajt.12968

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

R. Sapir-Pichhadze[1,2,3,*,†] K. Tinckam[1,4], K. Quach[1,5], A.G. Logan[1,2,3], A. Laupacis[2,6], R. John[4], J. Beyene[2,6,7] and S.J. Kim[1,2,3,8]

[1]*Division of Nephrology and The Kidney Transplant Program, Toronto General Hospital, University Health Network, Toronto, Ontario, Canada*
[2]*Institute of Health Policy, Management and Evaluation, University of Toronto, Toronto, Ontario, Canada*
[3]*Department of Medicine, Division of Nephrology, University of Toronto, Toronto, Ontario, Canada*
[4]*Departments of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada*
[5]*Department of Clinical Epidemiology & Biostatistics, McMaster University, Hamilton, Ontario, Canada*
[6]*Li KaShing Knowledge Institute of St. Michael's Hospital, University of Toronto, Toronto, Ontario, Canada*
[7]*Population Health Sciences, Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada*
[8]*Division of Nephrology and The Renal Transplant Program, St. Michael's Hospital, Toronto, Ontario, Canada*
[*]*Corresponding author: Ruth Sapir-Pichhadze, ruth.sapir-pichhadze@mcgill.ca*
[†]*Present address: Division of Nephrology and Multi-Organ Transplant Program, Department of Medicine, McGill University, Royal Victoria Hospital, McGill University Health Centre, Montreal, Quebec, Canada*

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

Received 16 April 2014, revised 04 August 2014 and accepted for publication 05 August 2014

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 (CoReTRIS) (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® HD SSO; 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 DRDQ 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 (53–57). 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

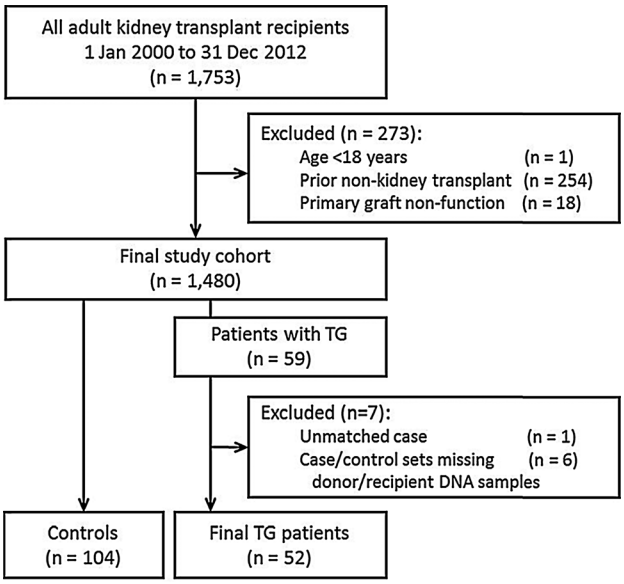

Figure 1: Study flow diagram.

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-DQB 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

	Cases (n = 52)	Controls (n = 104)	p-Value
Mean recipient age (years)	43.7 ± 13.5	49.7 ± 12.2	0.01
Recipient sex (%)			0.81
Male	65.4	63.5	
Female	34.6	36.5	
Recipient race (%)			0.15
African Canadian	3.9	12.5	
Non-African Canadian	96.1	87.5	
Cause of ESRD (%)			0.54
Diabetes	19.2	15.4	
Non-Diabetes	80.8	84.6	
Median peak panel-reactive antibodies (PRA)	8 (0, 80)	0 (0, 26)	0.12
Mean donor age (years)	43.38 ± 11.7	45.8 ± 14.3	0.40
Donor sex (%)			0.65
Male	40.4	44.2	
Female	59.6	55.8	
Donor type (%)			0.02
Deceased	36.5	56.7	
Living	63.5	43.3	
HLA antigen identical living-related donors (%)	0.0	6.7	0.10
HLA-A antigen mismatches			0.35
0 mismatches (%)	11.5	19.2	
1 mismatch (%)	46.2	43.3	
2 mismatches (%)	40.4	37.5	
Missing (%)	1.9	0.0	
HLA-B antigen mismatches			0.16
0 mismatches (%)	7.7	12.5	
1 mismatch (%)	32.7	43.3	
2 mismatches (%)	57.7	44.2	
Missing (%)	1.9	0.0	
HLA-DQ antigen mismatches[1]			0.61
0 mismatches (%)	23.0	24.0	
1 mismatch (%)	55.8	48.1	
2 mismatches (%)	21.2	27.9	
Median HLA-DQ eplet mismatches[2]	21 (12, 27)	15 (7, 29)	0.32
HLA-DR antigen mismatches[3]			0.24
0 mismatches (%)	7.7	17.3	
1 mismatch (%)	42.3	42.3	
2 mismatches (%)	50.0	40.4	
Median HLA-DR eplet mismatches[4]	17 (12, 27)	13 (5, 21)	0.01
HLA-DR + DQ antigen mismatches			0.22
0 mismatches (%)	5.8	15.4	
1 mismatch (%)	13.5	7.7	
2 mismatches (%)	34.6	35.6	
3 mismatches (%)	26.9	17.3	
4 mismatches (%)	19.2	24.0	
Median HLA-DR + DQ eplet mismatches	37 (27, 55)	34 (15, 49)	0.04
Transplant era (%)			1.00
2000–2004	53.9	53.9	
2005–2008	38.4	38.4	
2009–2012	7.7	7.7	
First transplant (%)	84.6	88.5	0.50
Induction therapy (%)			0.25
Lymphocyte depleting	63.5	53.9	
Nonlymphocyte depleting	36.5	46.1	
Maintenance immunosuppression (%)			0.70
Tacrolimus	53.1	45.7	
Cyclosporine	47.0	54.3	
mTOR inhibitor	3.9	3.0	
Delayed graft function (%)			0.17
Yes	21.1	31.7	

Table 1: Continued

	Cases (n = 52)	Controls (n = 104)	p-Value
No	78.9	68.3	
Acute rejection (%)[5]			<0.001
Yes	71.1	16.3	
No	28.9	83.7	
Acute antibody-mediated rejection (%)[6]			<0.001
Yes	57.7	4.8	
No	42.3	95.2	

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.
[1]Serologic equivalents of HLA-DQB1 chains were considered to quantify HLA-DQ antigen mismatches.
[2]The number of HLA-DQ eplet mismatches was determined using HLAMatchmaker from amino acid chains coded by HLA-DQA1 and HLA-DQB1 alleles.
[3]Serologic equivalents of HLA-DRB1 chains were considered to quantify HLA-DR antigen mismatches.
[4]The number of HLA-DR eplet mismatches was determined using HLAMatchmaker from amino acid chains coded by HLA-DRB1 and HLADRB3/4/5 alleles.
[5]Includes acute cellular rejection and acute antibody-mediated rejection diagnosed in accordance with the Banff classification (44,59,87,88).
[6]Diagnosed 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).

an OR of 2.84 (95% CI: 1.03, 7.84; p = 0.043) and 4.62 (95% CI: 1.51, 14.14; p = 0.007) in the presence of 27–43 and >43 HLA-DR + DQ related eplet mismatches versus <27 eplet mismatches, respectively.

Multivariable conditional logistic regression models also demonstrated an increase in the odds of TG across HLA-DR eplet load categories divided by tertiles. Model 4 showed an OR of 3.61 (95% CI: 1.33, 9.80; p = 0.012) and 5.61 (95% CI: 1.87, 16.93; p = 0.002) in the presence of 13–27 and >27 HLA-DR eplet mismatches versus <13 HLA-DR eplet mismatches. Multivariable conditional logistic regression models evaluating the risk of TG as a function of categories of HLA-DQ eplet load divided by tertiles demonstrated an OR of 3.72 (95% CI: 1.33, 10.43; p = 0.012) and 2.39 (95% CI: 0.79, 7.21; p = 0.124) in the presence of 11–21 and >21 HLA-DQ eplet mismatches (vs. <11 HLA-DQ eplet mismatches), respectively.

The relationship between TG and the number of eplet mismatches persisted when the eplet load was modeled as a binary variable using the median number of

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

	Model 1		Model 2		Model 3		Model 4	
Main exposure	OR [95% CI]	p-Value	OR [95% CI]	p-Value	OR [95% CI]	p-Value	OR [95% CI]	p-Value
HLA-DR + DQ eplet mismatch category								
<27	Referent		Referent		Referent		Referent	
27–43	2.59 [1.09, 6.17]	0.032	2.41 [0.94, 6.14]	0.064	3.00 [1.10, 8.14]	0.030	2.84 [1.03, 7.84]	0.043
>43	2.14 [0.88, 5.24]	0.095	3.54 [1.28, 9.80]	0.015	4.89 [1.61, 14.86]	0.005	4.62 [1.51, 14.14]	0.007
HLA-DQ eplet mismatch category								
<11	Referent		Referent		Referent		Referent	
11–21	2.90 [1.20, 6.97]	0.018	3.20 [1.23, 8.76]	0.018	3.98 [1.43, 11.10]	0.008	3.72 [1.33, 10.43]	0.012
>21	1.19 [0.48, 3.00]	0.713	1.85 [0.67, 5.12]	0.242	2.52 [0.84, 7.51]	0.100	2.39 [0.79, 7.21]	0.124
HLA-DR eplet mismatch category								
<13	Referent		Referent		Referent		Referent	
13–27	3.33 [1.43, 7.75]	0.005	3.14 [1.25, 7.86]	0.015	3.71 [1.37, 10.04]	0.010	3.61 [1.33, 9.80]	0.012
>27	3.44 [1.43, 8.28]	0.006	4.18 [1.61, 10.83]	0.003	6.20 [2.05, 18.73]	0.001	5.61 [1.87, 16.93]	0.002

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

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

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 + DQ, 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 + DQ, 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 + DQ, 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 + DQ, 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 + DQ, 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

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

	Model 1		Model 2		Model 3		Model 4	
Main exposure	OR [95% CI] per 10 eplet mismatches	p-Value	OR [95% CI] per 10 eplet mismatches	p-Value	OR [95% CI] per 10 eplet mismatches	p-Value	OR [95% CI] per 10 eplet mismatches	p-Value
HLA-DR + DQ eplet mismatches	1.11 [0.96, 1.29]	0.157	1.25 [1.05 1.50]	0.012	1.37 [1.12, 1.68]	0.003	1.25 [1.04, 1.50]	0.019
HLA-DQ eplet mismatches	1.07 [0.85, 1.35]	0.575	1.29 [0.98, 1.71]	0.076	1.44 [1.05, 1.98]	0.025	1.27 [0.95, 1.69]	0.109
HLA-DR eplet mismatches	1.38 [1.02, 1.87]	0.034	1.65 [1.16, 2.35]	0.005	1.91 [1.28, 2.85]	0.002	1.63 [1.14, 2.33]	0.008

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 5: Sensitivity analyses

Main exposure	HLA-DR + DQ eplet mismatches		HLA-DQ eplet mismatches		HLA-DR eplet mismatches	
	Parsimonious model OR [95% CI]	Adjustment for CNI OR [95% CI]	Parsimonious model OR [95% CI]	Adjustment for CNI OR [95% CI]	Parsimonious model OR [95% CI]	Adjustment for CNI OR [95% CI]
Eplet mismatch categories (tertiles)						
Category 2 vs. referent	2.81 [1.04, 7.60][1]	2.68 [1.00, 7.42][2]	3.62 [1.35, 9.72][3]	3.50 [1.27, 9.63][2]	4.28 [1.61, 11.39][4]	3.58 [1.29, 9.95][2]
Category 3 vs. referent	4.27 [1.43, 12.81][1]	4.61 [1.49, 14.21][2]	1.99 [0.71, 5.52][3]	2.27 [0.76, 6.80][2]	6.89 [2.27, 20.94][4]	5.83 [1.89, 17.99][2]
Threshold of eplet mismatch (median)	3.17 [1.36, 7.40][5]	3.07 [1.31, 7.18][2]	2.30 [0.99, 5.33][5]	2.27 [1.00, 5.29][2]	3.77 [1.48, 9.58][6]	3.77 [1.42, 9.96][2]
10 eplet mismatches	1.37 [1.12, 1.68][1]	1.24 [1.03, 1.49][2]	1.36 [1.00, 1.85][5]	1.24 [0.93, 1.65][2]	1.92 [1.29, 2.86][7]	1.67 [1.15, 2.42][2]

CNI, calcineurin inhibitor; CI, confidence interval; PRA, panel reactive antibodies; OR, odds ratio.
[1]Model covariates: recipient age, recipient race, peak PRA and donor type.
[2]Model covariates: recipient age, recipient sex, peak PRA, recipient race, donor type, induction and CNI.
[3]Model covariates: recipient race and donor type.
[4]Model covariates: peak PRA and donor type.
[5]Model covariates: recipient age, recipient race, donor type and induction.
[6]Model covariates: recipient race, peak PRA, donor type and induction.
[7]Model covariates: recipient race, peak PRA and donor type.

Table 6: Odds ratio of transplant glomerulopathy as a function of HLA-DRB1 and HLA-DRB3/4/5 eplet mismatches in multivariable conditional logistic regression models

Main exposure	OR [95% CI]	p-Value
HLA-DRB1		
Eplet mismatch categories (tertiles)		
<8	Referent	
8–13	5.70 [1.92, 16.94]	0.002
>13	4.89 [1.53, 15.62]	0.007
Threshold of eplet mismatch (median)		
<9	Referent	
≥9	2.95 [1.23, 7.12]	0.016
Per eplet mismatch	1.07 [1.01, 1.14]	0.034
HLA-DRB3/4/5		
Eplet mismatch categories (tertiles)		
<2	Referent	
2–6	2.79 [1.13, 6.90]	0.027
>6	3.35 [1.09, 10.30]	0.035
Threshold of eplet mismatch (median)		
<3	Referent	
≥3	6.03 [2.08, 17.47]	0.001
Per eplet mismatch	1.09 [0.98, 1.22]	0.124

CI, confidence interval; OR, odds ratio.
Model covariates: 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

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 Kosmoliaptsis et al (61), who reported that, in addition to the number of polymorphic amino acid mismatches, physiochemical 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 pre-formed 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 variables). 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 proteinuria). 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 immunologically 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 process. 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 understanding 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.

Acknowledgments

We would like to thank Yanhong Li, Segun Famure and the students of the Multi-Organ Transplant Student Research Training Program for collecting, entering and auditing data for the Comprehensive Renal Transplant Research Information System (CoReTRIS) at the Toronto General Hospital, University Health Network. We would also like to thank Marilyn Marrari for her assistance with HLAMatchmaker. RS-P is funded by the Eliot Phillipson Clinician Scientist Training Program and the Edward Christie Stevens Fellowship, Department of Medicine, University of Toronto, Toronto, Ontario, Canada. AL is the recipient of a Canada Research Chair in Health Policy and Citizen Engagement. This study was funded by The Physicians' Services Inc. Foundation, Ontario, Canada.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

References

1. Matas AJ, Smith JM, Skeans MA, et al. OPTN/SRTR 2012 annual data report: Kidney. Am J Transplant 2014; 14: 11–44.
2. Kaplan B, Meier-Kriesche HU. Death after graft loss: An important late study endpoint in kidney transplantation. Am J Transplant 2002; 2: 970–974.
3. Laupacis A, Keown P, Pus N, et al. A study of the quality of life and cost-utility of renal transplantation. Kidney Int 1996; 50: 235–242.
4. Cosio FG, Gloor JM, Sethi S, Stegall MD. Transplant glomerulopathy. Am J Transplant 2008; 8: 492–496.
5. Husain S, Sis B. Advances in the understanding of transplant glomerulopathy. Am J Kidney Dis 2013; 62: 352–363.
6. Sis B, Campbell PM, Mueller T, et al. Transplant glomerulopathy, late antibody-mediated rejection and the ABCD tetrad in kidney allograft biopsies for cause. Am J Transplant 2007; 7: 1743–1752.
7. El-Zoghby ZM, Stegall MD, Lager DJ, et al. Identifying specific causes of kidney allograft loss. Am J Transplant 2009; 9: 527–535.
8. Haas M, Sis B, Racusen LC, et al. Banff 2013 meeting report: Inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. Am J Transplant 2014; 14: 272–283.
9. Haas M. Transplant glomerulopathy: It's not always about chronic rejection. Kidney Int 2011; 80: 801–803.

10. Gloor JM, Sethi S, Stegall MD, et al. Transplant glomerulopathy: Subclinical incidence and association with alloantibody. Am J Transplant 2007; 7: 2124–2132.
11. Habib R, Broyer M. Clinical significance of allograft glomerulopathy. Kidney Int Suppl 1993; 43: S95–S98.
12. Sijpkens YW, Joosten SA, Wong MC, et al. Immunologic risk factors and glomerular C4d deposits in chronic transplant glomerulopathy. Kidney Int 2004; 65: 2409–2418.
13. Suri DL, Tomlanovich SJ, Olson JL, Meyer TW. Transplant glomerulopathy as a cause of late graft loss. Am J Kidney Dis 2000; 35: 674–680.
14. Kim MG, Kim YJ, Kwon HY, et al. Outcomes of combination therapy for chronic antibody-mediated rejection in renal transplantation. Nephrology (Carlton) 2013; 18: 820–826.
15. Stegall MD, Diwan T, Raghavaiah S, et al. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. Am J Transplant 2011; 11: 2405–2413.
16. Jordan SC, Reinsmoen N, Lai CH, Vo A. Novel immunotherapeutic approaches to improve rates and outcomes of transplantation in sensitized renal allograft recipients. Discov Med 2012; 13: 235–245.
17. Bartel G, Schwaiger E, Bohmig GA. Prevention and treatment of alloantibody-mediated kidney transplant rejection. Transpl Int 2011; 24: 1142–1155.
18. Lee PC, Terasaki PI, Takemoto SK, et al. All chronic rejection failures of kidney transplants were preceded by the development of HLA antibodies. Transplantation 2002; 74: 1192–1194.
19. Gaston RS. Our evolving understanding of late kidney allograft failure. Current Opin Organ Transplant 2011; 16: 594–599.
20. Gaston RS, Cecka JM, Kasiske BL, et al. Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. Transplantation 2010; 90: 68–74.
21. Gilks WR, Bradley BA, Gore SM, Klouda PT. Substantial benefits of tissue matching in renal transplantation. Transplantation 1987; 43: 669–674.
22. Worthington JE, Martin S, Al-Husseini DM, Dyer PA, Johnson RW. Posttransplantation production of donor HLA-specific antibodies as a predictor of renal transplant outcome. Transplantation 2003; 75: 1034–1040.
23. Eng HS, Bennett G, Chang SH, et al. Donor human leukocyte antigen specific antibodies predict development and define prognosis in transplant glomerulopathy. Hum Immunol 2011; 72: 386–391.
24. Nair R, Fraer M, Agrawal N, Suneja M. Acute transplant glomerulopathy is associated with antibody-mediated rejection and poor graft outcome. Transplant Proc 2010; 42: 3507–3512.
25. Willicombe M, Brookes P, Sergeant R, et al. De-novo DQ donor-specific antibodies are associated with a significant risk of antibody-mediated rejection and transplant glomerulopathy. Transplantation 2012; 94: 172–177.
26. Ting A, Morris PJ. Powerful effect of HL-DR matching on survival of cadaveric renal allografts. Lancet 1980; 2: 282–285.
27. Freedman BI, Thacker LR, Heise ER, Adams PL. HLA-DQ matching in cadaveric renal transplantation. Clin Transplant 1997; 11: 480–484.
28. Hsia S, Tong JY, Parris GL, et al. Molecular compatibility and renal graft survival—The HLA DRB1 genotyping. Transplantation 1993; 55: 395–399.
29. Colvin RB, Hirohashi T, Farris AB, Minnei F, Collins AB, Smith RN. Emerging role of B cells in chronic allograft dysfunction. Kidney Int Suppl 2010; 119: S13–S17.
30. Issa N, Cosio FG, Gloor JM, et al. Transplant glomerulopathy: Risk and prognosis related to anti-human leukocyte antigen class II antibody levels. Transplantation 2008; 86: 681–685.
31. Duquesnoy RJ. A structurally based approach to determine HLA compatibility at the humoral immune level. Hum Immunol 2006; 67: 847–862.
32. Brooks EG, MacPherson BR, Fung MK. Validation of HLAMatchmaker algorithm in identifying acceptable HLA mismatches for thrombocytopenic patients refractory to platelet transfusions. Transfusion 2008; 48: 2159–2166.
33. Claas FH, Dankers MK, Oudshoorn M, et al. Differential immunogenicity of HLA mismatches in clinical transplantation. Transpl Immunol 2005; 14: 187–191.
34. Duquesnoy RJ. ''Match and treat'': An effective strategy for transplanting highly sensitized pediatric transplant candidates? Pediatr Transplant 2007; 11: 3–4.
35. Duquesnoy RJ, Marrari M. Determination of HLA-A,B residue mismatch acceptability for kidneys transplanted into highly sensitized patients: A report of a collaborative study conducted during the 12th International Histocompatibility Workshop. Transplantation 1997; 63: 1743–1751.
36. Duquesnoy RJ, Askar M. HLAMatchmaker: A molecularly based algorithm for histocompatibility determination. V. Eplet matching for HLA-DR, HLA-DQ, and HLA-DP. Hum Immunol 2007; 68: 12–25.
37. Duquesnoy RJ, Awadalla Y, Lomago J, et al. Retransplant candidates have donor-specific antibodies that react with structurally defined HLA-DR,DQ,DP epitopes. Transpl Immunol 2008; 18: 352–360.
38. Duquesnoy RJ, Marrari M. Correlations between Terasaki's HLA class I epitopes and HLAMatchmaker-defined eplets on HLA-A, -B and -C antigens. Tissue Antigens 2009; 74: 117–133.
39. Marrari M, Duquesnoy RJ. Correlations between Terasaki's HLA class II epitopes and HLAMatchmaker-defined eplets on HLA-DR and -DQ antigens. Tissue Antigens 2009; 74: 134–146.
40. Duquesnoy RJ, Marrari M. HLAMatchmaker-based definition of structural human leukocyte antigen epitopes detected by alloantibodies. Curr Opin Organ Transplant 2009; 14: 403–409.
41. Wiebe C, Pochinco D, Blydt-Hansen TD, et al. Class II HLA epitope matching—A strategy to minimize de-novo donor-specific antibody development and improve outcomes. Am J Transplant 2013; 13: 3114–3122.
42. Sapir-Pichhadze R, Wang Y, Famure O, Li Y, Kim SJ. Time-dependent variability in tacrolimus trough blood levels is a risk factor for late kidney transplant failure. Kidney Int 2014; 85: 1404–1411.
43. Famure O, Phan N, Kim S. Health information management for research and quality assurance: The Comprehensive Renal Transplant Research Information System (CoReTRIS). Health Care Manage Forum 2014; 27: 30–36.
44. Solez K, Colvin RB, Racusen LC, et al. Banff '05 Meeting Report: Differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). Am J Transplant 2007; 7: 518–526.
45. Rothman KJ, Greenland S, Lash TL. *Modern epidemiology*. Philadelphia, PA: Lippincott Williams & Wilkins, 2008.
46. Cano P, Klitz W, Mack SJ, et al. Common and well-documented HLA alleles: Report of the Ad-Hoc committee of the american society for histocompatiblity and immunogenetics. Hum Immunol 2007; 68: 392–417.
47. Tinckam K. Histocompatibility methods. Transplant Rev (Orlando) 2009; 23: 80–93.

48. Mack SJ, Cano P, Hollenbach JA, et al. Common and well-documented HLA alleles: 2012 update to the CWD catalogue. Tissue Antigens 2013; 81: 194–203.
49. Cano P, Mack SJ, Fernandez-Vina M. The CWD allele catalog in China. Hum Immunol 2012; 73: 60.
50. Maiers M, Gragert L, Klitz W. High-resolution HLA alleles and haplotypes in the United States population. Hum Immunol 2007; 68: 779–788.
51. Robinson J, Waller MJ, Parham P, et al. IMGT/HLA and IMGT/MHC: Sequence databases for the study of the major histocompatibility complex. Nucleic Acids Res 2003; 31: 311–314.
52. Gonzalez-Galarza FF, Christmas S, Middleton D, Jones AR. Allele frequency net: A database and online repository for immune gene frequencies in worldwide populations. Nucleic Acids Res 2011; 39: D913–D919.
53. Navarrete C, Jaraquemada D, Fainboim L, et al. Genetic and functional relationship of the HLA-DR and HLA-DQ antigens. Immunogenetics 1985; 21: 97–101.
54. Fernandez-Vina M, Moraes JR, Moraes ME, Miller S, Stastny P. HLA class II haplotypes in Amerindians and in black North and South Americans. Tissue Antigens 1991; 38: 235–237.
55. Fuggle SV, Carter C, Watts F, Kirkley J, Morris PJ. Monoclonal antibody definition of multiple polymorphic epitopes on human leukocyte antigen-DRw52. Hum Immunol 1987; 20: 249–264.
56. Klitz W, Maiers M, Spellman S, et al. New HLA haplotype frequency reference standards: High-resolution and large sample typing of HLA DR-DQ haplotypes in a sample of European Americans. Tissue Antigens 2003; 62: 296–307.
57. Fernandez-Vina MA, Gao XJ, Moraes ME, et al. Alleles at four HLA class II loci determined by oligonucleotide hybridization and their associations in five ethnic groups. Immunogenetics 1991; 34: 299–312.
58. Biesheuvel CJ, Vergouwe Y, Oudega R, Hoes AW, Grobbee DE, Moons KG. Advantages of the nested case–control design in diagnostic research. BMC Med Res Methodol 2008; 8: 48.
59. Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: Updates and future directions. Am J Transplant 2008; 8: 753–760.
60. Haririan A, Fagoaga O, Daneshvar H, et al. Predictive value of human leucocyte antigen epitope matching using HLAMatchmaker for graft outcomes in a predominantly African-American renal transplant cohort. Clin Transplant 2006; 20: 226–233.
61. Kosmoliaptsis V, Sharples LD, Chaudhry AN, Halsall DJ, Bradley JA, Taylor CJ. Predicting HLA class II alloantigen immunogenicity from the number and physiochemical properties of amino acid polymorphisms. Transplantation 2011; 91: 183–190.
62. Kraus ES, Parekh RS, Oberai P, et al. Subclinical rejection in stable positive crossmatch kidney transplant patients: Incidence and correlations. Am J Transplant 2009; 9: 1826–1834.
63. Iniotaki-Theodoraki AG, Boletis JN, Trigas G, Kalogeropoulou HG, Kostakis AG, Stavropoulos-Giokas CG. Humoral immune reactivity against human leukocyte antigen (HLA)-DQ graft molecules in the early posttransplantation period. Transplantation 2003; 75: 1601–1603.
64. Tambur AR, Leventhal JR, Friedewald JJ, Ramon DS. The complexity of human leukocyte antigen (HLA)-DQ antibodies and its effect on virtual crossmatching. Transplantation 2010; 90: 1117–1124.
65. Devos JM, Gaber AO, Knight RJ, et al. Donor-specific HLA-DQ antibodies may contribute to poor graft outcome after renal transplantation. Kidney Int 2012; 82: 598–604.
66. Kobayashi T, Maruya E, Niwa M, et al. Significant association between chronic antibody-mediated rejection and donor-specific antibodies against HLA-DRB rather than DQB in renal transplantation. Hum Immunol 2011; 72: 11–17.
67. Tambur AR, Rosati J, Roitberg S, Glotz D, Friedewald JJ, Leventhal JR. Epitope analysis of HLA-DQ antigens: What does the antibody see? Transplantation 2014; 98: 157–166.
68. Tambur AR, Claas FH. Toward HLA epitope matching in clinical transplantation. Am J Transplant 2013; 13: 3059–3060.
69. Haas M, Mirocha J. Early ultrastructural changes in renal allografts: Correlation with antibody-mediated rejection and transplant glomerulopathy. Am J Transplant 2011; 11: 2123–2131.
70. Gebel HM, Bray RA, Nickerson P. Pre-transplant assessment of donor-reactive, HLA-specific antibodies in renal transplantation: Contraindication vs. risk. Am J Transplant 2003; 3: 1488–1500.
71. Mohan S, Palanisamy A, Tsapepas D, et al. Donor-specific antibodies adversely affect kidney allograft outcomes. J Am Soc Nephrol 2012; 23: 2061–2071.
72. McKenna RM, Takemoto SK, Terasaki PI. Anti-HLA antibodies after solid organ transplantation. Transplantation 2000; 69: 319–326.
73. Wiebe C, Nickerson P. Posttransplant monitoring of de-novo human leukocyte antigen donor-specific antibodies in kidney transplantation. Curr Opin Organ Transplant 2013; 18: 470–477.
74. Wiebe C, Gibson IW, Blydt-Hansen TD, et al. Evolution and clinical pathologic correlations of de-novo donor-specific HLA antibody post kidney transplant. Am J Transplant 2012; 12: 1157–1167.
75. Liefeldt L, Brakemeier S, Glander P, et al. Donor-specific HLA antibodies in a cohort comparing everolimus with cyclosporine after kidney transplantation. Am J Transplant 2012; 12: 1192–1198.
76. Rosenberg WM, Bushell A, Higgins RM, et al. Isolated HLA-DP mismatches between donors and recipients do not influence the function or outcome of renal transplants. Hum Immunol 1992; 33: 5–9.
77. Pfeiffer K, Vogeler U, Albrecht KH, Eigler FW, Buchholz B, Grosse-Wilde H. HLA-DP antibodies in patients awaiting renal transplantation. Transpl Int 1995; 8: 180–184.
78. Qiu J, Cai J, Terasaki PI, El-Awar N, Lee JH. Detection of antibodies to HLA-DP in renal transplant recipients using single antigen beads. Transplantation 2005; 80: 1511–1513.
79. Perkowska-Ptasinska A, Ciszek M, Chmura A, Galazka Z, Paczek L, Durlik M. Transplant glomerulopathy: Clinical and pathological correlations. Transplant Proc 2009; 41: 141–149.
80. Kuypers DR, Vanrenterghem Y. Time to reach tacrolimus maximum blood concentration, mean residence time, and acute renal allograft rejection: An open-label, prospective, pharmacokinetic study in adult recipients. Clin Ther 2004; 26: 1834–1844.
81. Kuypers DR, de Jonge H, Naesens M, Vanrenterghem Y. Effects of CYP3A5 and MDR1 single nucleotide polymorphisms on drug interactions between tacrolimus and fluconazole in renal allograft recipients. Pharmacogenet Genomics 2008; 18: 861–868.
82. Kuypers DR, Claes K, Evenepoel P, Maes B, Vanrenterghem Y. The rate of gastric emptying determines the timing but not the extent of oral tacrolimus absorption: Simultaneous measurement of drug exposure and gastric emptying by carbon-14-octanoic acid breath test in stable renal allograft recipients. Drug Metab Dispos 2004; 32: 1421–1425.
83. van Gelder T. Drug interactions with tacrolimus. Drug Saf 2002; 25: 707–712.
84. Toda F, Tanabe K, Ito S, et al. Tacrolimus trough level adjustment after administration of fluconazole to kidney recipients. Transplant Proc 2002; 34: 1733–1735.
85. Takahashi K, Yano I, Fukuhara Y, et al. Distinct effects of omeprazole and rabeprazole on the tacrolimus blood concentration

in a kidney transplant recipient. Drug Metab Pharmacokinet 2007; 22: 441–444.
86. Park SI, Felipe CR, Pinheiro-Machado PG, et al. Tacrolimus pharmacokinetic drug interactions: Effect of prednisone, mycophenolic acid or sirolimus. Fundam Clin Pharmacol 2009; 23: 137–145.
87. John R, Herzenberg AM. Our approach to a renal transplant biopsy. J Clin Pathol 2010; 63: 26–37.
88. Racusen LC, Solez K, Colvin RB, et al. The Banff 97 working classification of renal allograft pathology. Kidney Int 1999; 55: 713–723.

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.