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Prediction of HLA-DQ in Deceased Donors and its Clinical Significance in Kidney Transplantation

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Background: HLA-DQ typing in deceased donors is not mandatory in Korea. Therefore, when patients develop DQ antibodies after kidney transplantation (KT) from deceased donor, it is impossible to determine whether they are donor-specific antibodies (DSA). We developed DQ prediction programs for the HLA gene and evaluated their clinical utility.

Methods: Two HLA-DQ prediction programs were developed: one based on Lewontin's linkage disequilibrium (LD) and haplotype frequency and the other on an artificial neural network (ANN). Low-resolution HLA-A, -B, -DR, and -DQ typing data of 5,603 Korean patients were analyzed in terms of haplotype frequency and used to develop an ANN DQ prediction program. Predicted DQ (pDQ) genotype accuracy was analyzed using the typed DQ data of 403 patients. pDQ DSA agreement, sensitivity, specificity, and false-negative rate was evaluated using 1,970 single-antigen bead assays performed on 885 KT recipients. The clinical significance of DQ and pDQ DSA was evaluated in 411 KT recipients.

Results: pDQ genotype accuracies were 75.4% (LD algorithm) and 75.7% (ANN). When the second most likely pDQ (LD algorithm) was also considered, the genotype accuracy increased to 92.6%. pDQ DSA (LD algorithm) agreement, sensitivity, specificity, and false-negative rate were 97.5%, 97.3%, 98.6%, and 2.4%, respectively. The antibody-mediated rejection treatment frequency was significantly higher in DQ or pDQ DSA-positive patients than in DQ or pDQ DSA-negative patients ($P<0.001$).

Conclusions: Our DQ prediction programs showed good accuracy and could aid DQ DSA detection in patients who had undergone deceased donor KT without donor HLA-DQ typing.

Key Words: Artificial neural network, Donor-specific antibody, HLA-DQ, Kidney transplantation, Linkage disequilibrium

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INTRODUCTION

The clinical significance of HLA-DQ antibodies in kidney transplantation (KT) has been highlighted by the development of molecular HLA typing and single-antigen bead (SAB) assay. It is known that 15%–30% of patients develop donor-specific antibodies (DSA) within three years of KT, and 33%–77% of them develop DQ DSA, which is associated with poor graft outcome [1–7]. Given its clinical significance, HLA-DQ typing for deceased donors is required by Eurotransplant (Austria, Belgium, Croatia, Germany, Hungary, Luxembourg, the Netherlands, and Slovenia) and the United Network for Organ Sharing (USA) [8, 9].

However, HLA-DQ typing for deceased donors is not yet required in Korea. Furthermore, the Korean Network for Organ

Sharing has no regulations regarding deceased donor specimen storage for additional HLA typing, making retrospective HLA typing nearly impossible. Therefore, when a patient develops DQ antibodies after deceased donor KT, it is impossible to determine whether these antibodies are donor-specific or not.

HLA shows typical haplotype characteristics according to individual ethnic groups [10, 11]. Therefore, HLA-DQ can be predicted using HLA haplotype frequency and linkage disequilibrium (LD) data. We developed a DQ prediction program based on HLA-A, -B, and -DR and analyzed the clinical significance of the predicted DQ (pDQ) in KT recipients. To the best of our knowledge, this is the first study to predict HLA-DQ using an artificial neural network (ANN) and to evaluate its clinical significance.

MATERIALS AND METHODS

Data source and study population

HLA typing data, SAB assay data, and patients' medical records were retrospectively reviewed. To develop the DQ prediction program and evaluate its accuracy, low-resolution HLA-A, -B, -C, -DR, -DQ typing data of 6,006 Korean patients registered at Asan Medical Center, Seoul, Korea from October 2005 to April 2019 were used. The data of 5,603 patients were analyzed in terms of allele and haplotype frequencies and used to train an ANN.

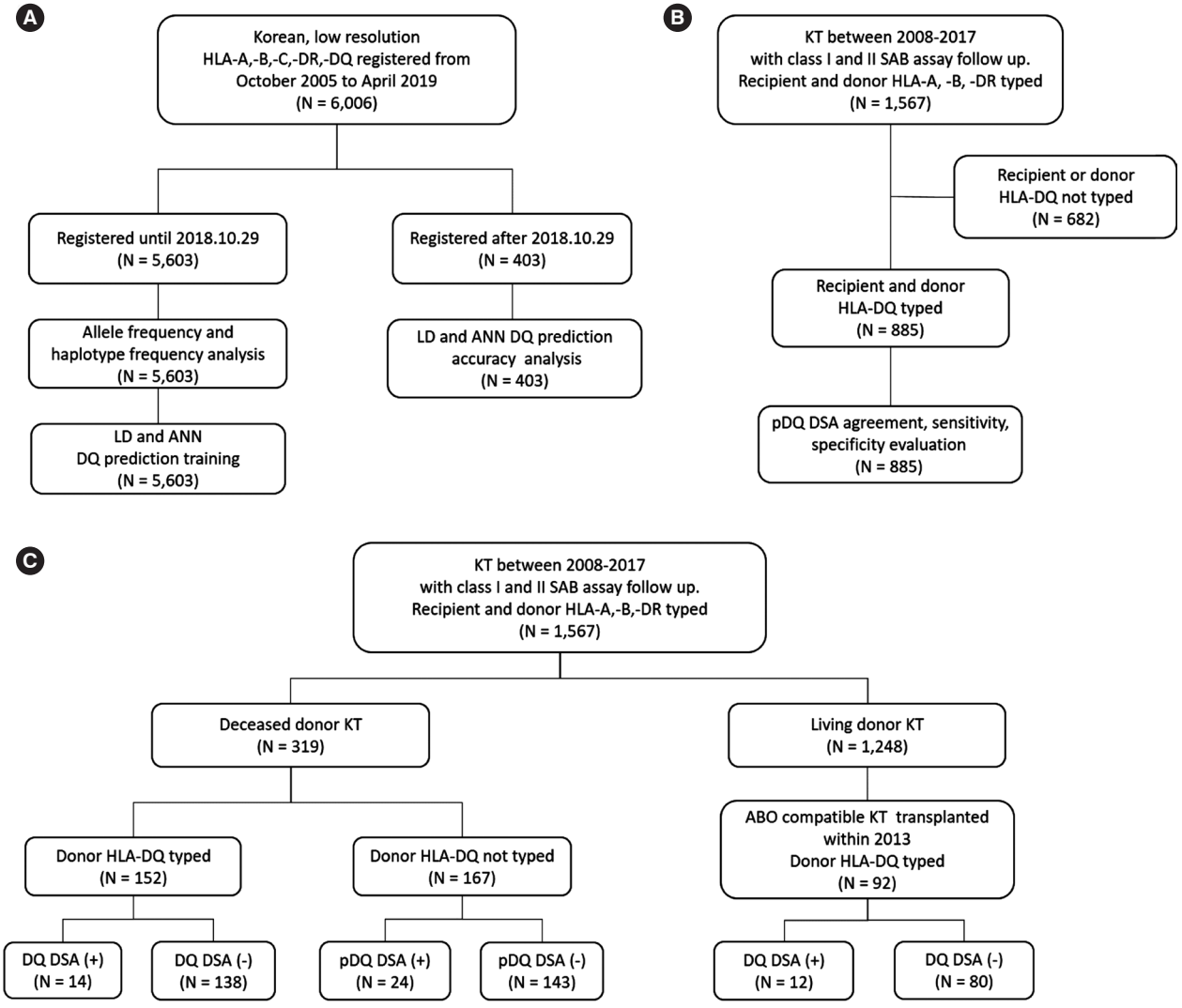

Fig. 1. Patient and data selection flowchart. (A) HLA-DQ prediction program development and evaluation. (B) Evaluation of pDQ DSA agreement, sensitivity, and specificity. (C) Evaluation of DQ and pDQ DSA clinical significance.
Abbreviations: ANN, artificial neural network; DSA, donor specific antibody; KT, kidney transplantation; LD, linkage disequilibrium; pDQ, predicted DQ; SAB, single-antigen bead.

The data of the remaining 403 patients were used to evaluate the accuracy of the HLA-DQ prediction program (Fig. 1A).

To evaluate pDQ DSA agreement, sensitivity, specificity, and clinical significance, we enrolled Korean patients who underwent KT between January 2008 and December 2017, whose recipient/donor HLA-A, -B, -DR typing data were available, and who had undergone follow-up class I and II SAB assays. Patients who underwent kidney re-transplantation or any other type of transplantation were excluded. A total of 1,567 patients met the inclusion criteria. For pDQ DSA agreement, sensitivity, and specificity analyses, recipients/donors with unknown HLA-DQ were excluded. In total, 885 recipients/donors with available HLA-DQ typing data were included (Fig. 1B). To evaluate the clinical significance of pDQ DSA, previously selected 1,567 patients were divided into the deceased donor KT and living donor KT groups. The deceased donor KT group (N=319) was subdivided according to donor HLA-DQ availability and DQ or pDQ DSA positivity. In the living donor KT group (N=1,248), 92 patients who had ABO-compatible KT in 2013 were selected and subdivided according to DQ DSA positivity (Fig. 1C). This study was approved by the Institutional Review Board of Asan Medical Center (S2019-0353-0001).

HLA typing and DSA detection

The HLA-A, -B, -C of KT recipients and living donors were analyzed using AVITA Cross sequence-based typing (SBT) (Biowithus, Seoul, Korea), while their HLA-DR and -DQ were analyzed using AVITA SBT (Biowithus). HLA-A, -B, -C, -DR of deceased donors were typed using a PCR/sequence-specific primer (SSP) kit (BioSewoom Inc., Seoul, Korea). Since November 2015, deceased donor HLA-DQ has been typed using AVITA SBT (Biowithus). HLA DSA was screened using LABScreen Single Antigen assay (One Lambda, Canoga Park, CA, USA). Normalized mean fluorescence intensity (MFI) was calculated using an HLA fusion software (One Lambda), and an MFI of 1,000 was set as a cutoff.

Development of HLA-DQ prediction programs

Two DQ prediction programs were developed, one based on LD and the other on a multilayer perceptron ANN.

The LD algorithm used Lewontin's *D´* to predict HLA-DQ using HLA-B and -DR information [12]. In each case, possible HLA-B-DR haplotypes were generated, and the frequency of each haplotype was calculated. The *D´* was then calculated between possible HLA-B-DR haplotypes and every DQ type in our database. The HLA-DQ showing the highest *D´*, and the most frequent HLA-B-DR-DQ haplotype was chosen as the pDQ. The second most likely pDQ, showing the second-highest D' or the second most frequent HLA-B-DR-DQ haplotype, was also generated by the LD algorithm. The second most likely pDQ was only applied for pDQ accuracy evaluation.

The ANN model predicted the HLA-DQ with HLA-A, -B, and -DR information using feed-forward neural network. In the input layer, the categorical data allele was encoded into a sparse vector, a one hot vector. A total of six one hot vectors are converted for each sample to represent HLA-A, -B, and -DR alleles. The embedding layer multiplied the one hot sparse vector with the embedding matrix to convert it into dense vectors. Hidden layers consisted of multiple layers, and performed linear transformation and activation functions repeatedly. Finally, the output layer consisted of a classifier model with two heads. In each head, the input vector was converted to a one hot vector through a linear transformation and a soft-max function, and converted to a categorical value, DQ alleles.

pDQ accuracy and pDQ DSA agreement, sensitivity, and specificity

pDQ genotype accuracy and allele accuracy were evaluated by comparing the pDQ and typed DQ data. DSA was reanalyzed with recipient/donor pDQ instead of typed DQ from preexisting SAB assay data. Any DQ DSA detected through the reanalysis of the SAB assay using donor and recipient pDQ data was labeled pDQ DSA.

pDQ DSA agreement was determined by comparing the presence of DQ DSA and pDQ DSA in the 1,970 SAB assay results from 885 recipients. If pDQ DSA was detected in DQ DSA-positive case or not detected in DQ DSA-negative case, it was considered as an agreement. The sensitivity (number of SAB assay results with pDQ DSA among DQ DSA-positive ones), specificity (number of SAB assay results without pDQ DSA among DQ DSA-negative ones), false-positive, and false-negative rates of pDQ DSA were estimated using the presence of the SAB assay DQ DSA as a standard.

Clinical significance of pDQ DSA

The post-transplantation outcomes were classified into five groups: biopsy-proven antibody-mediated rejection (ABMR) [13], biopsy-suspicious ABMR, clinically suspicious ABMR, biopsy-proven non-ABMR, and clinically non-ABMR. pDQ DSA was also regarded as serological evidence of DSA.

Statistical analysis

The ALLELE and HAPLOTYPE procedures of SAS (version 9.4;

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SAS Institute Inc., Cary, NC, USA) were used to analyze the allele and haplotype frequencies, respectively. Differences in clinical course among the patient groups were analyzed using the chi-square test. Two-sided $P<0.05$ was considered statistically significant. Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA) and SPSS Statistics for Windows version 19.0 (IBM Corp., Armonk, NY, USA) were also used for the analyses.

Table 1. HLA-DQ genotype accuracy predicted by the LD algorithm and ANN

HLA-DQ genotype	Patients (N)	LD	ANN
		Accurately predicted N (%)	Accurately predicted N (%)
DQ2, DQ2	1	1 (100.0)	1 (100.0)
DQ2, DQ4	9	9 (100.0)	6 (66.7)
DQ2, DQ5	4	2 (50.0)	2 (50.0)
DQ2, DQ6	20	19 (95.0)	20 (100.0)
DQ2, DQ7	8	7 (87.5)	7 (87.5)
DQ2, DQ8	12	6 (50.0)	10 (83.3)
DQ2, DQ9	7	6 (85.7)	6 (85.7)
DQ4, DQ4	9	7 (77.8)	8 (88.9)
DQ4, DQ5	14	11 (78.6)	11 (78.6)
DQ4, DQ6	29	20 (69.0)	18 (62.1)
DQ4, DQ7	14	5 (35.7)	7 (50.0)
DQ4, DQ8	13	7 (53.9)	10 (76.9)
DQ4, DQ9	16	11 (68.8)	11 (68.8)
DQ5, DQ5	10	10 (100.0)	10 (100.0)
DQ5, DQ6	29	24 (82.8)	26 (89.7)
DQ5, DQ7	17	12 (70.6)	11 (64.7)
DQ5, DQ8	16	6 (37.5)	7 (43.8)
DQ5, DQ9	10	10 (100.0)	10 (100.0)
DQ6, DQ6	27	27 (100.0)	27 (100.0)
DQ6, DQ7	33	23 (69.7)	21 (63.6)
DQ6, DQ8	33	25 (75.8)	20 (60.6)
DQ6, DQ9	26	23 (88.5)	22 (84.6)
DQ7, DQ7	8	7 (87.5)	7 (87.5)
DQ7, DQ8	11	6 (54.6)	6 (54.6)
DQ7, DQ9	8	7 (87.5)	5 (62.5)
DQ8, DQ8	3	2 (66.7)	3 (100.0)
DQ8, DQ9	9	4 (44.4)	6 (66.7)
DQ9, DQ9	7	7 (100.0)	7 (100.0)
Total	403	304 (75.4)	305 (75.7)

Abbreviations: ANN, artificial neural network; LD, linkage disequilibrium.

RESULTS

pDQ accuracy

pDQ genotype accuracies were 75.4% and 75.7% using the LD algorithm and ANN, respectively (Table 1). For 99 patients with an incorrect LD pDQ, the second most likely LD pDQ was compared with the typed DQ, and DQ was correctly predicted in 69 patients. When the LD pDQ and the second most likely LD pDQ were considered together, LD pDQ genotype accuracy increased to 92.6% (373/403).

Allele accuracies were 87.1% and 87.3% using the LD algorithm and ANN, respectively (Table 2). The lowest accuracy was observed for DQ8 for both prediction programs.

Table 2. HLA-DQ allele accuracy predicted by the LD algorithm and ANN

HLA-DQ allele	HLA-DQ allele (N)	LD	ANN
		Accurately predicted N (%)	Accurately predicted N (%)
DQ2	62	62 (100.0)	62 (100.0)
DQ4	113	85 (75.2)	88 (77.9)
DQ5	110	105 (95.5)	100 (90.9)
DQ6	224	219 (97.8)	219 (97.8)
DQ7	107	85 (79.4)	80 (74.8)
DQ8	100	62 (62.0)	74 (74.0)
DQ9	90	84 (93.3)	81 (90.0)
Total	806	702 (87.1)	704 (87.3)

Abbreviations: ANN, artificial neural network; LD, linkage disequilibrium.

Table 3. False-negative DQ DSA predicted by the LD algorithm and ANN

HLA-DQ	False-negative LD pDQ DSA N (%) (N=885)	False-negative ANN pDQ DSA N (%) (N=885)	Korean allele frequency (%)
DQ2	0 (0.0)	0 (0.0)	8.8
DQ4	2 (0.2)	2 (0.2)	12.7
DQ5	2 (0.2)	1 (0.1)	15.6
DQ6	2 (0.2)	5 (0.6)	28.1
DQ7	10 (1.1)	6 (0.7)	14.0
DQ8	4 (0.5)	7 (0.8)	9.6
DQ9	1 (0.1)	2 (0.2)	11.2
Total	21 (2.4)	23 (2.6)	100.0

Abbreviations: ANN, artificial neural network; DSA, donor-specific antibody; LD, linkage disequilibrium; pDQ, predicted DQ.

Table 4. Clinical presentation of KT recipients according to the presence of DQ DSA

Post-transplantation outcome*	Living donor KT 2013 (N=92)		Deceased donor KT (N=319)			
			Donor DQ typed (N=152)		Donor DQ not typed (N=167)	
	DQ DSA (+) (N=12)	DQ DSA (-) (N=80)	DQ DSA (+) (N=14)	DQ DSA (-) (N=138)	pDQ DSA (+) (N=24)	pDQ DSA (-) (N=143)
With ABMR treatment†	10 (83.3)	6 (7.5)	6 (42.9)	9 (6.5)	16 (66.7)	13 (9.1)
Biopsy-proven ABMR	7 (58.3)	3 (3.8)	4 (28.6)	5 (3.6)	14 (58.3)	8 (5.6)
Biopsy-suspicious ABMR	3 (25.0)	0 (0.0)	2 (14.3)	3 (2.2)	1 (4.2)	5 (3.5)
Clinically suspicious ABMR	0 (0.0)	3 (3.8)	0 (0.0)	1 (0.7)	1 (4.2)	0 (0.0)
Without ABMR treatment	2 (16.7)	74 (92.5)	8 (57.1)	129 (93.5)	8 (33.3)	130 (90.9)
Biopsy-proven non-ABMR	1 (8.3)	62 (77.5)	8 (57.1)	88 (63.8)	4 (16.7)	92 (64.3)
Clinically non-ABMR	1 (8.3)	12 (15.0)	0 (0.0)	41 (29.7)	4 (16.7)	38 (26.6)

Data are presented as N (%).
*Post-transplantation outcomes were classified into five groups: (1) biopsy-proven ABMR (patients who met the Banff 2017 ABMR criteria [13]), (2) biopsy-suspicious ABMR (patients who did not meet the Banff 2017 ABMR criteria but were clinically suspected as having ABMR; the patients underwent ABMR treatment, including rituximab, plasma exchange, intravenous immunoglobulin, or bortezomib), (3) clinically suspicious ABMR (a kidney biopsy was not performed, but the patients were clinically suspected as having ABMR and underwent ABMR treatment), (4) biopsy-proven non-ABMR (patients who had undergone a kidney biopsy and did not meet the Banff 2017 ABMR criteria), and (5) clinically non-ABMR (a kidney biopsy was not conducted and ABMR was not clinically suspected); †ABMR treatment frequency was significantly higher in DQ or pDQ DSA-positive recipients than in DQ or pDQ DSA-negative recipients. Deceased donor KT (donor DQ not typed): pDQ DSA-positive recipients vs. pDQ DSA-negative recipients [66.7% (16/24) vs. 9.1% (13/143), $P<0.001$]. Deceased donor KT (donor DQ typed): DQ DSA-positive recipients vs. DQ DSA-negative recipients [42.9% (6/14) vs. 6.5% (9/138), $P<0.001$]. Living donor KT: DQ DSA-positive recipients vs. DQ DSA-negative recipients [83.3% (10/12) vs. 7.5% (6/80), $P<0.001$]. Overall DQ or pDQ DSA-positive recipients vs. DQ or pDQ DSA-negative recipients (64.0% (32/50) vs. 7.8% (28/361), $P<0.001$). No significant difference in ABMR treatment frequency was observed between the DQ DSA-positive and pDQ DSA-positive groups [61.5% (16/26) vs. 66.7% (16/24), $P=0.706$] and between the DQ DSA-negative and pDQ DSA-negative groups [6.9% (15/218) vs. 9.1% (13/143), $P=0.443$].
Abbreviations: ABMR, antibody-mediated rejection; DSA, donor-specific antibody; KT, kidney transplantation; pDQ, predicted DQ.

pDQ DSA agreement, sensitivity, and specificity

LD pDQ DSA agreement was 97.5%; sensitivity, 97.3%; and specificity, 98.6%. The ANN pDQ DSA agreement was 96.6%; sensitivity, 96.4%; and specificity, 98.6%. The MFI of the false-positive pDQ DSAs was <5,000.

Table 3 shows the false-negative pDQ DSAs results. DQ7 (1.1%) and DQ8 (0.8%) had the highest false-negative rates using the LD algorithm and ANN, respectively.

Clinical significance of pDQ DSA

Of the patients who underwent deceased donor KT without donor DQ typing, ABMR treatment frequency was significantly higher in pDQ DSA-positive patients than in pDQ DSA-negative patients (Table 4). Of the patients who underwent living donor KT or deceased donor KT with donor DQ typing, the ABMR treatment frequency was significantly higher in DQ DSA-positive patients than in DQ DSA-negative patients.

Of the 24 patients who underwent deceased donor KT (without donor DQ typing) and developed pDQ DSA, one patient had pre-transplantation pDQ DSA and was not subjected to a follow-up SAB assay. The remaining 23 patients had post-transplantation pDQ DSA. Among the 167 patients who underwent deceased donor KT (without donor DQ typing), 5.4% (9/167) had pDQ DSA only, 2.4% (4/167) had pDQ DSA and other DSAs (MFI<3,000), and 6.0% (10/167) had pDQ and other DSAs (MFI≥3,000).

Five patients in the pDQ-only DSA group had biopsy-proven ABMR (Table 5, patients 1 to 5); the pDQ DSA was first detected in these patients at 4 years (median) after transplantation, and the mean MFI was 5,747 at the time of first detection. One patient (patient 4) had undergone steroid and tacrolimus treatment without plasma exchange or rituximab. The details of the four patients with pDQ+other DSA (MFI<3,000) are provided in Table 5 (patients 6 to 9). Three patients had biopsy-proven ABMR and received ABMR treatment, while one patient did not undergo kidney biopsy or ABMR treatment.

DISCUSSION

It was thought that if the HLA-DR was compatible serologically, HLA-DQ would not affect KT outcomes, as HLA-DR and HLA-DQ show strong LD [14, 15]. However, molecular typing has revealed that HLA-DQ is discordant in 15%–26% of HLA-DR-matched patients [16, 17].

We developed two different DQ prediction programs using the

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Table 5. Characteristics of KT recipients who developed pDQ-only DSA and were confirmed as having ABMR by kidney biopsy (patients 1 to 5) and KT recipients who developed pDQ + other DSA (MFI < 3,000) (patients 6 to 9)

Patient	KT date	SAB assay or biopsy date	Other DSA (MFI)	pDQ DSA (MFI)	Kidney biopsy result	ABMR Tx*
1	2008.04	2015.11	None	DQ9 (9,315)	v0, g1, cg0, ptc2 (diffuse), c4d1 (< 5%)	Yes
		2016.01	None	DQ9 (13,102)	NT	
		2017.04	None	DQ9 (12,505)	NT	
		2018.08	None	DQ9 (15,205)	v0, g2, cg3, ptc3 (diffuse), c4d1 (5%)	Yes
2	2008.11	2015.11	None	DQ6 (4,596)	v0, g2, cg0, ptc2 (focal), c4d0 (0%)	Yes
3	2010.08	2013.06	None	None	NT	
		2014.09	None	DQ5 (5,129)	v0, g1, cg0, ptc1 (diffuse), c4d0 (0%)	Yes
		2017.01	None	None	NT	
		2018.09	None	None	NT	
4†	2011.06	2014.08	None	DQ7 (5,260)	v0, g1, cg1, ptc1 (diffuse), c4d0 (0%)	
5	2017.06	2017.12	None	DQ7 (4,437)	v1, g2, cg1, ptc3 (focal), c4d0 (0%)	Yes
6	2010.08	2017.01	A2 (1,019)	DQ7 (10,249)	NT	
		2018.06	None	DQ7 (14,907)	NT	
7	2010.09	2014.04	DR13 (1,772)	DQ9 (14,351)	v0, g1, cg0, ptc0, c4d0 (0%)	
		2014.05	NT	NT	v0, g3, cg0, ptc3 (diffuse), c4d0 (0%)	Yes
		2018.06	None	DQ9 (5,322)	v0, g2, cg0, ptc3 (diffuse), c4d1 (7%)	Yes
		2018.07	None	DQ9 (3,648)	NT	
		2018.09	None	DQ9 (2,848)	NT	
		2018.11	None	DQ9 (3,683)	NT	
		2019.02	None	DQ9 (3,961)	v1, g1, cg0, ptc2 (focal), c4d0	Yes
8	2012.02	2016.02	A33 (1,494)	DQ2 (11,189), DQ6 (7,504)	v0, g1, cg1, ptc3 (diffuse), c4d3 (60%)	Yes
		2016.04	None	DQ2 (9,413), DQ6 (5,139)	v0, g3, cg1, ptc3 (diffuse), c4d3 (70%)	Yes
		2016.06	None	DQ2 (11,386), DQ6 (5,504)	NT	
		2016.07	None	DQ2 (8,848), DQ6 (3,772)	v0, g1, cg2, ptc3 (diffuse), c4d3 (70%)	
		2016.09	NT	NT	v0, g1, cg1, ptc2 (focal), c4d1 (5%)	Yes
		2017.04	None	DQ2 (12,985), DQ6 (2,834)	NT	
9	2015.09	2017.06	B51 (1,654)	DQ6 (2,798)	v0, g2, cg1, ptc3 (diffuse), c4d1 (5%)	Yes

*ABMR treatment includes therapeutic plasma exchange, rituximab, bortezomib, and intravenous immunoglobulin; †Patient 4 had undergone steroid and tacrolimus treatment without ABMR treatment.
Abbreviations: ABMR, antibody-mediated rejection; cg, Banff chronic glomerulopathy score; DSA, donor-specific antibody; g, Banff glomerulitis score; KT, kidney transplantation; MFI, mean fluorescence intensity; NT, not tested; pDQ, predicted DQ; ptc, Banff peritubular capillaritis score; SAB, single-antigen bead; Tx, treatment; v, Banff intimal arteritis score.

LD algorithm and an ANN. The genotype accuracies of LD pDQ and ANN pDQ were over 75%. When the second most likely pDQ was included, the LD pDQ genotype accuracy increased to 92.6%. The accuracy was poorest for DQ8. This is partly due to the LD distribution of the HLA-B-DR-DQ haplotype with which DQ8 is associated. We evaluated the LD between HLA-B-DR and DQ of 74 HLA-B-DR-DQ8 haplotypes in Korean patients. Among the HLA-B-DR haplotypes associated with DQ8, 61 (82.4%) had the same or higher D' with DQ, except for DQ8 (data not shown).

The SAB assay results were reanalyzed using pDQ. Both LD and ANN pDQ DSA showed agreement, sensitivity, and specificity over 95%. The MFI of all false-positive pDQ DSAs was <5,000. Previous studies have shown that patients with allograft loss had a significantly higher DQ DSA MFI (6,000–16,000) than those without allograft loss [3, 18]. Thus, antibodies with an MFI <5,000 detected as false positives by the DQ prediction program were considered to be of low clinical significance.

In this study, ABMR incidence was significantly higher in DQ or pDQ DSA-positive patients than in DQ or pDQ DSA-negative

patients (64.0% vs. 7.8%, $P<0.001$). ABMR incidence was similar to that in the study by DeVos, *et al.* [2], wherein ABMR incidence in KT recipients without DSA, recipients with DQ-only DSA, and recipients with DQ + other DSA was 11% (31/285), 21% (7/33), and 67% (10/15), respectively. No significant difference in ABMR incidence was observed between the DQ DSA- and pDQ DSA-positive groups and between the DQ DSA- and pDQ DSA-negative groups. These results suggest that DQ DSA and pDQ DSA have similar clinical characteristics.

Of the patients who underwent a deceased donor KT with unknown donor DQ, patient 1 had pDQ9 DSA MFI increased from 9,315 to 15,205 during the follow-up period, with worsening glomerulopathy and peritubular capillaritis (Table 5). The clinical course of the patient and the accuracy of the DQ prediction program suggest that pDQ9 is likely an actual DSA. Although the pDQ9 DSA MFI exceeded 10,000 in 2016 and 2017, the patient did not receive ABMR treatment at that time. Patient 4 had normal but increasing creatinine levels at three yrs post-transplantation (Table 5). DQ7 antibody was identified but not reported as a DSA, as the donor DQ was not available. The patient received only steroid treatment, and his creatinine level remained normal. In this case, it might have been helpful to follow up the SAB assay and review the MFI trends of the DQ7 antibody.

Patients 2, 3, 4, and 5 were C4d-negative through kidney biopsy. ABMR can be diagnosed based on kidney biopsy results and the presence of DSA; however, when DSA results are not available, the C4d status can serve as an alternative for ABMR diagnosis [13]. Unfortunately, C4d has low sensitivity and, even among microvascular injury and DSA, 20% of the patients are C4d-negative [19]. Therefore, the revised Banff 2017 strongly recommends DSA analysis [13]. pDQ DSA analysis would be particularly useful for patients with unclear biopsy results, such as those who are C4d-negative, in the context of donors who have not been HLA-DQ typed.

Patient 6 had a pDQ DSA with an MFI persistently exceeding 10,000. This patient had an elevated creatinine level and underwent steroid treatment. As kidney biopsy was not available for this patient, it was difficult to determine whether the pDQ DSA was a true DSA. Given that the pDQ DSA MFI exceeded 10,000 and increased during the follow-up period, additional assays might be needed to diagnose ABMR.

Taken together, our results suggest that if DQ antibodies are detected in the SAB assay of a patient with an unknown donor DQ, the DQ prediction program can help diagnose and treat ABMR. If the patient presents pDQ DSA, we recommend performing a SAB assay periodically and follow up pDQ DSA MFI, in addition to tests such as kidney scan and biopsy. More active treatment should be considered, if the pDQ DSA MFI exceeds 5,000 or other DSA is also present. In addition, even if pDQ DSA is not detected, if the same DQ antibody is repeatedly identified by the SAB assay, the possibility of an incorrect DQ prediction should be considered. In such cases, the SAB assay can be performed using the second most likely pDQ according to the LD algorithm.

Our study had several limitations. First, pDQ DSA agreement does not indicate an actual match between pDQ DSA and DQ DSA, but a comparison of the presence of a pDQ and DQ DSA. To assess the clinical significance of DQ or pDQ DSA, the ABMR treatment frequency was analyzed. Long-term observation of cases for ABMR-free survival or graft survival rates would better reflect the clinical course. Lastly, usage of the HLA-DQ prediction programs is limited to our institution.

In conclusion, we developed HLA-DQ prediction programs using the LD algorithm and ANN. Although experts agree that HLA-DQ typing is necessary for deceased donors, cost remains an issue, and it will take time for existing policies to address this issue. As there were some inaccuracies in the prediction programs developed in this study, a policy of HLA-DQ typing for deceased donors should be established as promptly as possible in Korea.

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AUTHOR CONTRIBUTIONS

HO designed the study; SKK and JJY collected and analyzed the data; SKK wrote the paper; SH, HS, SS and SYK contributed to the conception. All authors have accepted responsibility for the entire content of this manuscript and have approved the submission.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this study are reported.

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