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ORIGINAL ARTICLE

LIMS1 risk genotype and T cell–mediated rejection in kidney transplant recipients

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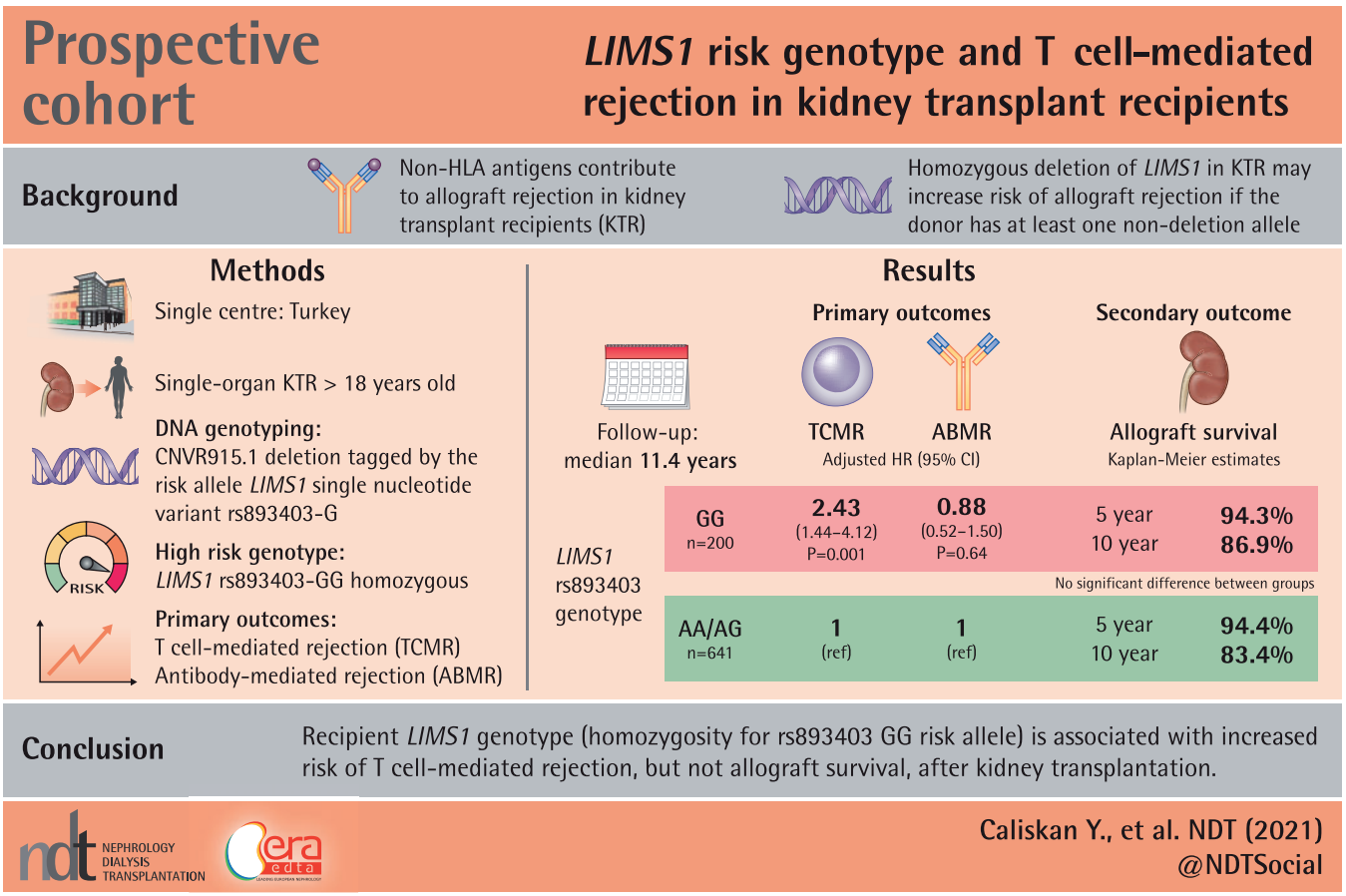
GRAPHICAL ABSTRACT



ABSTRACT

Background. This study aims to examine the association of LIM zinc finger domain containing 1 (*LIMS1*) genotype with allograft rejection in an independent kidney transplant cohort.

Methods. We genotyped 841 kidney transplant recipients for the *LIMS1* rs893403 variant by Sanger sequencing followed by polymerase chain reaction confirmation of the deletion. Recipients who were homozygous for the *LIMS1* rs893403 genotype GG were compared with the AA/AG genotypes. The primary outcome was T cell–mediated or antibody-mediated rejection (TCMR or ABMR, respectively) and secondary outcome was allograft loss.

Results. After a median follow-up of 11.4 years, the rate of TCMR was higher in recipients with the GG genotype ($n = 200$) compared with the AA/AG genotypes ($n = 641$) [25 (12.5%) versus 35 (5.5%); $P = 0.001$] while ABMR did not differ by genotype [18 (9.0%) versus 62 (9.7%)]. Recipients with the GG genotype had 2.4 times higher risk of TCMR than those who did not have this genotype [adjusted hazard ratio2.43 (95% confidence interval 1.44–4.12); $P = 0.001$]. A total of 189 (22.5%) recipients lost their allografts during follow-up. Kaplan–Meier estimates of 5-year (94.3% versus 94.4%; $P = 0.99$) and 10-year graft survival rates (86.9% versus 83.4%; $P = 0.31$) did not differ significantly in the GG versus AA/AG groups.

Conclusions. Our study demonstrates that recipient *LIMS1* risk genotype is associated with an increased risk of TCMR after kidney transplantation, confirming the role of the *LIMS1* locus in allograft rejection. These findings may have clinical implications for the prediction and clinical management of kidney transplant rejection by pretransplant genetic testing of recipients and donors for *LIMS1* risk genotype.

Keywords: genetics, kidney transplantation, LIMS1, rejection, T cell–mediated rejection

INTRODUCTION

Kidney transplantation is the preferred modality of renal replacement therapy and is the most cost-effective treatment option for eligible patients with kidney failure [1, 2]. Advances in immunosuppression, donor preparation, human leucocyte antigen (HLA) matching and antibody screening have contributed to improved kidney allograft survival. However, all grafts are still at risk of rejection, which remains the most significant barrier to sustained long-term graft survival [3–5]. HLA matching between donor and recipient has long been recognized as critical to graft survival, but it does not account for the entire risk of rejection; recent advances have implicated important roles for non-HLA mismatching in post-transplant rejection risk [6, 7].

KEY LEARNING POINTS

What is already known about this subject?

- Recent advances have provided new insights into the pathogenesis of allograft rejection caused by non-human leucocyte antigens (non-HLAs). The risk of kidney transplant rejection is increased if a recipient carries a homozygous risk genotype (deletion) in LIM zinc finger domain containing 1 (*LIMS1*) but receives an allograft from a donor who has at least one non-deletion allele.
- To date, there is only one study describing the association of the *LIMS1* genotype with allograft rejection, thus there is a need for an independent validation of these findings in more diverse cohorts and transplant practice settings.
- Beyond independent replication, our study addressed some of the limitations of the previous study, providing detailed analysis of rejection types associated with the *LIMS1* risk genotype, donor-specific anti-HLA antibodies, responses to therapy and long-term outcomes.

What this study adds?

- This study provides independent evidence for the role of the *LIMS1* genotype in determining kidney allograft rejection outcomes.
- Our data support the *LIMS1* locus as an important factor in determining the risk of T cell–mediated alloimmunity. We also demonstrate the association of the *LIMS1* risk genotype with tubular inflammation.
- We found no association of the *LIMS1* risk genotype with *de novo* donor-specific anti-HLA antibodies or long-term allograft survival.

What impact this may have on practice or policy?

- These findings may have implications for the clinical management of kidney transplant rejection, potentially including pretransplant genetic testing of recipients and donors for the *LIMS1* risk genotype.
- Our results advance the understanding of the role of *LIMS1* in determining kidney transplant outcomes, facilitating identification and characterization of suitable treatments to overcome allograft rejection in individuals at genetic risk.

Genetic variants in both the kidney donor genome and recipient genome might contribute to transplantation outcomes [8]. Recent advances have provided new insights into the pathogenesis of allograft rejection caused by additional antigens associated with the genetic risk variant of the LIM zinc finger domain containing 1 (*LIMS1*) gene and suggest an increased risk of rejection in the allograft recipients with homozygous deletion near *LIMS1* [7, 8]. This common 1.5-kb deletion (CNVR915.1) is downstream of *LIMS1* and tagged by the risk allele rs893403-G, which is frequent in persons of European and African ancestry. There is a strong linkage disequilibrium between this tag single-nucleotide variant (SNV) rs893403-G and the deletion CNVR915.1 ($r^2 = 0.98$ in the HapMap European population). In the initial study by Steers *et al.* [7], recipients who were homozygous for the rs893403 GG genotype had ∼63% higher risk of rejection than those who did not have this genotype across four international transplant cohorts. To date, this is the only study examining the association of the homozygous *LIMS1* rs893403 genotype with allograft rejection and there is a need to characterize the histologic and serologic patterns of rejection associated with the *LIMS1* mismatch and test the impact of the risk genotypes on responsiveness to immunosuppressive therapy and long-term allograft function.

In this study we performed *LIMS1* genotyping among kidney transplant recipients to examine the relationship between the recipient *LIMS1* genotype and the risk of allograft rejection and survival. We also assessed a detailed description of rejection episodes and the relationship of recipient *LIMS1* risk genotype status with post-transplant donor-specific antibodies (DSAs) and long-term allograft function. Donor–recipient genotype mismatching was assessed in a subcohort with available living donor DNA for *LIMS1* genotyping.

MATERIALS AND METHODS

Study population

Renal transplant recipients who were ≥18 years of age and followed at the Istanbul School of Medicine Transplant Clinic, had DNA available for genotyping ($n = 905$) and provided research consent were included in the study. Exclusion criteria were having multi-organ transplantation ($n = 14$), missing follow-up data ($n = 47$), suffering from cognitive impairment or being unable to provide consent ($n = 3$). The study cohort included 841 prevalent kidney transplant recipients who consented for genetic studies; 41 living kidney transplant recipients were genotyped with their living donors as pairs for *LIMS1* rs893403 variant sequencing. Information on recipient demographic and clinical characteristics [age, sex, ethnicity, primary cause of kidney failure, kidney replacement treatment (KRT) modalities, KRT duration and pretransplant sensitization history including blood transfusions, pretransplant pregnancy, history of previous transplantation, panel reactive antibody (PRA) levels, DSAs, HLA mismatch and date of transplant] were recorded from medical records.

All study procedures were conducted according to good medical and laboratory practices and the recommendations of the Declaration of Helsinki on biomedical research involving human subjects. This study was approved by the Istanbul School of Medicine Clinical Studies Board (approval 2011/483-480). All those enrolled in the study provided written informed consent.

Definition of immunosuppressive regimens

Induction therapy [antithymocyte globulin (ATG) Fresenius, 2 mg/kg/day, for 3–7 days] was used in all transplantations from deceased donors, whereas ATG, interleukin-2 receptor (IL-2R) blocking antibodies or no induction were applied to living donor transplant recipients. No induction therapy was given to living related transplant recipients when the number of HLA mismatches was ≤1A, 1B, 1DR and pretransplant class I and class II PRA were 0% per our centres protocol. Induction use in the data is recorded as a binary indication (given or not), but information on days of treatment is not available. There were no HLA identical transplantations.

All the patients received intra-operative methylprednisolone bolus injection at a dose of 500 mg and afterward were treated by a triple maintenance immunosuppressive regimen including a calcineurin inhibitor (CNI) [cyclosporine or tacrolimus (Tac)], an antiproliferative drug [azathioprine (AZA) or mycophenolic acid derivative mycophenolate mofetil (MMF)/sodium] and prednisolone (Pred). Target blood levels of cyclosporine (C0) and Tac after transplantation were, respectively, 200–300 and 8–12 ng/mL for the first 3 months and 50–150 and 4–8 ng/mL for subsequent months. MMF and AZA were administered at a dose of 2 g/day (1440 mg/day for mycophenolate sodium) and 1.5 mg/kg/day, respectively. On postoperative Day 1, patients received methylprednisolone beginning with a dose of 120 mg/day, with a rapid taper and reaching a maintenance dose of 10 mg/day within the first month and 5 mg/day within the first year. Alterations were made in treatment strategies per immunologic risk and post-transplant complications, if necessary.

Molecular analyses

Genomic DNA was extracted from peripheral blood for a total of 841 prevalent kidney transplant recipients and 41 living donors. Genotyping for *LIMS1* SNV rs893403 was performed by Sanger sequencing in the Medical Biology Laboratory. We defined risk genotype according to a recipient's homozygosity for the *LIMS1* rs893403 GG genotype. *LIMS1* high-risk mismatch was defined by a specific living donor–recipient genotype combination in which a recipient homozygous for a *LIMS1* rs893403 GG genotype received a transplant from a non-homozygous donor.

Detailed methods regarding the polymerase chain reaction (PCR)-based CNVR915.1 deletion confirmation, HLA genotyping, anti-HLA antibody screening and biochemical tests are provided in the Supplementary Methods section in the Supplementary data.

Follow-up principles

Patients were followed at the transplantation clinic at weekly intervals in the beginning; the follow-up intervals were gradually increased to every 3 months over time. Laboratory

data including serum creatinine, albumin, complete blood count, quantitative proteinuria and DSA measurements were retrieved from patients' charts. The estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration equation.

Allograft biopsy was performed when there was a persistent, unexplained increase in serum creatinine level and/or proteinuria $\geq$1 g/day. None of the patients received empiric anti-rejection treatment prior to biopsy, per our centre's protocol. The follow-up period was considered as the time interval between kidney transplantation and the last outpatient visit, allograft failure or death.

Study outcomes

The primary outcome was defined as biopsy-confirmed allograft rejection by Banff criteria [9] and categorized as T cell–mediated rejection (TCMR) or antibody-mediated rejection (ABMR). The secondary outcome was graft loss in a time-to-event analysis. Graft loss was defined as a return to dialysis, retransplantation or death with a functioning graft.

Histopathological evaluation for classifying rejection

Adequate kidney biopsy specimens, defined as having seven or more glomeruli with at least two arteries, were evaluated. Sections of 3–4 μm were used for all histochemical and immunohistochemical staining. About 0.4–0.6 cm of unfixed tissue was frozen with liquid nitrogen for immunofluorescence staining [immunoglobulin G (IgG), IgM, IgA, C1q, C3, fibrinogen and kappa and lambda light chains]. The remaining tissues were fixed in Hollande's fixative, embedded in paraffin and processed routinely for light microscopic evaluation (haematoxylin and eosin, periodic acid–Schiff, methenamine silver–periodic acid, Masson trichrome and Congo red). Banff criteria were used when evaluating the specimens by light microscopy [9, 10]. C4d staining was performed by immunohistochemistry on paraffin-embedded tissue blocks. Linear and circumferential staining in peritubular capillaries were regarded as positive (C4d >0) [9]. Rejections were classified as TCMR or ABMR, as defined by the Banff 2013 criteria [9].

Statistical analyses

Statistical analyses were performed using SPSS software for Windows (version 25.0; IBM, Armonk, NY, USA) and R version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria) [11]. Data were expressed as mean ± SD when normally distributed or as median [interquartile range (IQR)] otherwise. Parametric and non-parametric tests were used according to the distribution pattern of the data. Comparisons of continuous variables between the GG and AA/AG genotype groups or *LIMS1* high-risk and non-risk donor recipient matching groups were assessed by *t*-test or the Mann–Whitney *U*-test, where appropriate. The differences in the proportions were compared by the Fisher's exact test.

Recipient deletion-tagging alleles were examined under a recessive model in which the risk genotype was defined according to a recipient's homozygosity for a deletion-tagging allele. The impact of the recipient and donor variables on the primary and secondary outcomes was analysed. Univariate Cox models were fitted for variables for each of the study outcomes. The adjusted association of the *LIMS1* rs893403 genotype with primary and secondary outcomes {adjusted hazard ratio [95% confidence interval (CI)]}after transplant was assessed using multivariable Cox proportional hazards regression analysis. The full models were adjusted for recipient, donor and transplant factors including age at transplant, sex, pre-transplant dialysis modality, sensitization events including previous kidney transplant, pregnancy, blood transfusions, donor type, donor age, donor sex, number of HLA mismatches, PRA levels at transplant and induction treatment at transplant. Variables were selected by backward elimination using likelihood ratio tests. Allograft survival and rejection-free survival for the GG and AA/AG genotypes were analysed by the Kaplan–Meier method and the allograft survival time for each patient was computed from the transplantation time to the last follow-up or the primary outcome. All statistical tests were two-sided and the level of statistical significance was set at $P < 0.05$.

RESULTS

Genotyping results

A total of 841 prevalent kidney transplant recipients (59.4% men; 76.1% living donor recipients; mean age 34 ± 12 years) were genotyped for the *LIMS1* rs893403 variant by Sanger sequencing. Alleles A and G were in Hardy–Weinberg equilibrium with respective frequencies $p = 0.52$ and $q = 0.48$. The *LIMS1* rs893403 GG, AG and AA genotypes were observed in 23.8% ($n = 200$), 48.5% ($n = 408$) and 27.7% ($n = 233$) of recipients, respectively. Recipients were grouped according to the *LIMS1* rs893403 variant genotype: risk genotype group included 200 recipients who were homozygous for the *LIMS1* rs893403 GG genotype and the non-risk genotype group included 641 recipients with the *LIMS1* rs893403 AA or AG genotype.

The CNVR915.1 deletion was confirmed by quantitative PCR in participant samples with rs893043-AG [405/408 (99.3%)] and GG [198/200 (99%)] genotypes based on the presence of a PCR product between close primer pairs.

Clinical, histopathologic and therapeutic features

The study groups were similar in age; sex; pretransplant dialysis modality; history of previous kidney transplant; pretransplant pregnancy; pretransplant PRA level; donor age, gender and type; number of HLA mismatches and induction and maintenance immunosuppressive treatments (Table 1). Pretransplant DSAs were negative in all study group patients before kidney transplantation.

Follow-up and outcomes

Overall, the median follow-up time after transplantation was 11.4 years (IQR 8.7–15.3). There was no statistically significant difference in follow-up times between the groups (Table 2). The mean eGFR and serum albumin levels and median proteinuria level at the last follow-up visit were not significantly different between the study groups ($P = 0.73$, $P = 0.23$ and $P = 0.09$,

Table 1. Demographic, clinical and therapeutic features of study groups based on *LIMS1* gene rs893403 genotype

Characterisitcs	GG genotype ($n = 200$)	AA/AG genotype ($n = 641$)	P-value
Age (years), mean ± SD	33 ± 13	35 ± 12	0.06
Gender (male/female), *n* (%)	119 (60)/81 (40)	381 (59)/260 (41)	0.99
Pretransplant dialysis, *n* (%)			
Pre-emptive	28 (14)	69 (11)	
HD	136 (68)	477 (74)	
PD	20 (10)	54 (8)	0.35
HD + PD	16 (8)	41 (7)	
Previous kidney transplant, *n* (%)	1 (0.5)	21 (3.3)	0.03
Pretransplant pregnancy, *n* (%)	30 (37)	121 (46)	0.21
Most current PRA level, *n* (%)			
<10	197 (98.5)	621 (96.9)	
10–79	3 (1.5)	16 (2.5)	0.38
≥80	–	4 (0.6)	
Donor age (years), mean ± SD	44 ± 13	45 ± 13	0.40
Donor gender (male/female), *n* (%)	104 (52)/96 (48)	355 (55)/286 (45)	0.40
Donor type (living/deceased), *n* (%)	156 (78)/44 (22)	484 (76)/157 (24)	0.47
HLA mismatches, mean ± SD	3.0 ± 1.3	3.1 ± 1.3	0.30
Induction treatment, *n* (%)			
ATG	45 (23)	165 (26)	0.59
IL-2R antibodies	34 (17)	113 (18)	
No induction	121 (60)	363 (56)	
Maintenance immunosuppression, *n* (%)			
Tac + MMF/AZA + Pred	88 (44)	289 (45)	
CsA + MMF/AZA + Pred	72 (36)	221 (35)	
mTORi-based	20 (10)	59 (9)	0.98
AZA/MMF + Pred	11 (5.5)	40 (6)	
CNI + MMF/AZA	6 (3)	18 (3)	
CNI + Pred	3 (1.5)	14 (2)	

ATG, antithymocyte globulin (rabbit); CsA, cyclosporin A; HD, hemodialysis; mTORi, mammalian target of rapamycin inhibitor; PD, peritoneal dialysis.

Table 2. Follow-up outcomes of study groups based on *LIMS1* rs893403 genotype

Outcomes	GG genotype ($n = 200$)	AA/AG genotype ($n = 641$)	P-value
Follow-up period (years), median (IQR)	11.3 (8.9–15.2)	11.5 (8.7–15.3)	0.67
Post-transplant DSA, *n* (%)	29/82 (35.4)	115/269 (42.8)	0.23
MFI of DSA, median (IQR)	8075 (2367–15 750)	11 869 (5815–20 020)	0.32
Proteinuria at last follow-up (g/day), median (IQR)	0 (0–0.12)	0 (0–0.2)	0.16
Serum albumin at last follow-up (g/dL), mean ± SD	4.46 ± 0.35	4.37 ± 0.43	0.09
eGFR at last follow-up (mL/min/1.73 m^2), mean ± SD	52 ± 29	51 ± 29	0.73
Overall biopsy confirmed rejection, *n* (%)	36 (18)	87 (13.6)	0.12
TCMR, *n* (%)	25 (12.5)	35 (5.5)	0.001
Banff tubulitis score (*t*), mean ± SD	1.53 ± 0.84	1.17 ± 0.69	0.01
ABMR, *n* (%)	18 (9)	62 (9.7)	0.78
Overall graft failure (non-censored for death), *n* (%)	40 (20)	149 (23)	0.34
Overall death-censored graft failure, *n* (%)	36 (18)	122 (19)	0.74

eGFR, estimated glomerular filtration rate; MFI, mean fluorescence intensity.

respectively) (Table 2). Overall, *de novo* development of post-transplant DSAs was evaluated in all patients with pretransplant anti-HLA antibodies and an unexplained increase in serum creatinine level and/or proteinuria ≥1 g/day after transplantation {351 [41.7%] patients; GG group [$n = 82$ (41%)], AA/AG group [$n = 269$ (42%)]}. There was no significant difference regarding *de novo* DSA development after transplant between the study groups {GG group [$n = 29$ (35.4%) versus AA/AG group [$n = 115$ (42.8%)]; P = 0.23}. When development of *de novo* class I {GG group [$n = 17$ (20.7%)] versus AA/AG group [$n = 64$ (23.8%)]} and class II DSAs {GG group [$n = 23$ (28%)] versus AA/AG group [$n = 93$ (34.6%)]} were compared separately between study groups, there were also no significant differences between the study groups (P = 0.565 and P = 0.272, respectively).

Study outcomes

Primary outcomes. A total of 60 (7.1%) recipients developed TCMR after a median follow-up time of 5.5 months (IQR 1–28). The overall risk of TCMR was significantly higher in recipients with the GG compared with the AG/AA genotype

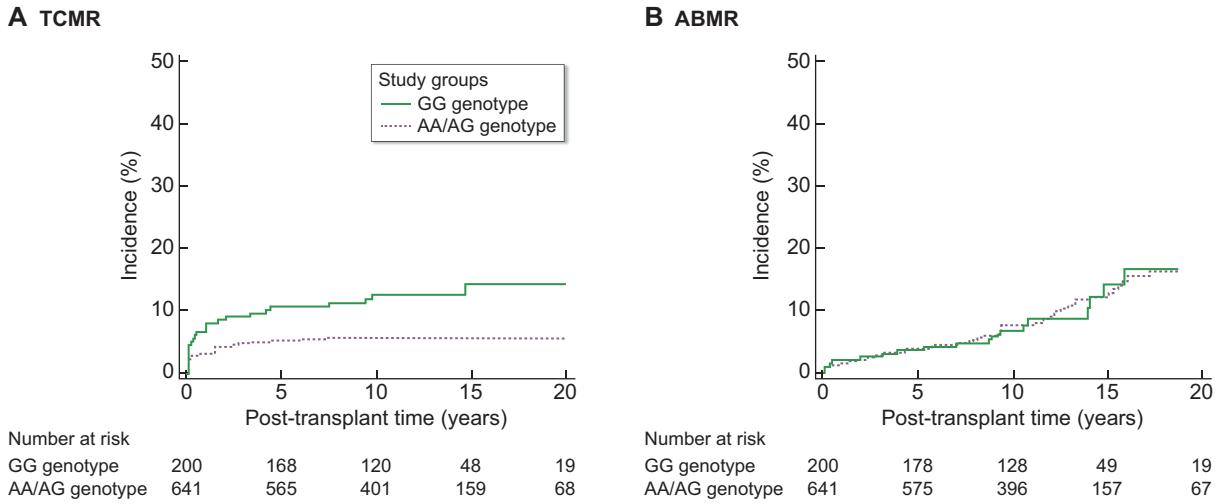


FIGURE 1: (**A**) Overall TCMR incidences in the *LIMS1* rs893403 GG and AA/AG genotypes groups (P = 0.001). (**B**) Overall ABMR incidences in the *LIMS1* rs893403 GG and AA/AG genotypes groups (P = 0.777).

[25 (12.5%) versus 35 (5.5%); HR 2.29 (95% CI 1.41–3.73); P = 0.001] (Table 2 and Figure 1A). The Banff tubulitis score was significantly higher in the GG group compared with the AA/AG group (1.53 ± 0.84 versus 1.17 ± 0.69; P = 0.01).

To explore the correlation of the *LIMS1* rs893403 genotype with TCMR, the Cox proportional hazard regression model was employed to analyse prognostic factors. Univariate Cox regression analysis showed that the *LIMS1* rs893403 GG genotype [HR 2.34 (95% CI 1.40–3.91); P = 0.001], history of previous kidney transplant [HR 2.10 (95% CI 1.03–4.27); P = 0.04], history of pretransplant blood transfusion [HR 1.76 (95% CI 1.06–2.91); P = 0.03] and female donors [HR 1.70 (95% CI 1.02–2.85); P = 0.04] were significantly associated with TCMR (Table 3). In the multivariable Cox regression analysis, only the *LIMS1* rs893403 GG genotype [aHR 2.43 (95% CI 1.44–4.12); P = 0.001] and a history of pretransplant blood transfusion [aHR 1.85 (95% CI 1.07–3.21); P = 0.03] were associated with TCMR. Age; gender; pretransplant dialysis modality; time on dialysis; history of kidney transplant; pretransplant pregnancy; donor type, gender and age; number of HLA mismatches; pretransplant PRA levels and induction treatment were not associated with TCMR in multivariable Cox regression analysis (Table 3).

A total of 80 (9.5%) recipients developed ABMR after a median follow-up time of 8.5 years (IQR 2.6–12.6). There was no significant difference in ABMR rates between patients with the GG and AA/AG genotype [18 (9.0%) versus 62 (9.7%); P = 0.777] (Table 2 and Figure 1B). In the multivariable Cox regression analysis, recipient age [aHR 0.97 (95% CI 0.94–0.99); P = 0.01] and number of HLA mismatches [aHR 1.46 (95% CI 1.11–1.93); P = 0.007] were the predictors of ABMR (Supplementary data, Table S1). *LIMS1* rs893403 genotype; gender; pretransplant dialysis modality; time on dialysis; history of kidney transplantation; pretransplant blood transfusion; pretransplant pregnancy; donor type, age and gender; pre-transplant PRA levels and induction treatment did not predict ABMR in multivariable Cox regression analysis (Supplementary data, Table S1).

Secondary outcomes. A total of 189 (22.5%) recipients lost their allografts after a median post-transplant follow-up time of 9.9 years (IQR 5.2–15.5). Kaplan–Meier estimates of 5-year (94.3% versus 94.4%; P = 0.99) and 10-year graft survival rates (86.9% versus 83.4%; P = 0.31) did not differ significantly in the GG versus AA/AG groups (Figure 2A). During follow-up, 31 (3.7%) patients died with a functioning graft and there were no significant differences regarding death with functioning grafts according to the *LIMS1* genotype (P = 0.15). Kaplan–Meier estimates of 5-year (95.3% versus 94.7%; P = 0.73) and 10-year death-censored graft survival rates (88% versus 85.4%; P = 0.42) also did not differ significantly by genotype (Figure 2B).

In the multivariable Cox regression analysis, time on dialysis [aHR 1.01 (95% CI 1.00–1.01; P < 0.001], female gender [aHR 0.62 (95% CI 0.42–0.92); P = 0.02], primary disease of congenital abnormalities of the kidney and urinary tract [CAKUT; aHR 0.35 (95% CI 0.20–0.62); P < 0.001], history of pretransplant blood transfusion [aHR 1.45 (95% CI 1.05–2.02); P = 0.03], donor age [aHR 1.03 (95% CI 1.01–1.04); P < 0.001] and number of HLA mismatches [aHR 1.35 (95% CI 1.14–1.61); P = 0.001] predicted allograft loss (Supplementary data, Table S2). Recipient *LIMS1* rs893403 GG genotype, age, pretransplant pregnancy, pretransplant dialysis modality, donor gender, pretransplant PRA levels and induction treatment did not predict allograft loss in the multivariable Cox regression analysis (Supplementary data, Table S2).

Recipient donor pairs

A total of 41 prevalent living donor kidney transplant recipient [24 (59%) males; mean age 34 ± 13 years] and donor [15 (37%) males; mean age 47 ± 13 years] pairs were genotyped for the *LIMS1* rs893403 variant by Sanger sequencing.

Table 3. Univariate and multivariate Cox regression analysis to explore the correlation of recipient and donor factors with TCMR

Variables	Univariate correlates of TCMR ($n = 841$) HR (95% CI)	P-value	Adjusted correlates of TCMR ($n = 841$) aHR (95% CI)	P-value
LIMS genotype				
AA/AG	Reference		Reference	
GG	2.34 (1.40–3.91)	**0.001**	2.43 (1.44–4.12)	**0.001**
Age (years)	0.99 (0.96–1.01)	0.20	0.99 (0.96–1.02)	0.54
Gender				
Male	Reference		Reference	
Female	1.10 (0.66–1.86)	0.71	0.76 (0.39–1.51)	0.43
Cause of kidney failure				
Glomerulonephritis	Reference		Reference	
Hypertension	0.50 (0.12–2.12)	0.35	0.54 (0.12–2.43)	0.42
Polycystic kidney disease	0.46 (0.06–3.39)	0.46	0.52 (0.07–4.04)	0.53
CAKUT	0.86 (0.38–1.95)	0.72	0.69 (0.30–1.60)	0.38
Other	0.81 (0.38–1.72)	0.58	0.71 (0.33–1.55)	0.39
Unknown	0.87 (0.46–1.63)	0.66	0.92 (0.48–1.75)	0.80
Pretransplant dialysis				
Pre-emptive	Reference		Reference	
HD	0.80 (0.48–1.33)	0.39	0.90 (0.40–2.04)	0.80
PD	1.11 (0.57–2.18)	0.76	1.44 (0.51–4.07)	0.49
HD + PD	1.50 (0.77–2.92)	0.23	0.84 (0.23–3.11)	0.80
Time on dialysis	1.00 (0.99–1.00)	0.45	1.00 (0.99–1.01)	0.75
Previous kidney transplant				
No	Reference		Reference	
Yes	2.10 (1.03–4.27)	**0.04**	1.23 (0.29–5.25)	0.78
Previous pregnancy				
No	Reference		Reference	
Yes	1.17 (0.82–1.67)	0.40	1.16 (0.48–2.85)	0.74
History of transfusion				
No	Reference		Reference	
Yes	1.76 (1.06–2.91)	**0.03**	1.85 (1.07–3.21)	**0.03**
Pre-transplant PRA level (%)				
<10	Reference		Reference	
10–79	1.57 (0.38–6.43)	0.53	1.24 (0.28–5.43)	0.78
≥80	0.00 (0.00–0.00)	0.97	0.00 (0.00–0.00)	0.97
Donor age (years)	1.02 (0.99–1.04)	0.11	1.01 (0.99–1.04)	0.23
Donor gender				
Male	Reference		Reference	
Female	1.70 (1.02–2.85)	**0.04**	1.39 (0.80–2.41)	0.24
Donor type				
Living	Reference		Reference	
Deceased	0.56 (0.28–1.13)	0.11	0.58 (0.24–1.42)	0.23
HLA mismatches	1.02 (0.84–1.25)	0.84	1.25 (0.92–1.70)	0.16
Induction treatment				
No induction	Reference		Reference	
ATG	0.53 (0.26–1.08)	0.08	0.59 (0.24–1.46)	0.25
IL2rAb	0.74 (0.36–1.52)	0.42	0.65 (0.22–1.88)	0.43

aHR, adjusted hazard ratio; ATG, antithymocyte globulin (rabbit); CAKUT, congenital abnormalities of kidney and urinary tract; HD, hemodialysis; HLA, human leukocyte antigen; IL2rAb, interleukin-2 (IL-2) receptor–blocking antibodies; PD, peritoneal dialysis; PRA, panel reactive antibody; TCMR, T-cell mediated rejection. Bold indicates statistically significant associations ($p < 0.05$).

Five recipients homozygous for the *LIMS1* rs893403 GG genotype received a transplant from a non-homozygous donor described as a high-risk *LIMS1* mismatch. There were no differences between recipients with a high-risk *LIMS1* mismatch ($n = 5$) and recipients without a high-risk *LIMS1* mismatch ($n = 36$) regarding demographic factors, duration of dialysis, pretransplant PRA, HLA mismatch, immunosuppressive protocols and follow-up time (Supplementary data, Table S3). After a median follow-up of 10.3 years (IQR 8.7–12.6), recipients with a high-risk *LIMS1* mismatch had a significantly higher risk of biopsy-confirmed allograft rejection compared with recipients without a high-risk *LIMS1* mismatch [60% versus 13.9%; HR 4.32 (95% CI 1.46–12.76); P = 0.015] (Supplementary data, Table S4). TCMR was higher in recipients with a high-risk *LIMS1* mismatch compared with recipients without a high-risk *LIMS1* mismatch (40% versus 11.1%; P = 0.087). Two of the recipients without a high-risk *LIMS1* mismatch had biopsy-confirmed ABMR mixed with concomitant TCMR. There were no significant differences between patients with and without a high-risk *LIMS1* mismatch in post-transplant DSA, ABMR and allograft failure. The mean eGFR levels at the last follow-up were also similar

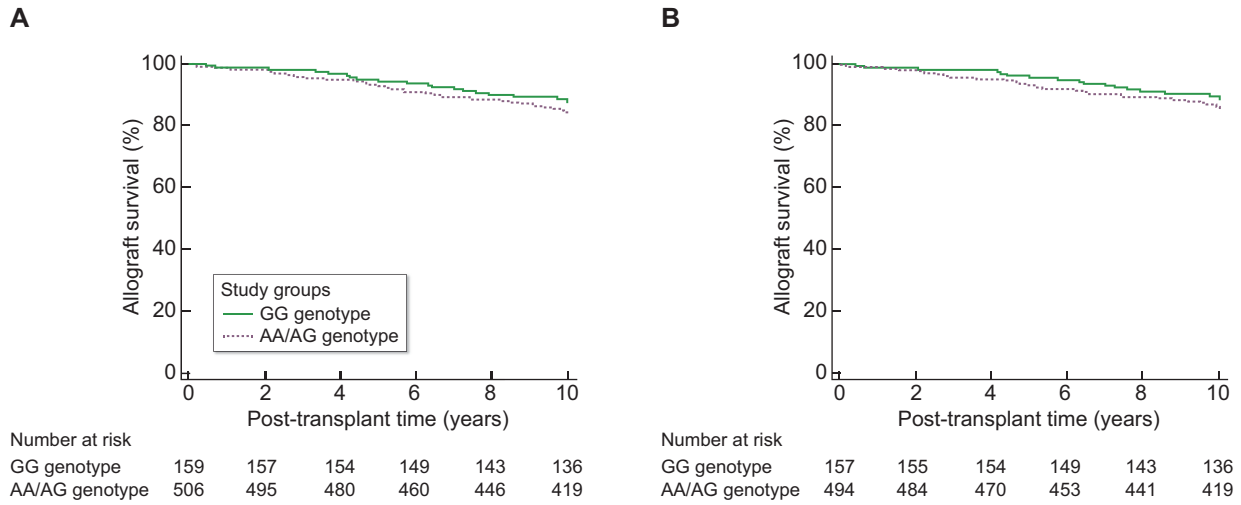


FIGURE 2: (**A**) Kaplan–Meier estimates of allograft survival in the *LIMS1* rs893403 GG and AA/AG genotypes groups. (**B**) Kaplan–Meier estimates of death-censored allograft survival in the *LIMS1* rs893403 GG and AA/AG genotypes groups.

among recipients with and without a high-risk *LIMS1* mismatch.

DISCUSSION

In this study we showed that the *LIMS1* risk genotype rs893403-GG in a kidney transplant recipient was significantly associated with TCMR compared with a non-risk *LIMS1* genotype, while ABMR and allograft failure rates were similar in both groups. This finding is consistent with results published recently by Steers *et al.* [7] and suggests the effect of the *LIMS1* locus was driven primarily by cellular rejection events.

Donor and recipient HLA matching has long been recognized as critical to graft survival; however, recent studies point to an important role of non-HLAs [6, 7], including studies using genetic approaches to identify these antigens [7, 8, 12–18]. Such approaches are particularly challenging, because the relevant outcomes are often determined by composite effects of genetic interactions between donor and recipient genomes and multiple medical and surgical factors have additional large effects on outcomes. Despite these challenges, prior candidate gene studies have suggested several non-HLA mismatches that may cause ligand–receptor incompatibilities between donor and recipient or production of antibodies against non-HLAs [7, 8, 12–18].

A recent study carried out by the Wellcome Trust Case Control Consortium with 2094 kidney donor–recipient pairs in the discovery phase and an additional 5866 pairs in the replication phase aimed to identify genetic loci associated with rejection and allograft failure [8, 12]. In recipient and donor-only genome-wide association studies as well as in donor–recipient interaction models there were no statistically significant loci associated with time to acute rejection and graft failure except for HLA [12]. However, one of the major limitations of this study was that the phenotypes were assessed retrospectively and were not homogeneously defined [12]. A consortium called the International Genetics and Translational Research in Transplantation Network was developed to specifically address these limitations by including well-phenotyped patients [13, 14].

Using a discovery cohort of 705 transplant recipients and a replication cohort of 2004 kidney donor–recipient pairs, Steers *et al.* [7] recently reported that genomic mismatch at the *LIMS1* locus was associated with an increased risk of allograft rejection [7]. In addition, anti-LIMS1 antibodies were detected by protein arrays and confirmed by enzyme-linked immunosorbent assay in patients with allograft rejection and a high-risk *LIMS1* genotype [7]. The risk variant was associated with lower kidney messenger RNA levels of LIMS1, a protein expressed in the distal tubules and in the glomerular and vascular endothelium [7, 19]. The cell surface expression of LIMS1 appeared to be induced by hypoxia [7, 19, 20], supporting the hypothesis that anti-LIMS1 response may be directed against ischaemia-induced LIMS1 in genetically predisposed recipients who normally express only low protein levels.

In this study, the *LIMS1* risk genotype was associated with allograft rejection, but only with TCMR, not ABMR. TCMR can develop in ∼10–12% of kidney transplant patients with current immunosuppressive therapy at any time, as early as a week or as late as years after transplantation [21]. This study is different from the study by Steers *et al.* [7] with respect to the ethnic composition of the study cohort, detailed data on each rejection episode and the ability to test the *LIMS1* risk genotype against DSAs, response to therapy and long-term allograft function. Accurate patient phenotyping is critical in genetic association studies and we include detailed histopathologic analysis in the evaluation of genetic effects at the tissue level. Allograft kidney biopsy is the gold standard for the diagnostic and prognostic information after transplantation [21–23]. Anti-LIMS1 antibodies would be expected to lead to microcirculation inflammation, the histologic hallmark of ABMR, however, detailed histopathological data were missing in the study by Steers *et al.* [7]. Under normal conditions, intrarenal LIMS1 expression occurs mainly in tubular epithelial cells. Tubulitis is a histopathological marker of TCMR and is scored as t1–t3 according to the number of cells present per tubular cross-section in non-atrophic

tubules [21]. In this study, recipients with a homozygous *LIMS1* deletion had an increased rate of TCMR and higher tubulitis scores compared with recipients without homozygous deletion. This is the first study that shows an association between *LIMS1* risk genotype and the severity of tubulitis. Notably, the effect estimates for TCMR [HR 2.43 (95% CI 1.44–4.12)] were slightly higher compared with the original report by Steers *et al.* [7], but we also note that our 95% CI is wider due to a smaller cohort size and smaller number of rejection events. Additional differences between these two studies also relate to the fact that post-transplant DSA information was not analyzed in the study by Steers *et al.* [7].

This is important, since any immune response to an allograft may trigger the formation of DSAs, and the role of DSA in the setting of a high-risk *LIMS1* genotype is unknown. In this study we observed no significant differences in the development of post-transplant *de novo* class I and class II DSAs between groups with high-risk versus non-risk *LIMS1* genotypes. The study by Steers *et al.* [7] also did not analyse the effect of different immunosuppressive regimens. Based on integrated analyses of multiple national-level databases, allograft rejection rates, outcomes, key morbidity events and mortality vary as a function of immunosuppressive treatment regimens after kidney transplant [24, 25]. Therefore, in this study we examined the treatment data in detail. However, the choice of induction treatment and maintenance immunosuppressive regimens was not associated with the outcomes of TCMR, ABMR and allograft survival in our dataset.

The effects of mismatch in a high-risk *LIMS1* genotype between donors and recipients on outcomes of living donor kidney transplants were also examined in a smaller number of donor–recipient pairs. We observed a trend for higher rates of TCMR in high-risk mismatched donor–recipient pairs, consistent with the association observed in recipient-only analyses. There are several limitations of our study. Although hypoxia is shown to induce LIMS1 expression, we were unable to test for the effect of ischaemia time as a predictor of rejection in the recipients homozygous for a *LIMS1* deletion. To better understand the alloimmunity of *LIMS1*, it is also important to examine the occurrence of LIMS1 antibody in patients before and after transplantation, but our study was not prospectively designed to perform longitudinal serology tests. Study patients underwent clinical indication biopsies, and a rejection-free phenotype is not based on protocol biopsies, which may also cause a risk of bias.

Replication of genetic associations in diverse cohorts plays a prominent role in the process of validating genetic variants for complex traits. Steers *et al.* [7] and this study independently demonstrate the impact of a non-HLA genetic locus conveying a high risk of alloimmunity and rejection in the setting of kidney transplantation. These replicated findings support that pre-transplant genetic testing of recipients and donors might improve donor–recipient matching and may provide a means for immunological risk stratification relevant to the evaluation of individualized immunosuppressive regimens. Genetic testing combined with anti-LIMS1 response immunological surveillance could also be used to facilitate early detection of patients at risk of rejection.

In summary, this study provides additional evidence for the role of the *LIMS1* genotype in determining kidney allograft rejection outcomes. Our data support the *LIMS1* locus as an important factor in alloimmunity, and particularly determining the risk of TCMR. These findings may have clinical implications for the prediction and clinical management of kidney transplant rejection by pretransplant genetic testing of recipients and donors for the *LIMS1* risk genotype.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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AUTHORS' CONTRIBUTIONS

Y.C., G.K. and A.T. conceptualized the study design, acquired and analyzed the data, obtained regulatory approvals and wrote the paper. K.K., S.M., E.D., A.B.D., H.Y., M.S.S., A.G. and K.L.L. participated in the study design, data interpretation and writing of the paper. Y.O. reviewed all biopsy samples and participated in data interpretation and writing of the paper. G.K., F.O.S., S.U.A. and J.E. participated in genotyping, anti-HLA antibody testing, data interpretation and writing of the paper.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Collins AJ, Foley RN, Chavers B *et al.* US Renal Data System 2013 annual data report. *Am J Kidney Dis* 2014; 63(1 Suppl): A7
2. Axelrod DA, Schnitzler MA, Xiao H *et al.* An economic assessment of contemporary kidney transplant practice. *Am J Transplant* 2018; 18: 1168–1176
3. Lentine KL, Gheorghian A, Axelrod D *et al.* The implications of acute rejection for allograft survival in contemporary U.S. kidney transplantation. *Transplantation* 2012; 94: 369–376
4. Einecke G, Sis B, Reeve J *et al.* Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant* 2009; 9: 2520–2531
5. El-Zoghby ZM, Stegall MD, Lager DJ *et al.* Identifying specific causes of kidney allograft loss. *Am J Transplant* 2009; 9: 527–535
6. Li L, Sigdel T, Vitalone M *et al.* Differential immunogenicity and clinical relevance of kidney compartment specific antigens after renal transplantation. *J Proteome Res* 2010; 9: 6715–6721
7. Steers NJ, Li Y, Drace Z *et al.* Genomic mismatch at *LIMS1* locus and kidney allograft rejection. *N Engl J Med* 2019; 380: 1918–1928
8. Zanoni F, Kiryluk K. Genetic background and transplantation outcomes: insights from genome-wide association studies. *Curr Opin Organ Transplant* 2020; 25: 35–41

9. 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
10. Racusen LC, Colvin RB, Solez K *et al.* Antibody-mediated rejection criteria – an addition to the Banff 97 classification of renal allograft rejection. *Am J Transplant* 2003; 3: 708–714
11. R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing. 2013. http://www.R-project.org/ (25 July 2020, date last accessed)
12. Hernandez-Fuentes MP, Franklin C, Rebollo-Mesa I *et al.* Long- and short-term outcomes in renal allografts with deceased donors: a large recipient and donor genome-wide association study. *Am J Transplant* 2018; 18: 1370–1379
13. International Genetics and Translational Research in Transplantation Network. Design and implementation of the international genetics and translational research in transplantation network. *Transplantation* 2015; 99: 2401–2412
14. Fishman CE, Mohebnasab M, van Setten J *et al.* Genome-wide study updates in the International Genetics and Translational Research in Transplantation Network (iGeneTRAiN). *Front Genet* 2019; 10: 1084
15. Mesnard L, Muthukumar T, Burbach M *et al.* Exome sequencing and prediction of long-term kidney allograft function. *PLoS Comput Biol* 2016; 12: e1005088
16. Reindl-Schwaighofer R, Heinzel A, Kainz A *et al.* Contribution of non-HLA incompatibility between donor and recipient to kidney allograft survival: genome-wide analysis in a prospective cohort. *Lancet* 2019; 393: 910–917
17. Reindl-Schwaighofer R, Heinzel A, Gualdoni GA *et al.* Novel insights into non-HLA alloimmunity in kidney transplantation. *Transpl Int* 2020; 33: 5–17
18. Zhang Z, Menon MC, Zhang W *et al.* Genome-wide non-HLA donor-recipient genetic differences influence renal allograft survival via early allograft fibrosis. *Kidney Int* 2020; 98: 758–768
19. Braun A, Bordoy R, Stanchi F *et al.* PINCH2 is a new five LIM domain protein, homologous to PINCH and localized to focal adhesions. *Exp Cell Res* 2003; 284: 239–250
20. Manalo DJ, Rowan A, Lavoie T *et al.* Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood* 2005; 105: 659–669
21. Lusco MA, Fogo AB, Najafian B *et al.* AJKD atlas of renal pathology: acute T-cell-mediated rejection. *Am J Kidney Dis* 2016; 67: e29-30–e30
22. Williams WW, Taheri D, Tolkoff-Rubin N *et al.* Clinical role of the renal transplant biopsy. *Nat Rev Nephrol* 2012; 8: 110–121
23. Redfield RR, McCune KR, Rao A *et al.* Nature, timing, and severity of complications from ultrasound-guided percutaneous renal transplant biopsy. *Transpl Int* 2016; 29: 167–172
24. Dharnidharka VR, Schnitzler MA, Chen J *et al.* Differential risks for adverse outcomes 3 years after kidney transplantation based on initial immunosuppression regimen: a national study. *Transpl Int* 2016; 29: 1226–1236
25. Clayton PA, Lim WH, Wong G *et al.* Relationship between eGFR decline and hard outcomes after kidney transplants. *J Am Soc Nephrol* 2016; 27: 3440–3446

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