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Characteristics of mismatched eplets affecting de novo donor-specific antibody production and antibody-mediated rejection after kidney transplantation

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Abstract

De novo donor-specific antibody (dnDSA) generation is the most important marker of antibody-mediated rejection (AMR). However, not all dnDSAs induce AMR. The effects of mismatched eplets on dnDSA production and the occurrence AMR remain controversial. We analyzed 64 cases of dnDSA positive kidney transplantation that occurred between 2017 and 2021 at our center to reveal the relationships between mismatched eplet and dnDSA generation and the characteristics of antibody-specific and AMR associated mismatched eplets. Among the 64 dnDSA positive cases, 114 dnDSA were produced. Both the average production time and medium fluorescence index (MFI) value of human leukocyte antigen (HLA) II dnDSA were higher than those of HLA I (time, $p=0.024$; MFI, $p=0.032$). More HLA II dnDSAs were generated in the AMR group ($p<0.001$). The frequency of HLA II dnDSAs was higher in cases of longer antibody generation time, higher MFI, and AMR($p<0.05$). The differences in the numbers of mismatched HLA I and II eplets were statistically significant between the rejection and no rejection groups ($p=0.030$). dnDSA-specific and AMR associated mismatched eplets were strongly correlated ($p<0.0001$). The dominant mismatched eplets included 41 T, 163R, 25Q, 78 V, 47QL and 55PP. dnDSA-specific eplets accounted for majority of the total mismatched eplets of donors and recipients. The amino acids with increased proportions of dnDSA-specific eplets were mainly non-polarity amino acids ($p<0.0001$). AMR-associated mismatched eplets accounted for majority of the dnDSA-specific mismatched eplets. Arginine, histidine, glutamine, glutamate, lysine and asparagine levels increased significantly in the rejection group compared with the no rejection group ($p<0.001$). The amino acids with increased proportions of AMR-associated mismatched eplets were all polar ($p<0.0001$) and mainly positively charged ($p<0.0001$). The polarity and charge of amino acids in mismatched eplets may be the key factors affecting the occurrence of AMR after kidney transplantation.

Keywords Kidney transplantation, HLA, Eplet, dnDSA, AMR

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Introduction

Antibody-mediated rejection (AMR), especially chronic active AMR(caAMR), remains the main factor affecting the long-term survival of grafts [1]. Although intravenous immunoglobulin (IVIG) and plasmapheresis have been advocated as the standard of care, particularly in acute AMR, there are no effective treatments for caAMR that would prevent the gradual deterioration of graft function [2–4]. A means of avoiding caAMR is likely to be superior to any available cure [5–7]. De novo donor-specific antibody (dnDSA) serves as a potent biomarker linked to AMR and graft loss following kidney transplantation [8]. This is due to the fact that when dnDSA binds to the kidney graft, it triggers the activation of the complement, subsequently leading to transplant loss [9]. Although the development of dnDSAs remains among the most definitive known risk factors that promote AMR, not all dnDSAs promote AMR [5].

Several factors affect the generation of dnDSA and occurrence of AMR after kidney transplantation. Non-immune factors include donor organ quality, race, sex, body mass index (BMI) and other recipient demographic factors [10–13]. Recipients' sensitization status, post-transplant immune induction program, immunosuppression program of maintenance therapy, patient compliance and other immune factors affect recipients' immune system function [14, 15]. Regardless of the influencing factors, the most critical decision for the generation of dnDSA and the direct cause of AMR is donor-recipient human leukocyte antigen (HLA) mismatch [16–19].

The HLA system is recognized as one of the most intricate polymorphic systems in humans. Since the first HLA discovery, this system has become a key focus in the fields of immunogenetics, immunobiology, and biochemistry [20–22]. HLA typing, which assesses donor-recipient mismatches, is now a critical tool for evaluating the risk of allogeneic HLA recognition [23, 24]. Structural studies of crystallized antibody-HLA complexes reveal that binding specificity is shaped by a small group of amino acids on the HLA, known as the functional epitope. Matching HLA eplets, which are also referred to as 'eplets' in HLAMatchmaker, has become central to discussions around organ transplantation [25, 26], as it is linked to improved allograft outcomes and reduced risk of dnDSA formation [27, 28]. Additionally, antibody-verified mismatched eplets can predict the likelihood of kidney transplant failure [29]. However, not every HLA eplet mismatch triggers an immune response or rejection, making it crucial to identify non-immunogenic eplets that do not lead to rejection. This remains a significant challenge in donor selection for transplantation [30, 31].

In this study, donor-recipient HLA mismatched eplets, dnDSA specific and AMR associated mismatched eplets were analyzed in 64 cases of kidney transplantation with dnDSA. To determine whether the rejection reaction occurred, the time of postoperative dnDSA generation, medium fluorescence index (MFI) value, position of mismatched eplets in HLA molecules, and eplets involved in amino acid physicochemical properties were analyzed. The purpose of this study was to reveal the influence of HLA eplets matching on dnDSA generation and AMR occurrence after transplantation and to provide a basis for individualized and accurate HLA eplets matching between donors and recipients.

Materials and methods

Study participants

This retrospective single-center study included 64 deceased donor kidney recipients who underwent dnDSA post-kidney transplant from January 2017 to December 2020. Patient characteristics and outcomes (dnDSA and rejections) were analyzed with respect to donor and recipient HLA eplets matching. Clinical data were retrieved from the electronic patient database of the First Affiliated Hospital of Xi'an Jiaotong University. Kidney grafts were allocated using the Chinese organ transplant response system. This study was approved by the institutional review board/ethics of the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China (ethics approval number: XJTU1AF2015LSL-058) and was conducted in accordance with the principles of the Declaration of Helsinki.

HLA typing and eplet mismatching identification

HLA typing (HLA-A, B, C and HLA-DRβ1, DQα1/β1,) was performed using sequence-specific oligonucleotide probes technology (LAB Type XR SSO; One Lambda, Canoga Park, CA, USA). Samples without certain determination of alleles at the 2-field typing level, were retested using sequence-based typing (SBT) technology (TBG, Medigen Biotechnology, Taipei, Taiwan). Single-antigen beads (SAB) assays were used to rule out dnDSA at the time of transplantation to ensure that dnDSA was de novo. HLAMatchmaker software (version 3.1, http://www.epitopes.net/downloads.html, University of Pittsburgh Medical Center, USA) was used to define eplet mismatching between the HLA alleles of donors and those of recipients; and to analyze dnDSA-specific eplets. All eplets analyzed in this study were antibody-verified epitopes obtained from the HLA Epitope Registry list (http://www.EpRegistry.com.br).

Immunosuppression and postoperative management

Induction treatment with an intravenous administration of interleukin-2 receptor blocker or anti-thymocyte globulin was performed in all cases. The total dose

of Interleukin-2 receptor blocker was 40 mg in two doses. 20mg at a time. The first 20 mg was administered within 2 h before transplant, and the second 20 mg was administered 4 days after transplant. Rabbit anti-human thymocyte globulin (rATG) [1.25–1.50 mg/ (kg·d), intravenously] was administered on the day of surgery and tapered until discontinuation on postoperative day 5. Methylprednisolone was administered on the day of surgery, tapered along with rATG (500 mg, 250 mg, 120 mg, and 80 mg after surgery, and then replaced with prednisone (10mg/d). The basic immunosuppressive regimen was tacrolimus (0.06–0.08mg/kg/day) or cyclosporine A (4.0–4.5mg/kg/day), mycophenolate mofetil (2000 mg/day), and prednisone (10mg/day).

Anti-HLA antibody monitoring

Posttransplant surveillance for dnDSA was instituted in all patients who underwent kidney transplant. Serum samples were collected at 0, 1, 3, 6, 9, and 12 months and then yearly or at the time of biopsy for graft dysfunction as a routine clinical practice in our program since 2017. dnDSA screening was performed using flow panel of reactive antibody (PRA) beads representing HLA-A, HLA-B, HLA-Cw, HLA-DR, and HLA-DQ antigens (One Lambda). HLA antibody specificities were validated using LABScreen single-antigen beads (One Lambda) at a threshold MFI $\geq$ 1000.

Biopsy pathology

Clinically indicated allograft biopsies were performed on patients in whom dnDSAs appeared or proteinuria was > 0.5 g/d or serum creatinine rose above 25% from baseline due to an unknown cause. Ultrasound-guided percutaneous biopsy was performed using an 18G puncture needle to puncture two tissues. All tissues were routinely fixed, embedded, sliced, and treated with immunofluorescence antibodies, hematoxylin and eosin, periodic acid–Schiff, Masson, periodic Schiff methenamine, and immunohistochemical staining. Two experienced renal transplant pathologists (G.H. L and S.Y.X) evaluated the histology using the Banff criteria 2017 [32].

Definitions

dnDSA is a donor-specific antibody produced after kidney transplantation [33]. Rejection reactivity, including AMR and TCMR, was identified on biopsy and classified according to the Banff 2017 criteria [32].

Statistical analysis

The data were analyzed using SPSS for Windows (version 20.0; IBM Corp., Armonk, NY, USA). Unless otherwise stated, the results are expressed as numerical values and percentages for categorical variables and as mean ± standard deviation for continuous variables. Differences in the clinical characteristics of recipients and donors were examined using Student's *t*-test if the data matched a normal distribution and homogenous variance. The Mann–Whitney U test was used if the data were non-normally distributed. Correlation analysis was conducted using Pearson or Spearman correlation analysis. The chi-square test was used for statistical analysis of the measurement data. A two-sided p value of 0.050 was considered statistically significant.

Results

Cohort characteristics

As illustrated in Fig. 1, a total of 1314 adult patients underwent deceased donor kidney transplantation at our center between 2017 and 2020. We observed and followed up 214 patients for the study. We excluded patients without pathological biopsy and without SAB assay results, those who underwent ABO-incompatible transplants, those with comorbidities (infection, hepatitis, diabetes, autoimmune disease, and tumor) or PRA positivity before transplantation, and those without dnDSA. The final cohort consisted of 64 patients, 39 (60.9%) of whom experienced a rejection reaction. The patient characteristics are listed on Table 1. there were no significant differences in recipient age, sex, body mass index (BMI), dialysis type and duration, induction therapy and maintenance immunosuppression between the rejection and non-rejection groups. Additionally, no significant differences in donor age, sex, BMI, cause of death, terminal creatinine levels, and hypertension history were observed.

Characteristics of dnDSA generation time and MFI value after renal transplantation

A total of 114 types of dnDSAs were produced among the 64 patients. Analysis of dnDSA generation time after kidney transplantation showed that 67.6% of dnDSA was generated within 90 days and 32.4% after 90 days. The average production time (days) of dnDSA against HLA Class II was longer than that of HLA Class I (296.1 ± 64.2 vs. 136.4 ± 34.5, F = 5.206, p = 0.024) (Fig. 2A), especially for HLA-DQ (401.9 ± 96.3, p < 0.05) (Fig. 2B). The ratio of HLA class II dnDSA was significantly higher than that of HLA class I dnDSA in cases in which dnDSA occurred > 90 days post-transplantation (43.1% vs. 23.3%, Z = -2.211, p = 0.027, Z-test) (Fig. 2C), especially for HLA-DQ (Fig. 2D). In addition, the average MFI value of HLA Class II dnDSA was higher than that of HLA Class I dnDSA (10,520.3 ± 1017.9 vs. 7822.0 ± 755.1, F = 4.694, p = 0.032) (Fig. 2E), and the MFI value of HLA-DQ dnDSA was the highest (Fig. 2F). When stratification was performed according to dnDSA MFI

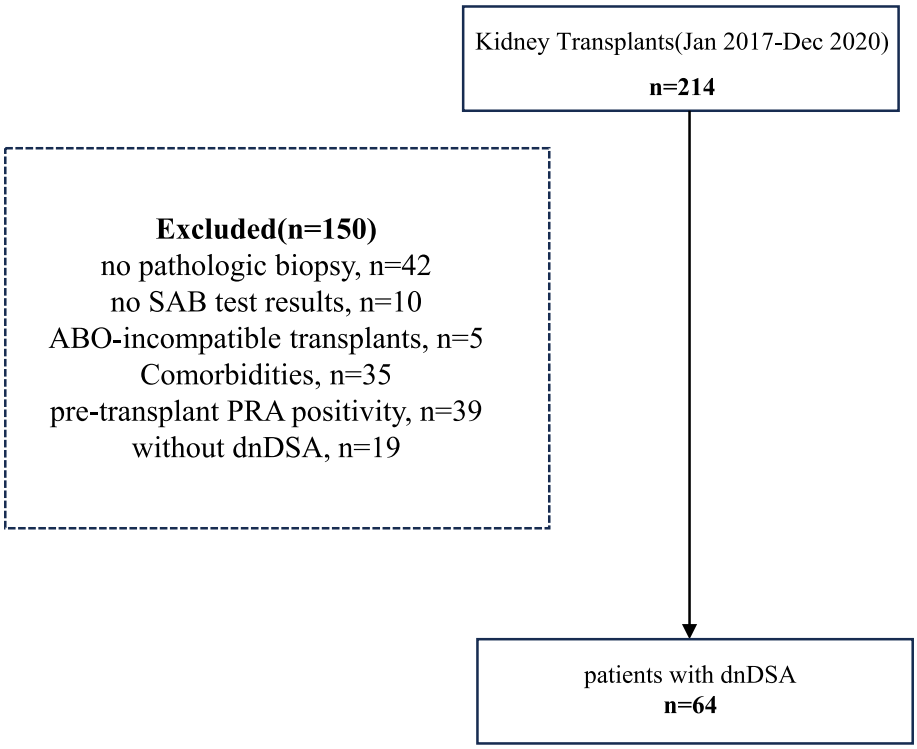

Fig. 1 Flow chart of the study. dnDSA, de novo donor-specific antibody

values (MFI1: ≤ 3000, MFI2: 3000–6000, MFI3: ≥ 6000), we found that the ratio of MFI 3 in HLA Class II dnDSA was significantly higher than that in HLA Class I dnDSA ($p = 0.039$, t-test) (Fig. 2G**).** MFI 3 had the highest ratio of dnDSA at all HLA loci except HLA-C (Fig. 2H).

Association of the generation time, types, and MFI value of dnDSA with rejection after renal transplantation

We found that the mean production time (days) of dnDSA in the rejection group was significantly higher than that in the no rejection group (270.2 ± 46.5 vs. 53.7 ± 28.3, F = 7.932, $p = 0.006$) (Fig. 3A). Among the three groups, the dnDSA generation time was longest in patients with AMR and TCMR (618.2 ± 93.1 vs 53.7 ± 28.3, F = 15.122, $p < 0.001$) (Fig. 3B). In addition, the MFI value of dnDSA in the rejection group was significantly higher than that in the no rejection group (10,520.3 ± 1017.9 vs. 4652.7.0 ± 541.7, F = 17.933, $p < 0.001$) (Fig. 3C), especially in patients with AMR (11,068.9 ± 819.5 vs. 4652.7 ± 541.7, F = 22.642, $p < 0.001$) (Fig. 3D). When stratification was performed according to dnDSA MFI values (MFI1: ≤ 3000, MFI2: 3000–6000, MFI3: ≥ 6000), the ratio of patients with dnDSA MFI ≥ 6000 in the rejection group was much higher than that in the no rejection group (67.5% vs 22.6%, Z = -4.510, $p < 0.001$) (Fig. 3E), especially in the pure AMR group (69.6% vs 22.6%, Z = -4.634, $p < 0.001$) (Fig. 3F). According to the DSA classification, the patients were divided into three groups DSA1 (HLA I dnDSA), DSA2 (HLA II dnDSA), and DSA3 (HLA I & II dnDSA). The proportion of HLA II dnDSA-positive and HLA I & II dnDSA-positive cases in the rejection group was much higher than that in the no rejection group (76.3% vs. 35.5%, Z = -3.864, $p < 0.001$) (Fig. 3G), especially in the pure AMR group (78.2% vs. 35.5%, Z = -3.938, $p < 0.001$) (Fig. 3H).

The association of recipient and donor HLA mismatched eplets with dnDSA generation time, MFI value, and rejection occurrence after renal transplantation

HLA I&II Ab VEps MM was significantly different between dnDSA occurred > 90 days post-transplantation and dnDSA occurred < 90 days post-transplantation groups (18 ± 3.44 vs 15 ± 3.50, F = 10.711, $p < 0.01$) (Fig. 4A). Donor and recipient HLA antibody-verified eplets mismatching (Ab VEps MM) had no significant influence on the MFI value of dnDSA after kidney transplantation (Fig. 4B). Sixty-four dnDSA-positive cases were divided into the rejection (AMR and AMR + TCMR) and no rejection groups according to clinical and pathological findings. Ab VEps MM analysis of HLA I molecules revealed that there was no significant difference between the rejection and no rejection groups (9.60 ± 4.12

Table 1 Clinical information of recipients with and without rejection among those with dnDSA ($n=64$)

	Rejection ($N=39$)	No rejection ($N=25$)	*p*	Test Statistic
Recipient related information				
Age	37.99 ± 11.21	37.69 ± 9.47	0.899	0.129[a]
Male/female	17/22	10/15	0.935	0.007[b]
BMI	20.48 ± 3.03	21.69 ± 3.47	0.138	1.581[a]
Dialysis type			0.318	0.999[b]
Hemodialysis	94.87%	88.00%		
Peritoneal dialysis	5.12%	12.00%		
Dialysis duration (month)	21.38 ± 7.24	20.32 ± 7.09	0.530	-0.628[c]
Induction therapy			0.652	0.204[b]
Anti-thymocyte globulin	79.49%	84.00%		
Basiliximab	20.51%	16.00%		
Maintenance immunosuppression			0.137	3.352[b]
FK506/MPA/Pred	92.31%	76.00%		
CsA/MPA/Pred	7.69%	24.00%		
Donor related information				
Age	51.44 ± 11.21	47.4 ± 9.47	0.141	1.490[a]
Male/female	30/9	21/4	0.492	0.471[b]
BMI	22.26 ± 2.60	23.09 ± 2.87	0.239	1.189[a]
Cause of death			0.898	-1.076[b]
Trauma	65.2%	34.8%		
Hematencephalon	54.8%	45.2%		
Hypoxic encephalopathy	75.0%	25.0%		
Tumor	66.7%	33.3%		
Others	60.9%	39.1%		
Terminal creatinine (μmol/L)	94.54 ± 9.61	78.40 ± 9.82	0.349	-0.936[c]
Hypertension	65.0%	35.0%	0.653	0.202[b]

BMI body mass index. [a]Student's t-test, [b]Chi-square test, [c]Mann–Whitney U

vs. 9.68 ± 2.94, F = 0.008, $p=0.928$) (Fig. 4C). However, HLA I Ab VEps MM in cases of AMR combined with TCMR were significantly higher than that in the pure AMR and no rejection groups (12.82 ± 5.23 vs. 9.09 ± 4.09, F = 7.969, $p=0.006$; 12.82 ± 5.23 vs 9.68 ± 2.94, F = 4.276, $p=0.016$) (Fig. 4D). HLA II Ab VEps MM was significantly different between the rejection and no rejection groups (8.49 ± 3.44 vs 6.06 ± 3.50, F = 10.711, $p=0.001$) (Fig. 4E), and that in the pure AMR group was markedly higher than that in the other two group (8.87 ± 3.50 vs. 6.27 ± 2.00, F = 6.318, p = 0.014; 8.87 ± 3.50 vs. 6.06 ± 3.50, F = 8.353, $p<0.001$). No difference was found between the AMR + TCMR and no rejection groups (Fig. 4F). Regarding total HLA I&II Ab VEps MM, statistically significant differences were found between the rejection and no rejection groups (18.20 ± 5.48 vs. 15.74 ± 4.52, F = 4.853, $p=0.030$) (Fig. 4G). Moreover, HLA I & II Ab VEps MM of the no rejection group was significantly lower than that of the AMR and AMR + TCMR groups (15.74 ± 4.52 vs. 18.05 ± 5.23, F = 5.192, $p=0.025$; 15.74 ± 4.52 vs. 19.09 ± 6.98, F = 3.547, $p=0.047$), and no difference was found between the mixed rejection and AMR groups (Fig. 4H).

Distribution frequency of recipient/donor all, dnDSA-specific and AMR-associated mismatched eplets

In total, 133 classes of eplets were included in this study: 70 HLA I, 32 HLA-DR, and 31 HLA-DQ eplets. There are 92 antibody-specific eplets for dnDSA, including 49 HLA I, 20 HLA-DR, and 23 HLA-DQ eplets. There were 89 AMR-associated eplets, including 46 HLA I, 20 HLA-DR, and 23 HLA-DQ eplets (Table 2). HLA I dnDSA specific and AMR associated mismatched eplets were highly correlated (r = 0.9193, $p<0.0001$). Among them, the dominant eplets (frequency > 0.03) were 41 T, 45KE, 80TLR, 81ALR, 82LR, 144QL, 163EW, 163R, 180E, especially 41 T and 163R. The dominant eplets were mainly from HLA B*13:01, HLA B*13:02, HLA B*40:01, HLA B*40:02, HLA B*07:02, HLA A*23:01, HLA A*23:02, HLA A*24:02, HLA A*66:02, HLA A*01:01,

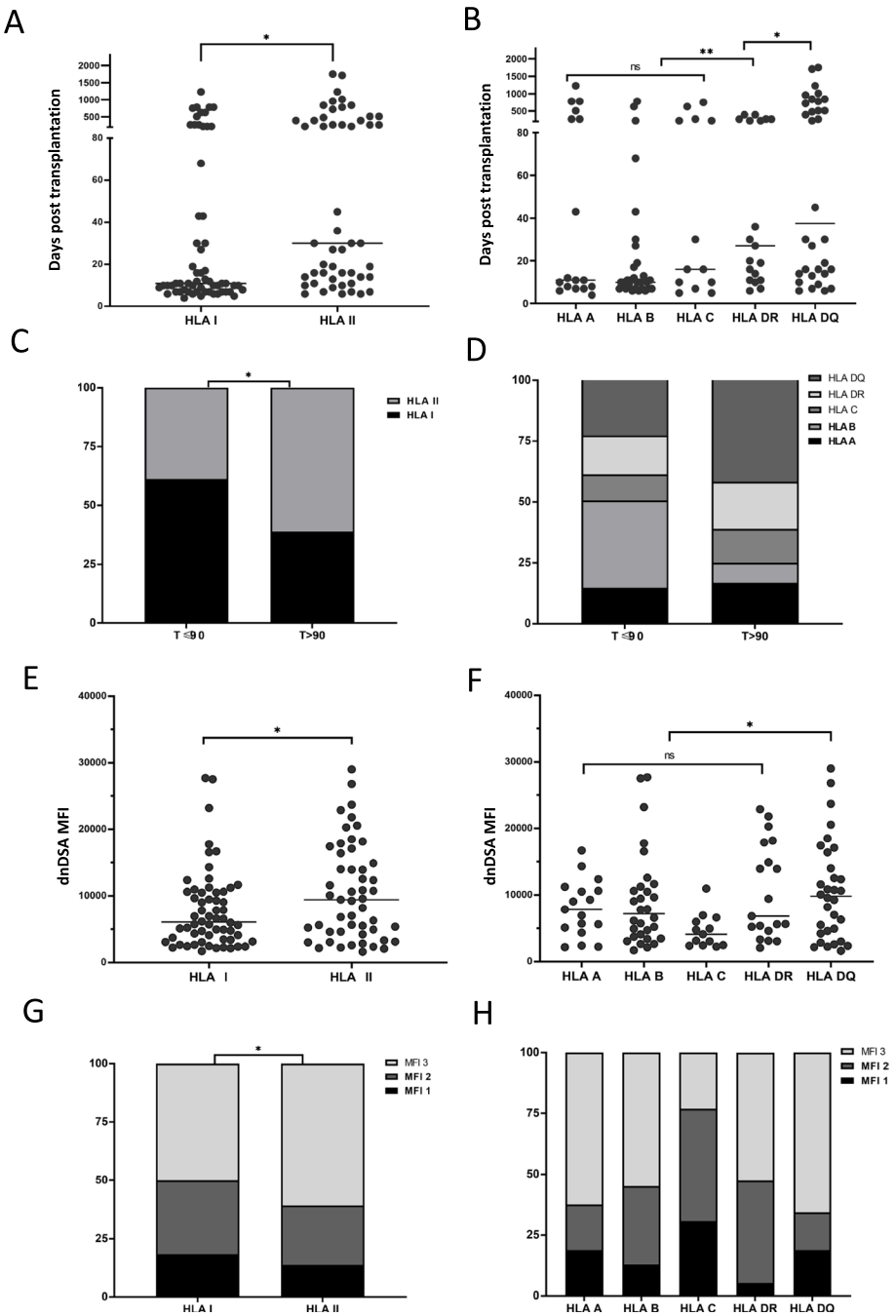

Fig. 2 Characteristics of dnDSA generation time and MFI value after renal transplantation. **A** The occurrence time of dnDSA in the different HLA class after renal transplantation. **B** The occurrence time of dnDSA in the different HLA loci after renal transplantation. **C** The proportion of dnDSA in the different HLA class within and after 90 days of transplantation. **D** The proportion of dnDSA in the different HLA loci within and after 90 days of transplantation. **E** The MFI of dnDSA in the different HLA class after renal transplantation. **F** The MFI of dnDSA in the different HLA loci after renal transplantation. **G** The difference in the proportion of dnDSA MFI stratification between HLA I and HLA II. **H** The difference in the proportion of dnDSA MFI stratification among all HLA loci. MFI1: ≤ 3000, MFI2: 3000–6000, MFI3: ≥ 6000. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; dnDSA, de novo donor specific antibody; HLA, human leukocyte antigen; MFI, mean fluorescence intensity

HLA A*11:01 (Fig. 5A). HLA-DR dnDSA specific and AMR associated mismatched eplets were highly correlated (r = 0.7941, $p < 0.0001$). Among them, the dominant eplets (frequency > 0.05) were 4Q, 25Q, 57 V, 70R, 78 V, 96Y, 181 M, especially 25Q and 78 V. These were mainly from HLA DRB1*07:01, HLA DRB1*09:01,

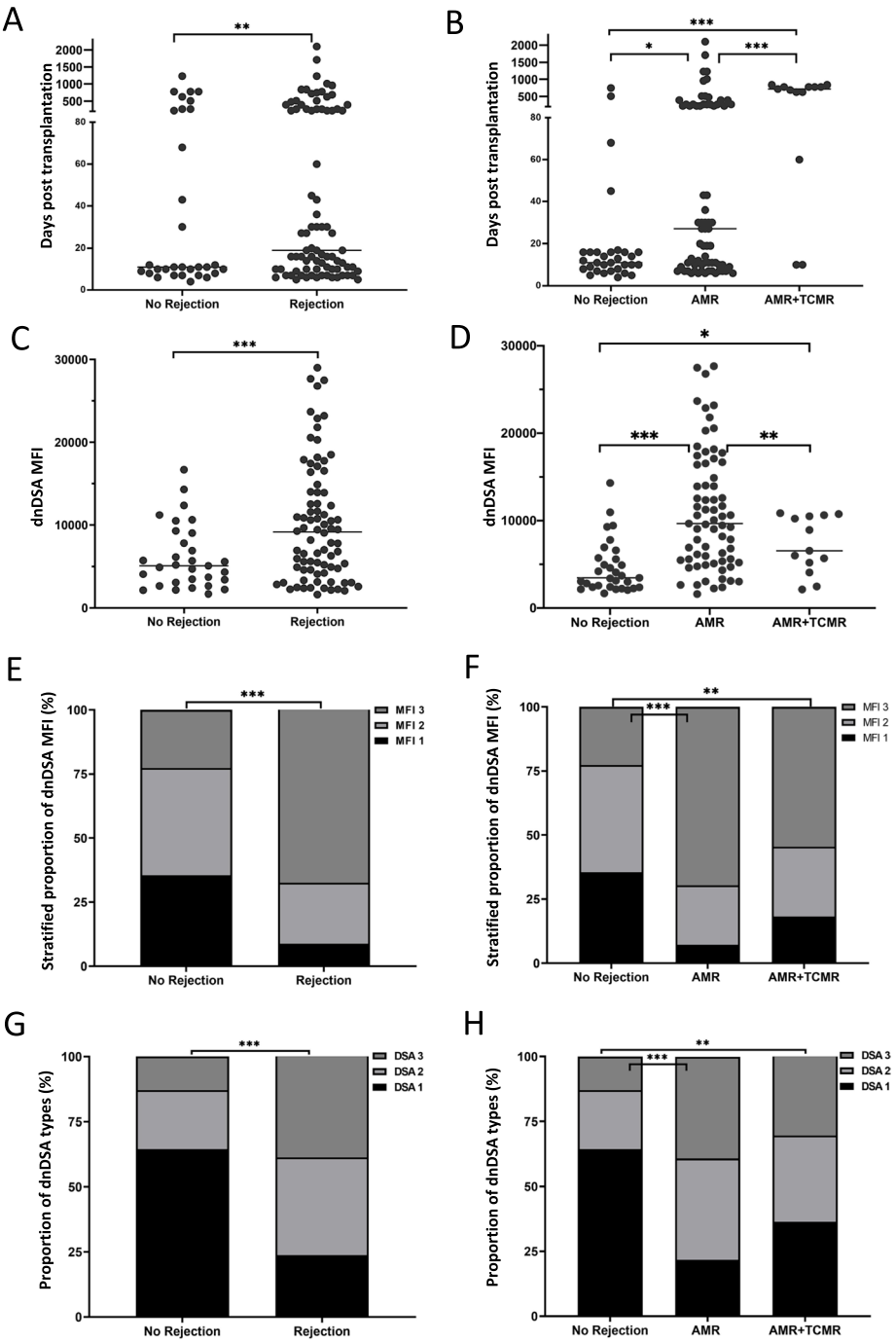

Fig. 3 The association of the generation time, types, and MFI value of dnDSA with rejection after renal transplantation. **A** Influence of time of dnDSA appearance on rejection after renal transplantation. **B** Influence of time of dnDSA appearance on rejection type after renal transplantation. **C** Influence of dnDSA MFI value on rejection after renal transplantation. **D** Influence of dnDSA MFI stratification on rejection after renal transplantation. **E** Influence of dnDSA MFI value on rejection type after renal transplantation. **F** Analysis of the proportion of dnDSA stratification in different types of rejection reactive. **G** Influence of dnDSA type on rejection after renal transplantation. **H** Influence of dnDSA type on rejection type after renal transplantation.$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; AMR, antibody mediated rejection; dnDSA, de novo donor-specific antibody; Eps, eplets; HLA, human leukocyte antigen; MFI, mean fluorescence intensity; TCMR, T cell-mediated rejection

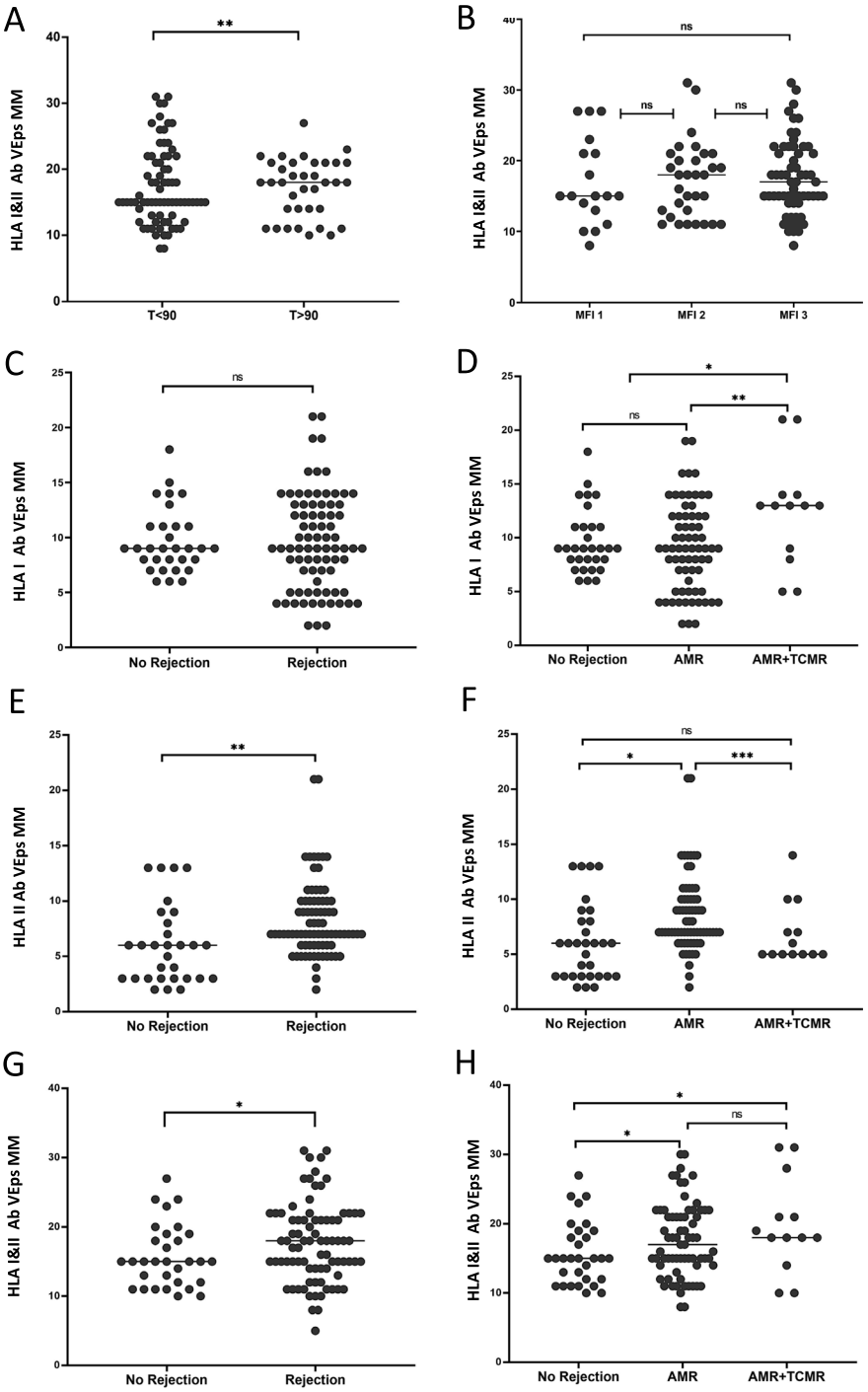

Fig. 4 The association of recipient and donor HLA eplets mismatching with dnDSA generation time and MFI value and AMR occurrence after renal transplantation. **A** Influence of HLA I & II Ab VEps on dnDSA generation time after renal transplantation. **B** Influence of HLA I & II Ab VEps on dnDSA MFI value after renal transplantation, MFI1: ≤ 3000, MFI2: 3000–6000, MFI3: ≥ 6000. **C** Influence of HLA I Ab VEps on rejection after renal transplantation. **D** Influence of HLA I Ab VEps on no rejection, AMR, and AMR + TCMR after renal transplantation. **E** Influence of HLA II Ab VEps on rejection after renal transplantation. **F** Influence of HLA II Ab VEps on no rejection, AMR, and AMR + TCMR after renal transplantation. **G** Influence of HLA I & II Ab VEps on rejection after renal transplantation. **H** Influence of HLA I & II Ab VEps on no rejection, AMR, and AMR + TCMR after renal transplantation. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; Ab VEps, antibody verified eplets; AMR, antibody mediated rejection; dnDSA, de novo donor-specific antibody; HLA, human leukocyte antigen; MFI, mean fluorescence intensity; MM, mismatch; TCMR, T cell-mediated rejection

Table 2 HLA mismatched eplets involved in the cases

	Donor-recipient all mismatched eplets	dnDSA specific mismatched eplets	AMR associated mismatched eplets
HLA I	1C(5), 21H(14), 41T(20), 44KM(2), 44RMA(15), 44RT(20), 45KE(15), 56R(11), 62EE(7), 62GE(11), 62LQ(2), 62QE(10), 62RR(14), 65GK(7), 65QIA(4), 65RNA(8), 69AA(8), 69TNT(8), 71ATD(2), 71TTS(12), 73AN(10), 73TVS(8), 76ANT(7), 76ESI(1), 76ESN(9), 76VRN(2), 79GT(1), 80I(15), 80K(14), 80N(1), 80TLR(16), 82LR(14), 90D(11), 107W(13), 127K(11), 131S(3), 138K(2), 138MI(5), 143S(8), 144K(5), 144KR(9), 144QL(17), 144TKH(13), 145KHA(14), 145RT(6), 149TAH(7), 150AAH(8), 151AHA(14), 156DA(8), 158T(7), 161D(3), 163EW(18), 163LS(6), 163LW(14), 163R(16), 166DG(7), 173K(13), 177KT(11), 180E(10), 193PL(15), 193PV(1), 219W(15), 248M(8), 253Q(9), 267QE(15)	41T(9), 44KM(1), 44RT(3), 45KE(6), 56R(2), 62EE(2), 62GE(2), 62GK(3), 62GRN(3), 62LQ(1), 65GK(3), 69TNT(2),70IAQ(2), 71ATD(1), 71TTS(2), 73AN(1), 73TVS(1), 76ANT(2), 76ESN(3), 80I(4), 80K(2), 80TLR(8), 81ALR(6), 82LR(6), 90D(3), 107W(3), 127K(2), 131S(1), 144KR(2),144TKH(2), 143S(3), 144QL(5), 145KHA(2), 145RT(1), 150AAH(1), 151AHA(2), 156DA(3), 163EW(5), 163LS(3), 163R(10), 166DG(1), 177KT(1), 180E(4), 193AV(3), 193PL(1), 219W(4), 248M(2), 253Q(2), 267QE(1)	41T(7), 44KM(1), 44RT(3), 45KE(5), 56R(2), 62EE(1), 62GE(2), 62GK(3), 62GRN(2), 62LQ(1), 65GK(1), 69TNT(1), 70IAQ(2), 71TTS(2), 73AN(1), 76ANT(2), 76ESN(3), 80I(2), 80K(1), 80TLR(5), 81ALR(4), 82LR(4), 90D(3), 107W(3), 127K(1), 131S(1), 143S(3), 144KR(2), 144QL(4), 144TKH(2), 145KHA(2), 145RT(1), 150AAH(1), 151AHA(1), 156DA(2), 163EW(5), 163LS(2), 163R(7), 166DG(1), 177KT(1), 180E(4), 193AV(3), 253Q(2)
HLA DR	4Q(22), 4R(8), 11STS(9), 13FE(19), 16Y(18), 25R(1), 25Q(10), 30RV(1), 37L(11), 37YV(15), 47F(16), 57DE(4), 57S(9), 57V(20), 58E(10), 67LQ(17), 70D(16), 70QT(3), 70R(16), 73A(1), 74R(4), 77N(4), 77T(1), 96EV(4), 96H(9), 96Y(12), 98E(21), 104A(21), 142M(10), 181M(3)	4Q(4), 4R(1), 13FE(1), 16Y(1), 25Q(4), 37L(2), 37YV(1), 40YD(1), 47F(1), 57DE(1), 57V(2), 67LQ(1), 70QT(1), 70R(4), 78V(5), 96H (1), 96Y(3), 98E(1), 142M(1), 181M(3)	4Q(4), 4R(1), 13FE(1), 16Y(1), 25Q(3), 37L(1), 37YV(1), 40YD(1), 47F(1), 57DE(1), 57V(2), 67LQ(1), 70QT(1), 70R(2), 78V(5), 96HK(1), 96Y(3), 98E(1), 142M(1), 181M(3)
HLA DQ	2D(18), 40E(20), 40GR(12), 45EV(16), 45GV(6), 46VY(5), 47KHL(4), 52LL(11), 52PQ(15), 55PP(18), 55R(10), 52SK(22), 56L(2), 57V(7), 61FT(10), 61GR(22), 74S(10), 75S(11), 75VT(2), 76V(20), 77R(13), 77T(6), 84QL(9), 87F(17), 87Y(12), 125SQ(7), 182S(12)	2D(3), 40E(4),40GR(4), 45EV(2), 45GV(5), 46VY(3), 47KHL(1), 47QL(11), 52LL(2), 52PL(5), 52PQ(5), 52SK(5), 55PP(10), 55RL(1), 61FT(3), 74SV(2), 75S(3), 77R(2), 77T(2), 84QL(3), 85VY(2), 87F(3), 140A(3)	2D(1), 40E(3), 40GR(4), 45EV(1), 45GV(3), 46VY(1), 47KHL(1), 47QL(10), 52LL(2), 52PL(4), 52PQ(3), 52SK(3), 55PP(7), 55RL(1), 61FT(2), 74SV(1), 75S(2), 77R(1), 77T(1), 84QL(2), 85VY(1), 87F(2), 140A(2)

Numbers in parentheses are the count of eplet in this study

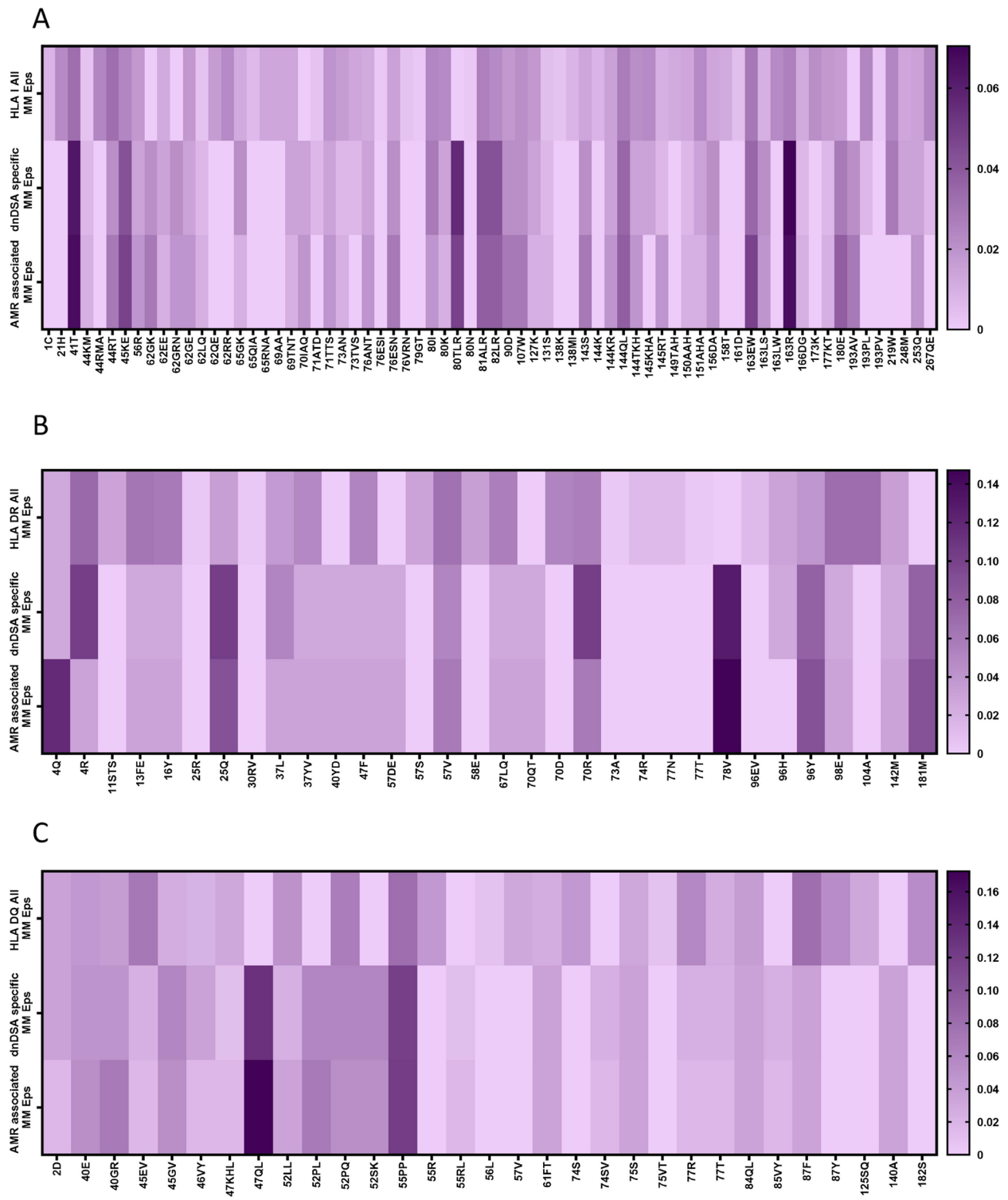

Fig. 5 The distribution frequency of recipient-donor all, dnDSA specific and AMR-associated mismatched eplets. **A** HLA I mismatched eplets. **B** HLA-DR mismatched eplets. **C** HLA-DQ mismatched eplets. AMR, antibody mediated rejection; dnDSA, de novo donor-specific antibody; MFI, mean fluorescence intensity; MM, mismatch

HLA DRB1*09:02, HLA DRB1*04:01, HLA DRB1*04:02 (Fig. 5B). HLA-DQ dnDSA-specific and AMR associated mismatched eplets showed a very high correlation (r = 0. 0.9637, $p < 0.0001$). Among them, the dominant eplets (frequency > 0.05) were 40E, 40GR, 45GV, 47QL, 52PL, 52PQ, 52SK, 55PP, especially the 47QL and 55PP. They were mainly from HLA DQB1*03:01, HLA DQB1*03:02, HLA DQB1*03:03, HLA DQA1*01:01, DQA1*01:02, DQA1*04:01, DQA1*04:02 (Fig. 5C). In addition, some dnDSA-specific eplets did not appear in the donor-recipient mismatched eplets or the HLA epitope registry list (62GK, 70IAQ, 81ALR, 40YD, 78 V, 47QL, 52PL, 55PL, 74SV) (Table 2).

Characteristics of dnDSA specific mismatched eplets

In this study, 72 types of dnDSA-specific and 52 types of non dnDSA-specific eplets were identified. The median amino acid positions of HLA-DQ mismatched eplets were significantly different between dnDSA-specific and non dnDSA-specific mismatched eplets [101(75, 153) vs 53(46, 76) (Z = -3.342, $p = 0.0004$), while the other loci were not different (Fig. 6A). dnDSA-specific eplets of HLA I, HLA-DR and HLA-DQ accounted for majority of the total number of donors and recipients eplets (Fig. 6B). Hydrophobic and neutral amino acids were common in both non dnDSA-specific and dnDSA-specific eplets, with no statistically significant difference between them (Fig. 6C). It shows the proportion of amino acids involved in non dnDSA-specific and dnDSA-specific eplets (Fig. 6D). According to the results shown, amino acids involved in dnDSA-specific eplets were divided into groups with no change in proportion (no change), an increase in proportion (up) and a decrease in proportion (down). The amino acids with increased proportions were mainly nonpolar amino acids ($X^2 = 99.46$, $p < 0.0001$) (Fig. 6E), whereas those with reduced and unchanged proportions were mainly positively charged and neutral amino acids, respectively (Fig. 6F).

Characteristics of AMR-associated mismatched eplets

All patients with dnDSA were divided into no rejection and rejection groups according to the occurrence of AMR. The median amino acid positions of dnDSA-specific mismatched eplets did not differ between the no rejection and rejection groups for any HLA locus. (Fig. 7A). AMR-associated mismatched eplets of HLA I, HLA-DR and HLA-DQ accounted for majority of dnDSA-specific mismatched eplets (Fig. 7B). Hydrophobic and neutral amino acids were more common in both the no rejection and rejection groups, and there was no statistically significant difference between them (Fig. 7C). According to the results in Fig. 7D, amino acids involved in AMR-associated eplets were divided into groups with no change in proportion (no change), an increase in proportion (up) and a decrease in proportion (down). The amino acids involved in all eplet-induced dnDSAs included all amino acids (except cysteine), among which leucine, arginine, glutamine and threonine were the most common amino acids (Fig. 7D). The proportion of arginine, histidine, glutamine, glutamate, lysine and asparagine were higher in the rejection group compared with the no rejection group ($p < 0.001$) (Fig. 7D). The amino acids with increased proportions were all polar amino acids ($X^2 = 128.1$, $p < 0.0001$) and mainly positively charged ($X^2 = 194.7$, $p < 0.0001$) (Fig. 7E), whereas those with reduced and unchanged proportions were mainly non-polar and neutral amino acids, respectively (Fig. 7F).

Discussion

Our study included 64 dnDSA-positive cases, involving a total of 114 types of dnDSA. HLA II dnDSA had a higher generation time and MFI value compared with HLA I dnDSA. dnDSA generation time and MFI values in the rejection group were also higher than those in the no rejection group. The number of eplet mismatches in the rejection group was considerably higher than that in the no rejection group. The difference in the proportion of amino acids in the mismatched eplets between the no rejection and rejection groups was statistically significant. AMR-associated mismatched eplets accounted for majority of the dnDSA-specific mismatched eplets. The amino acids with increased proportions of AMR-associated mismatched eplets were all polar and mainly positively charged.

In our previous study, we also found that early postoperative rejection was closely related to DGF (delayed graft function), HLA B eplets mismatch, and early DSA production. It has also been reported in other literature that ischemia in perfusion injury is one of the triggers of postoperative DSA and rejection [34]. Therefore, we hypothesise that the cause of early DSA generation is mainly due to ischaemia–reperfusion injury, which results in the release of a large amount of graft HLA antigens, which stimulates the recipient's immune system to have a higher chance of generating a reaction. The period of about 40 days postoperatively is also more consistent with the time when the immune system reacts to foreign antigens and produces antibodies. The factors affecting the production of DSA in the second 500–1500 days are complex, but of course, the first cause is the donor-recipient HLA epitope mismatch, and the second is the insufficient intensity of immunosuppression in the patient and the poor adherence to the procedure [35, 36]. In the later 500–1500 days of DSA production, there is an increase in the proportion of HLA class II antigens, especially DQ antigens, and we hypothesise that since HLA class II antigens are

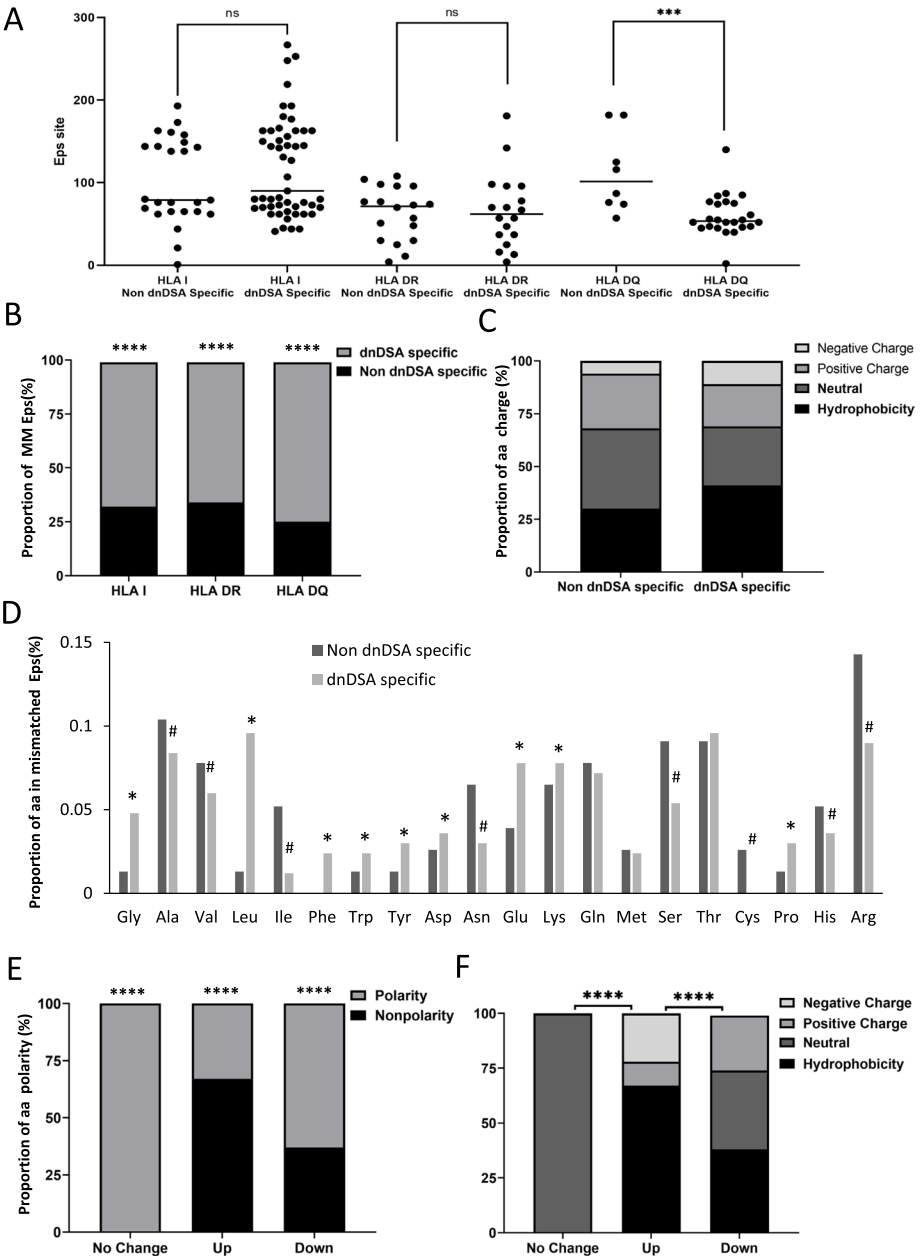

Fig. 6 Characteristics of dnDSA specific mismatched eplets. **A** Comparison of median amino acid positions for non dnDSA specific eplets and dnDSA specific eplets. **B** Ratio of non dnDSA specific eplets and dnDSA specific eplets of HLA. **C** Proportion of the charge of the amino acid involved in non dnDSA specific eplets and dnDSA specific eplets. **D** Frequency of the amino acids involved in non dnDSA specific and dnDSA specific mismatched eplets. **E** Proportion of the amino acids polarity in dnDSA specific MM eplets. **F** Proportion of the amino acids charge in dnDSA specific MM eplets. ***$p < 0.001$; ****$p < 0.0001$; Eps, eplets; HLA, human leukocyte antigen; MM, mismatch

mainly expressed in monocyte macrophages, activated lymphocytes such as T and B lymphocytes, and vascular endothelial cells, due to the transplantation of a small number of infiltrating donor-derived monocyte macrophages, and T and B lymphocytes, the early production of DSA is likely to result in a small number of infiltrating donor-origin macrophages, and T and B lymphocytes, B lymphocytes, there is a small chance of early production of antibodies against HLA class II antigens. As the transplantation progresses, vascular endothelial cell damage from various causes induces the expression of HLA class II antigens, which stimulates the recipient's immune system to respond and produce antibodies against HLA class II antigens.

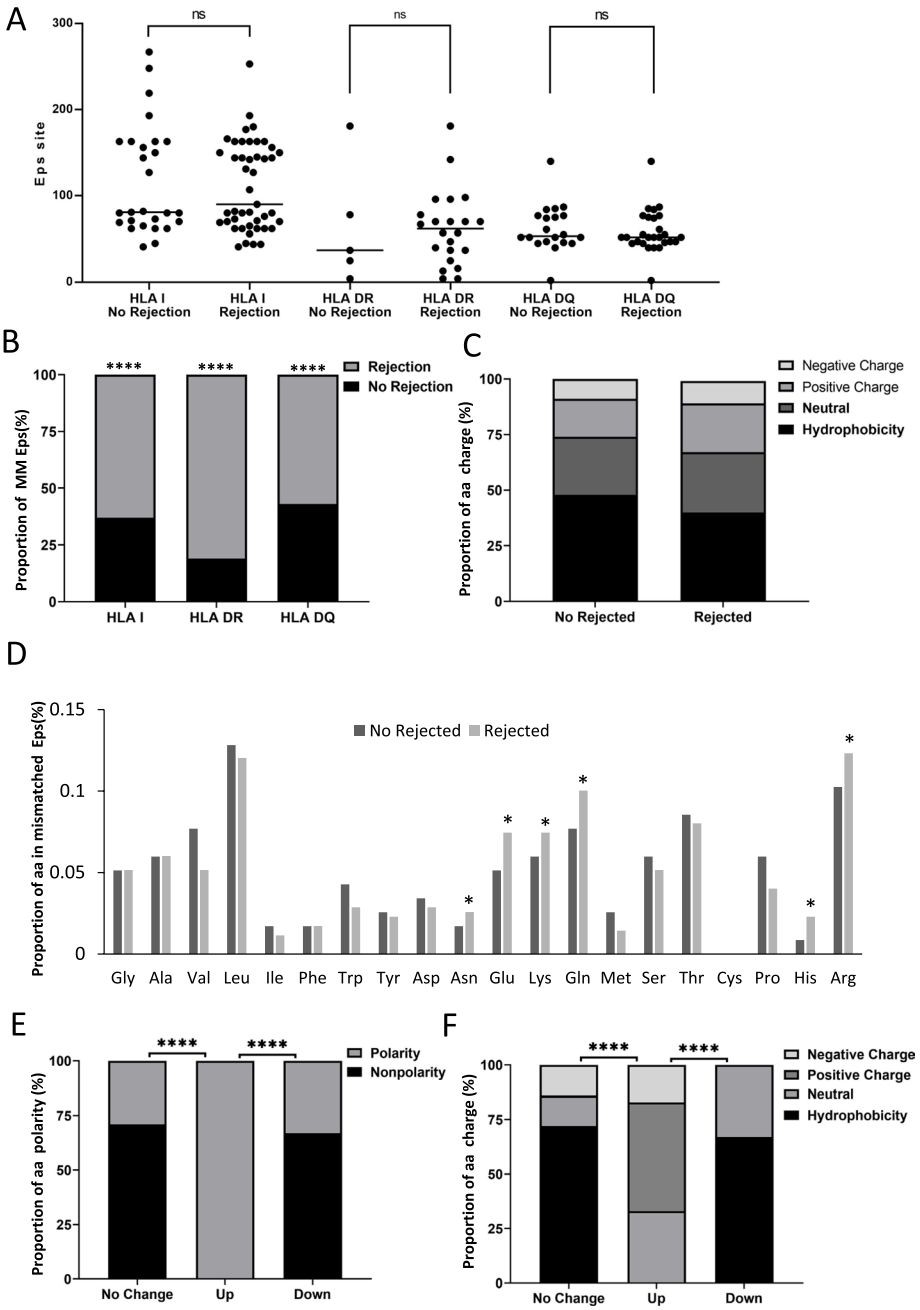

Fig. 7 Characteristics of AMR-associated mismatched eplets. **A** Comparison of median amino acid positions for no rejection-associated and rejection-associated mismatched eplets. **B** Ratio of no rejection-associated and rejection-associated mismatched eplets of HLA. **C** Proportion of the charge of the amino acid involved in no rejection-associated and rejection-associated mismatched eplets. **D** Frequency of the amino acids involved in no rejection-associated and rejection-associated mismatched eplets. **E** Proportion of the polarity of the amino acid in rejection-associated mismatched eplets. **F** Proportion of the amino acids charge in rejection-associated mismatched eplets. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$; Aa, Amino acid; dnDSA, de novo donor-specific antibody; HLA, human leukocyte antigen; MM, mismatch

We found that although donor and recipient HLA eplets mismatching was not directly related to the generation time of dnDSA after kidney transplantation or the MFI value, it was related (to an extent) to the occurrence of rejection after kidney transplantation. HLA I eplet mismatching had little influence on the occurrence of rejection reaction. However, HLA II eplet mismatching in patients with rejection was significantly higher than that in patients without rejection, although these patients also produced dnDSA. This may be because

HLA II mismatching is a risk factor for AMR, and the patients included in this study all had dnDSAs [37–40]. In addition, HLA I eplet mismatching in the mixed rejection group (AMR + TCMR) was significantly higher than that in the no rejection and AMR groups, whereas HLA II eplet mismatching in AMR was considerably higher than that in the no rejection and mixed rejection groups. According to the rejection mechanism, recipient antigen-presenting cells (APCs) promote proliferation and differentiation of CD4 + and CD8 + T cells. CD8 + T cells mediate cellular rejection in both intravascular and extravascular compartments, pathologically as endothelitis and tubulitis, respectively. CD4 + T cells stimulate B cell proliferation and ultimately antibody production [41]. Therefore, our findings suggest that HLA I eplet mismatching may be related to the occurrence of TCMR and have a synergistic effect with HLA II eplet mismatching to induce the simultaneous occurrence of TCMR and AMR.

All HLA dnDSA-specific mismatched eplets are closely correlated with AMR-associated mismatched eplets. All the dominant mismatched eplets, especially DQB1:03:01 (DQ7), DQB1:03:02 (DQ8) and DQB1:03:03 (DQ9), come from the common and well document HLA Alleles in China [42]. The epitopes of HLA-DQ are more complex because both their α and β chains are polymorphic. Analysis of HLA-DQ antibodies becomes even more complicated when considering the two possible forms of HLA-DQ antigens that can be expressed on the cell surface. Thus, HLA-DQ dnDSAs can theoretically be formed against a DQβ or DQα chain or both [43–45], which may explain why HLA-DQ dnDSA is more common. The HLA Epitope Registry has documented all theoretically defined HLA eplets along with their antibody verification status and has been the foundation for many clinical studies investigating eplet mismatch in transplantation. Up to now, the HLA Epitope Registry has a list of antibody-verified epitopes recorded this far for each locus, but the repertoire is still incomplete [46]. However, some dnDSA-specific eplets did not appear in the donor-recipient mismatched eplets or the HLA epitope registry list (62GK, 70IAQ, 81ALR, 40YD, 78V, 47QL, 52PL, 55PL, 74SV). we did not find a satisfactory explanation for this phenomenon and hope to accumulate more cases and find clues in follow-up studies.

Electrostatic disparity of donor HLA compared with recipient HLA molecules, as assessed by EMS-3D, was reportedly strongly associated with the risk of DSA development and electrostatic potential disparities were highest among HLA-DQ molecules, which were the most immunogenic alloantigens [47]. The ability of donor HLA to trigger alloantibody responses (HLA immunogenicity) relies on its structural recognition by receptors on recipient B cells initiating the immune response. Their structural recognition is related to amino acid polarity, electrostatic charge, aliphatic group, aromatic group and size [48]. We analyzed the amino acid properties of dnDSA-specific and AMR associated mismatched eplets, and found that hydrophobic and neutral amino acids were more common than charged amino acids. The amino acids with increased proportions in dnDSA-specific mismatched eplets were mainly nonpolar amino acids. The amino acids with increased proportions of AMR-associated mismatched eplets were all polar and mainly positively charged, whereas those with reduced and unchanged proportions were mainly non-polar and neutral amino acids, respectively. These results indicate that the polarity and charge of amino acids may be the key factors affecting eplets immunogenicity and immunoreactivity. In the future, we will continue to conduct further studies through in vivo and in vitro experiments and look forward to exploring the factors that influence dnDSA immunogenicity and AMR.

The reasons for dnDSA production without AMR in patients who have undergone transplantation are complex. There are many influencing factors, including dnDSA MFI value and immunoglobulin G subtype, complement activation pathway, and the number of effective immune cells [49–52]. The results of this study showed that the higher the MFI value of dnDSA, the longer the generation time after transplantation. HLA II dnDSA, especially HLA-DQ dnDSA, and the polarity and charge of amino acids involved in mismatched eplets, would affect the occurrence of AMR. In addition, owing to the limitations of sample size and follow-up duration, the results of this study need to be further verified by larger sample sizes and multicenter clinical studies.

All the rejection reactions in this study were AMR, and 13 of them were accompanied by TCMR, that is, mixed rejection reactions (AMR + TCMR), and there were no cases of TCMR alone. We first analyzed the differences in antibody production time and MFI values between the rejection groups. The results showed that DSA production time was significantly later in the mixed rejection group than in the AMR group, and MFI values were significantly lower than in the AMR group. We further compared the number of HLA epitope mismatches between the AMR group and the mixed rejection group, and the results of this study showed that the number of HLA class I epitope mismatches was significantly higher than the number of HLA class II epitope mismatches in the mixed rejection group (Fig. 3D), yet the number of HLA class II epitope mismatches was significantly lower than the number of HLA class II epitope mismatches in the mixed rejection group (Fig. 3F). Then, the total number of HLA I + II epitope

mismatches was not statistically significant between the AMR group and the mixed rejection group. However, due to the small number of cases in the mixed rejection group in this study and the fact that it was a single-center clinical study, it is uncertain whether the current results reflect the real situation. In order to compensate for the above shortcomings, we will subsequently increase the sample size by including samples from other centers in China for the analysis of multicenter samples, with the expectation that we will find out whether it is a specific mismatch of eplet characteristics associated with different rejection subtypes.

In summary, our study identified that the postoperative generation time and MFI value of HLA II dnDSAs were significantly higher than those of HLA I dnDSAs and that HLA II dnDSAs were more likely to appear in cases of longer antibody generation time, higher MFI value, and AMR. The difference in the number of mismatched eplets of HLA I and HLA II between the rejection and no rejection groups was statistically significant. dnDSA-specific and AMR associated mismatched eplets were highly correlated. The amino acids with increased proportions of dnDSA-specific eplets were mainly nonpolar, while those with increased proportions of AMR-associated mismatched eplets were all polar and mainly positively charged. The polarity and charge of amino acids in mismatched eplets may be the key factors influencing the occurrence of AMR after kidney transplantation. We will follow up with further exploration and validation through animal models and other means, in anticipation of providing more meaningful references for dnDSAs and the occurrence of AMR after kidney transplantation.

Authors' contributions

MHL and GXZ participated in the research design, writing of the paper, performance of the research and data analysis. PL,YXL and XZ participated in research and data analysis. PDK, YZ, YW, MYZ, and XMD participated in the research performance, while JZ and WJX participated in the research design and writing of the paper. All authors were responsible for providing intellectual content of critical importance to the work described and approved of the version to be published.

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Data availability

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding authors.

Declarations

Ethics approval and consent to participate

This study was approved by the institutional review board/ethics of the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China (ethics approval number: XJTU1AF2015LSL-058) and was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all study participants. Legally Authorized Representatives of illiterate participants provided informed consent for the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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