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Investigation of Killer Immunoglobulin-like Receptor (KIR) and HLA Genotypes to Predict the Occurrence of Acute Allograft Rejection after Kidney Transplantation

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ABSTRACT

After kidney transplantation, natural killer (NK) cells play a pivotal role in triggering the immune response to the allogeneic grafts primarily by their killer-cell immunoglobulin-like receptors (KIR). This process may be one mechanism that contributes to graft rejection. In this study, we have evaluated whether acute rejection after kidney transplantation was associated with predicted NK cell alloreactivity based on KIR gene and ligand along with KIR/HLA compound genotype analysis.

DNA from 65 patients with biopsy-proven acute kidney allograft rejection (AKAR), 61 clinically stable graft function (SGF) recipients and 176 healthy subjects were identified for the presence or absence of 10 variable KIR genes (both activating and inhibitory receptors) and their HLA ligands using polymerase chain reaction-sequence specific primers (PCR-SSP) assay.

Although no significant difference in the frequency of individual KIR genes, was found the gene content, and the haplotypic distribution between the three categories were detected, the frequency of the KIR3DL1+HLA-Bw4*A allele combination was significantly lower in AKAR patients compared to SGF recipients (p=0.004, OR=0.34, CI=0.16-0.72) and healthy subjects (p=0.019, OR=0.47, CI=0.25-0.89). Kaplan-Meier survival test showed that the KIR3DL1+HLA-Bw4*A allele combination could be considered protective for AKAR (p=0.04 by log-rank).

The results of this study suggest that KIR/HLA polymorphism may be a genetic susceptibility factor to alloreactivity dysfunction in the NK cells of patients with AKAR. It is likely that a KIR/HLA combinatorial study can be beneficial in predicting AKAR occurrence for the purpose of selecting donors appropriately.

Keywords: Human leukocyte antigen; Killer-cell immunoglobulin-like receptor (KIR); Kidney transplantation; Transplant rejection

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INTRODUCTION

Acute kidney allograft rejection (AKAR) occurs as a consequence of interactions between recipient immune cells within the transplanted organ.[1,2] Considerable evidence now exists demonstrating that adaptive immunity plays an important role in the occurrence of AKAR. However, recent studies have indicated the importance of innate immune effector cells such as natural killer (NK) cells in both graft rejection and tolerance following solid organ transplantation.[1,3-5]

NK cells are fundamental players during innate immune responses, which physically interact with other immune and non-immune cells.[6] These cells have been known to be associated with certain diseases. In the field of solid organ transplantation, significant strides have recently been made in the understanding of underlying cellular and molecular mechanisms in regulating the acquisition of effector functions by NK cells.[4] NK cells activation following engagement of their surface receptors induces the synthesis and secretion of mediators and the triggering of cell-mediated cytolysis, which is most closely associated with cell damage including destruction of transplanted cells.[7-10]

Among the cell surface receptors that regulate the function of NK cells, killer cell immunoglobulin-like receptors (KIRs) are the most important and polymorphic.[11] The human KIR gene family is less than 200 Kbp in length and located on chromosome 19 (19q13.4), the leukocyte receptor complex (LRC) region. The region encodes KIRs with inhibitory functions (iKIR; 3DL1−3, 2DL1−3, and 2DL5), activating functions (aKIR; 3DS1, 2DS1−5), or both (2DL4).[9,12,13] Furthermore, according to the number of inhibitory and activating genes, KIR genotypes can be further classified into A and B haplotypes.[14,15] Haplotype A, the most common haplotype in the human KIR gene, consists of mostly iKIR genes and also one aKIR gene. Haplotype B is characterized by the presence of more than one activatory KIR gene in addition to inhibitory genes.[14,16]

The KIRs recognize the presence of specific HLA type I ligands on the surface of potential target cells. HLA ligands have been described for some (but not all) KIR genes. KIR2DL1 and KIR2DS1 recognize a subset of HLA-Cw molecules with lysine at position 80 of the heavy chain (HLA-C2 group), whereas KIR2DL2/3 and KIR2DS2 interact with a subset of HLA-Cw molecules with asparagine at position 80 of the heavy chain (HLA-C1 group). KIR3DL1 and its activating counterpart 3DS1 bind to HLA-B and certain HLA-A allotypes that contain the Bw4 motifs (HLA-Bw4 alleles).[15,17-19]

Experimental studies have shown increased infiltration of host NK cells as well as enhanced ex vivo cytotoxicity of NK cells against donor cells in rejecting kidney allografts.[5,8] As previously mentioned, NK cell activation is critically determined by the balance between activating and inhibitory signals from KIR-HLA interactions.[17] It is likely that simultaneous polymorphisms in KIR and HLA can result in an increased frequency of alloreactive KIR^+ NK cells within the host NK cell population, which is consistent with the "missing self" hypothesis.[16,20,21] Regarding this hypothesis, it is considered that the genetic variability of KIRs and/or HLA ligands in individuals might help to determine the outcome of solid organ transplants.[4] In kidney transplantation, conflicting data exist regarding the role of KIR and KIR ligands in NK cell activation within the transplanted organ.[1,22,23] Therefore, we were interested in assessing the effect of KIR genes and their HLA ligands along with KIR/HLA compound genotypes on protection/susceptibility to AKAR.

MATERIALS AND METHODS

Study Population

A total of 126 allograft recipients who received a kidney transplant from living or cadaveric-unrelated donors between June 2007 and March 2013 at three transplant centers, Sina Hospital, Emam Khomeini Hospital both affiliated to Tehran University of Medical Sciences (TUMS) and Labbafi Nezhad Hospital, Shahid Beheshti University of Medical Sciences (SBMU) were included in this retrospective analysis. Recipients were classified into two groups according to the occurrence of biopsy-proven acute kidney allograft rejection (AKAR group) or having clinically stable graft function (SGF group) without any previous episode during five years of follow up. The AKAR group comprised of 65 patients receiving a kidney transplant, diagnosed with AKAR. Acute rejection was defined by clinical manifestations including increases in serum creatinine level and glomerular filtration rate (GFR) (Cr>1 mg/dL, GFR<60) and confirmed by biopsy protocol based on

Banff criteria.[24] The SGF group consisted of 61 patients who had no clinical manifestations of acute rejection during at least 18 months after transplantation. The immunosuppressant regimen for all recipients was adjusted conventional triple-drug therapy consisting of cyclosporine A or tacrolimus (FK506), mycophenolate mofetil (MMF) or azathioprine, and methyl prednisone as steroid. These recipients had not received antibody induction therapy.

Additionally, the KIR/ HLA genotyping data of 176 unrelated healthy volunteers from our previous publication were included in the study for better comparison.[15] All samples were collected with the written consent of the patients or of their legal guardians and the study was approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran. Genomic DNA was extracted from peripheral blood cells using the salting-out method as described by Miller and colleagues.[25] The quality and quantity of DNA was determined by UV spectrophotometry.

KIR and HLA-ligand Genotyping

Using the polymerase chain reaction-sequence specific primers (PCR-SSP) assay, DNA samples were genotyped for the presence or absence of 10 KIR genes including the KIR genes responsible for inhibitory signals (2DL1, 2DL2, 2DL3, 3DL1), those for activating signals (2DS1, 2DS2, 2DS3, 2DS4, 2DS5 and 3DS1), and their five major HLA class I ligands including HLA-C1, C2 and three allotypes with Bw4 motif: HLA-Bw4 A allele, HLA-Bw4 B allele with threonine at its position 80 (Bw4 Thr), and HLA-Bw4 B allele with isoleucine at its position 80 (Bw4 Ile).

Primer design and PCR thermal conditions for all the KIR genes and HLA ligand genes were adopted from the protocol by Vilches et al.[13] and Taiik et al.[18] respectively. An internal control primer was included in each PCR typing reaction. Positive control samples were a generous gift from Dr. D.Middleton, Liverpool University UK. All PCR reactions were amplified with programmable thermal cycler (Verity, ABI, USA). In each reaction, target DNA was amplified in a total volume of 20 μL containing 0.1-1.5 μM of each specific primers, 1.5 mM MgCl2, 1 μL of 10x PCR buffer, 0.5U of Taq DNA polymerase and 100 ng of the genomic DNA. For electrophoresis, the PCR products were run on 2% agarose gel capable of resolving 100–2000 base pair fragments of DNA.

Identification of Group A and B Haplotypes

To analyze haplotypic structures, samples were evaluated using the method of Rajalingam et al.[26] Briefly, individuals carrying sole 9 KIR genes including KIR2DL4, 2DS4, 2DL3, 2DL1, 2DP1, 3DP1, 3DL1-3 were typically assigned as carriers of AA genotype (the most frequent KIR genotype). Other individuals were regarded as carriers of Bx genotypes (AB+BB). Subjects without any of the four A haplotype-associated genes (KIR2DL3, 2DL1, 3DL1, and 2DS4) were considered as carrier for two copies of group B haplotype (BB genotype), and the remaining individuals were assigned as heterozygotes (AB genotype). Furthermore, according to locus region of KIR gene clusters, we analyzed individuals carrying the Bx genotype using model of Pyo and colleagues.[27] Based on this model, two distinct gene clusters were identified at centromeric and telomeric halves of the KIR gene complex. The centromeric cluster consisted of 2DS2, 2DL2, 2DL5B, 2DS3 genes, while the telomeric cluster consisted of KIR3DS1, 2DL5A, 2DS5, 2DS1 genes. Individuals carrying the Bx genotype can represent either centromeric cluster (we call it C4, in which C represents centromeric cluster with 4 genes), telomeric cluster (T4), or both (CT4).[15]

Statistical Analysis

The comparison of frequency between the groups was calculated using a two-tailed Fisher exact probability (p) test. Odds ratio (OR) with 95% confidence interval (CI) was considered for the groups. Additionally, probability that the graft is not rejected (postoperative survival) was calculated by the Kaplan-Meier method and the difference of survival curves was provided by the log-rank statistic (Mantel-Cox). The association of clinical (transplant characteristics, Table 1) and genetic variables with each outcome were evaluated by using multivariate survival analysis using Cox's regression model. $p<0.05$ was considered as statistically significant difference. All analyses were performed using SPSS software version 18.0 (IBM Corporation, New York, USA).

Results

The clinical parameters and general demographics for AKAR and SGF groups are listed in Table 1. Similar to the results of our previous study, SGF recipients had longer follow-up than AKAR group ($p=0.01$, Table1).[28] In the present study, HLA matching

was not considered, and all recipients received HLA-mismatched kidney transplants (1-6 mismatched) from living unrelated donors (61.9%), cadaveric donors (30.95%), and living related donors (7.15%). In terms of donor sources, there was no detectable difference between both recipient groups. Cytomegalovirus (CMV) infection was more prevalent in AKAR group than SGF group, but the difference was not statistically significant (p=0.4). Out of 65 patients with AKAR, 21 cases (32.3%) lost their kidney transplant and returned to dialysis or underwent a second attempt at kidney transplantation. The frequency of delayed graft function (DGF) was also higher in this group (29.3% vs 14.75% in SGF, p=0.052), with a higher level of serum creatinine during follow up ($p<0.01$).

Table 1. Demographic and clinical features of the kidney transplant patients with acute kidney allograft rejection (AKAR) and stable graft function (SGF) recipients group.

Parameters	AKAR group (n=65)	SGF group (n=61)	*p* value
Follow up duration (month)	42± 35.7	61.3± 22.4	0.01
Age (year)	34.02±13.5	31.4±10.9	0.278
Sex (Male/Female)	44 / 21	32 / 29	0.083
Donor Source			
Cadaver	24 (36.9%)	15 (24.6)	0.136
living unrelated	36 (55.4%)	42 (68.85)	0.121
living related	5 (7.7%)	4 (6.55)	0.805
CMV infection (Pos /Neg)	18 / 47	14 / 47	0.4
Rejection type			
Cell- mediated	54 (86.9%)	-	-
Antibody- mediated	10 (11.5%)	-	-
Cellular & humoral	1 (1.6%)	-	-
Graft loss (%)	21 (32.3%)	-	-
DGF (Yes /No)	19 / 46	9 / 52	0.052
Cr level	3.03± 1.37	1.08±0.13	0.000

CMV: Cytomegalovirus; DGF:Delayed graft function; Cr: creatinine. Data are presented as n (%).

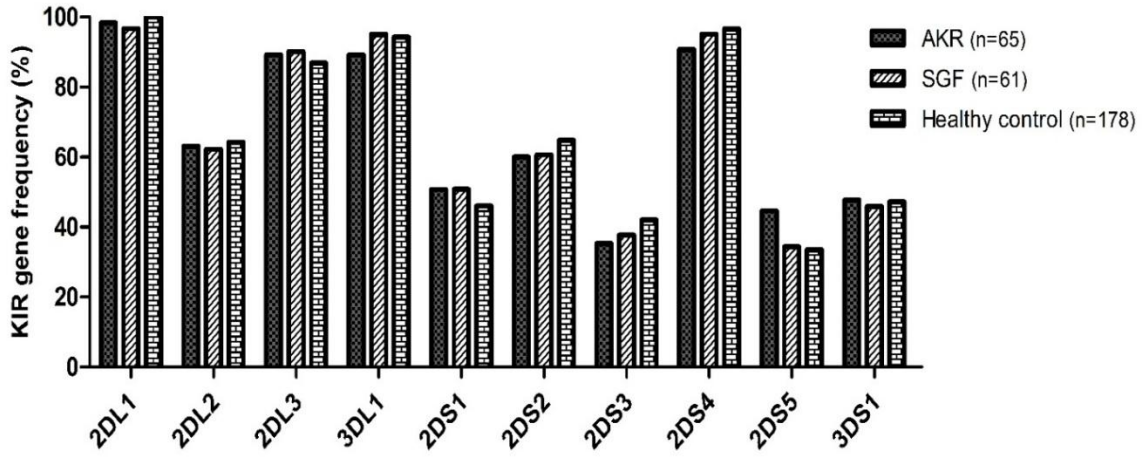

Figure 1. Patients with acute kidney allograft rejection (AKAR), stable graft function (SGF) recipients and healthy subjects have similar killer immunoglobulin-like receptor (KIR) gene frequencies. KIR2DL4,5, -3DL2, and -3DL3 were not typed because these genes are present in all individuals.

Table 2. Comparison of the genotype frequencies of killer immunoglobulin-like receptor (KIR) genes in patients with acute kidney allograft rejection (AKAR) and stable graft function (SGF) recipients. Each line represents one KIR genotype [gene presence (grey Box) or gene absence (white Box)]. C4: centromeric clustering, T4: telomeric clustering, CT4: centro-telomeric clustering

		Haplotype A-associated genes				Haplotype B-associated genes									
		2DL1	2DL3	3DL1	2DS4	2DS2	2DL2	2DS3	3DS1	2DS5	2DS1	Gene content	Genotype	Patients with AKAR (%)	SGF recipients (%)
1	AA											4		26.15	16.39
2												10	CT4	4.62	3.28
3												9	CT4	9.23	4.92
4												9	CT4	0.00	3.28
5												9	CT4	4.62	6.56
6												9	CT4	4.62	1.64
7												8	CT4	0.00	1.64
8												8	CT4	6.15	1.64
9												8	CT4	0.00	1.64
10												8	CT4	0.00	1.64
11												8	CT4	1.54	3.28
12												8	CT4	0.00	1.64
13												8	CT4	0.00	1.64
14												8	CT4	1.54	1.64
15												8	CT4	1.54	0.00
16												8	CT4	1.54	0.00
17												8	CT4	1.54	0.00
18												7	C4	9.23	4.92
19	Bx											7	T4	7.69	11.48
20												7	CT4	0.00	1.64
21												7	CT4	0.00	1.64
22												7	CT4	1.54	0.00
23												7	CT4	1.54	0.00
24												7	CT4	1.54	0.00
25												7	CT4	1.54	0.00
26												6	CT4	1.54	1.64
27												6	C4	0.00	4.92
28												6	C4	7.69	13.11
29												6	C4	0.00	1.64
30												6	T4	0.00	1.64
31												6	CT4	1.54	0.00
32												5	C4	0.00	3.28
33												5	T4	0.00	1.64
34												5	C4	1.54	0.00
35												5	T4	1.54	0.00
36												4	C4	0.00	1.64

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Figure 1 provides a comparison of the frequency of the different KIR genes among the study population. By excluding one patient in the case group and 2 recipients in SGF group, 2DL1 gene was observed in all recipients. KIR2DL2 and 2DL3 share a single locus and the entire study population had at least one copy of either gene (2DL2/2DL3 frequency =100%). There was no significant difference in the distribution of inhibitory and activating KIR genes between the groups.

Table 2 illustrates KIR genotype distribution among transplantation recipients with and without AKAR based on haplotype status. Among a total of 36 KIR genotypes delineated, 14 genotypes were solely found in SGF group; 10 genotypes were found in AKAR group, and all other genotypes were shared by both groups. Analysis of haplotype frequency did not show any significant difference between the groups. As shown in figure 2a, 26.1% of the AKAR group and 16.4% of the SGF recipients were carriers of the AA-zero genotype; however, this is a non-significant increment (p=0.183). Haplotype distribution in subjects with the Bx haplotype (with C4, T4, and CT4 clustering patterns) did not reveal a significant association between AKAR group and SGF recipients or between all transplant recipients and healthy subjects (Figure 2b). Meanwhile, in our study no significant increment or decrement in the genetic content of the KIR genes was seen between the groups.

Combinatorial frequency of KIR genes with their HLA class I ligands was also compared between the three groups. Comparison of compound KIR-HLA genotype showed that the frequency of the KIR3DL1+HLA-Bw4*A allele combination was significantly lower in AKAR patients compared to SGF recipients (p=0.004, OR= 0.34, CI=0.16-0.72) and healthy subjects (p=0.019, OR= 0.47, CI=0.25-0.89). In practice, when we analyzed the effects of KIR3DL1+HLA-Bw4*A allele combination on the outcome of kidney transplantation using the Kaplan-Meier test, we found that it had a significant protective effect against the occurrence of AKAR (p=0.04 by log-rank test, Figure 3). No other KIR/HLA combinations indicated significant susceptibility to or provided protection from AKAR (Table 3). Furthermore, Cox proportional hazard modeling showed that type of donor (hazard ratio [HR]=0.658, p= 0.04) was associated with an overall survival (OS) rate. We found no significant associations of other clinical parameters with OS rate.

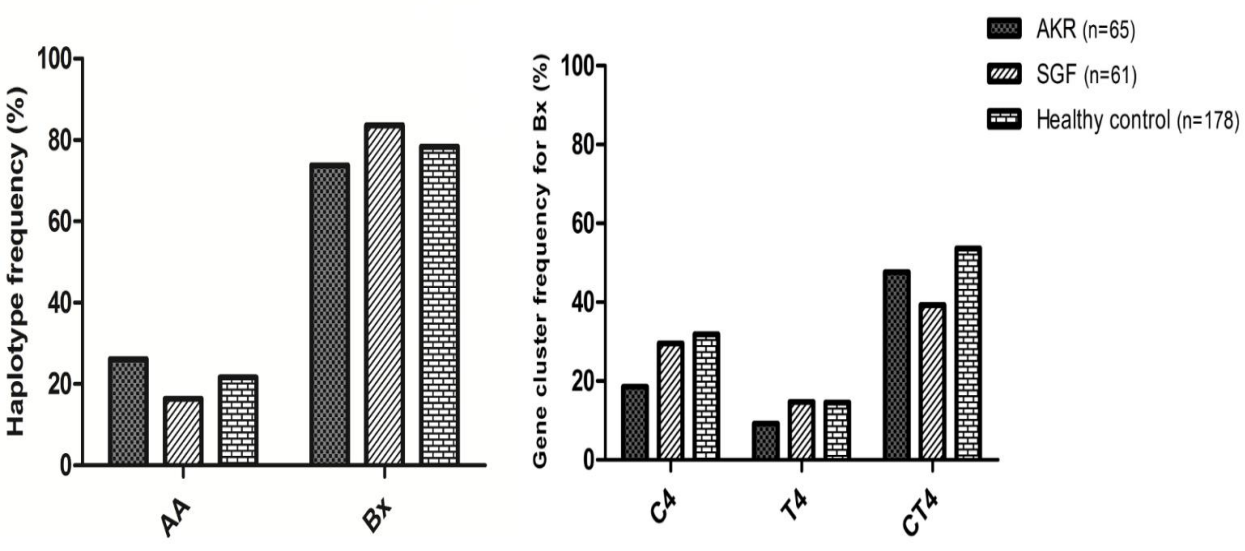

Figure 2. Haplotype frequencies (left) and gene cluster frequencies (for Bx haplotype) (right) in patients with acute kidney allograft rejection (AKAR), Stable graft function (SGF) recipients group and healthy subjects

Table 3. Killer immunoglobulin-like receptor (KIR) and their HLA ligand in susceptibility to acute kidney allograft rejection (AKAR)

	AKAR group (a) (n= 65)	SGF group (b) (n=61)	Healthy subjects (c) (n=176)	(a) vs. (b)	*p* value (a) vs. (c)	(b) vs. (c)
Genotype						
2DL1+/C2+	43 (66.1)	39 (63.9)	131 (74.4)	0.794	0.203	0.117
2DS1+/C2+	23 (35.3)	18 (29.5)	59 (33.5)	0.483	0.787	0.564
2DL1+/2DS1-/C2+	20 (30.7)	22 (36)	72 (40.9)	0.530	0.151	0.506
2DL1-/2DS1+/C2+	1(1.5)	1 (1.6)	0 (0)	0.964	0.099	0.088
2DL2+/C1+	29 (44.6)	24 (39.3)	79 (44.9)	0.550	0.970	0.452
2DL3+/C1+	41 (63)	37 (60.6)	112 (63.6)	0.661	0.936	0.678
2DS2+/C1+	27 (41.5)	23 (37.7)	81 (46.0)	0.530	0.535	0.26
2DL2+/2DS2-/C1+	2 (3)	4 (6.5)	0 (0)	0.361	0.019*	0.000*
2DL3+/2DS2-/C1+	18 (27.6)	20 (32.7)	43 (24.4)	0.535	0.606	0.204
2DL2-/2DS2+/C1+	0 (0)	2 (3.2)	2 (1.1)	0.142	0.391	0.263
2DL3-/2DS2+/C1+	4 (6.1)	5 (8.1)	12 (6.8)	0.657	0.854	0.719
3DL1+/ HLA-Bw4*A+	17 (26.1)	31 (50.8)	75 (42.6)	0.004	0.019	0.267
3DL1+/Bw4.Thr+	9 (13.8)	10 (16.3)	24 (13.6)	0.690	0.966	0.597
3DL1+/Bw4.Ile+	32 (49.2)	34 (55.7)	95 (54.0)	0.466	0.513	0.812
3DS1+/ HLA-Bw4*A+	12 (18.4)	16 (26.2)	35 (19.9)	0.296	0.804	0.299
3DS1+/Bw4.Thr+	5 (7.6)	4 (6.5)	15 (8.5)	0.805	0.836	0.626
3DS1+/Bw4.Ile+	15 (23)	15 (24.6)	36 (20.5)	0.986	0.658	0.499
3DL1+/3DS1-/ HLA-Bw4*A+	9 (13.8)	16 (26.2)	43 (24.4)	0.082	0.076	0.78
3DL1+/3DS1-/Bw4.Thr+	5 (7.6)	7 (11.4)	11 (6.3)	0.471	0.690	0.185
3DL1+/3DS1-/Bw4.Ile+	20 (30.7)	20 (32.7)	61 (34.7)	0.808	0.571	0.79
3DL1-/3DS1+/ HLA-Bw4*A+	4 (6.1)	1 (1.6)	3 (1.7)	0.196	0.068	0.972
3DL1-/3DS1+/Bw4.Thr+	1 (1.5)	1 (1.6)	2 (1.1)	0.964	0.803	0.762
3DL1-/3DS1+/Bw4.Ile+	3 (4.6)	0 (0)	2 (1.1)	0.090	0.093	0.404

AKAR= :Acute kidney allograft rejection; SGF= : graft function. Data are presented as n (%).

* The value is within the confidence interval and is not significant.

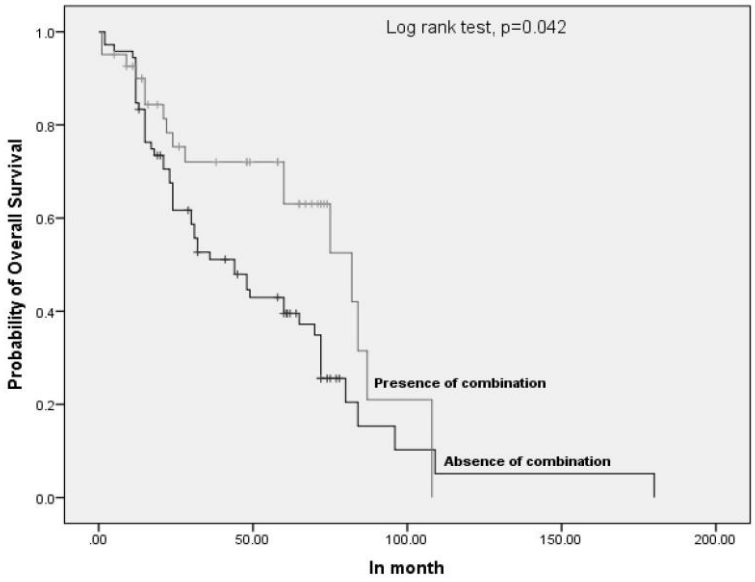

Figure 3. Kaplan-Meier graft survival curve. Graft survival rate based on the presence or absence of KIR3DL1+HLA-Bw4*A allele combination for patients with acute kidney allograft rejection (AKAR) and stable graft function recipients (SGF) recipients.

DISCUSSION

Acute kidney rejection is a potential post-transplantation event and is apparently linked with poor long-term outcomes after kidney transplantation. The immune system plays a vital role in the development of the disorder.[29] However, the exact mechanisms that have an impact on an individual transplant recipient's susceptibility to AKAR have not been well understood. In addition to T cells, NK cells may contribute to graft rejection via KIR-HLA interactions. These interactions seem to be involved in the pathophysiology of eliminating neoplastic and virus-infected cells,[9] and in the development of susceptibility to multiple autoimmune disease[30] and alloreactivity.[3] KIRs potentially regulate the function of NK cells through binding to their corresponding HLA ligands and adjust the balance between activation and inhibition of NK cells.[12] Recent studies have shown associations KIR genes and their HLA ligands with outcomes in kidney transplant patients.[1,20,23]

The comparison of the presence of single KIR gene between the groups has revealed no statistically significant difference with kidney graft survival and other studies have reported similar findings.[5,12,23,31] However, the group of Nowak et al. compared the frequency of 10 KIR genes and showed associations of KIR2DS4 variants and KIR2DS5 gene with increased probability of rejection and protection of the transplanted kidney, respectively. Blockage of KIR2DS5-ligand interaction through soluble KIR2DS4 could be the probable cause of susceptible role of KIR2DS4 in AKAR.[8] The results of Nowak et al. appear to conflict with a study carried out by Vampa et al., who reported that increased NK cell activity was associated with a greater proportion of recipient's activating KIR genes for donor HLA class I.[32] We recently demonstrated an unexpected increase in the distribution of activating KIR2DS5 gene in a healthy Northern Iranian population,with 41% showing activation of the gene (compared to 25.4% of the Southern Iranian population);[9] however, we could not identify a relation between kidney graft survival and the presence of single KIR gene.

Analysis of KIR haplotype status revealed no significant associations with the KIR inhibitory (A/A) haplotype versus the stimulatory (B/x) haplotype. Among the Bx haplotypes, the frequency of the C4, T4, or CT4 subtypes did not show a significant association in the acute rejection group compared to other subjects. In different populations, gene content might be associated with selective pressures caused by infections, epidemiological challenges, and genetic drift.[33] We presumed that KIR gene content (number of inhibitory and activating) could influence the activity of NK cells in a dose-dependent manner, although we detected no significant difference between groups.

The interesting finding of our study is the association between the KIR3DL1+HLA-Bw4*A allele combination and transplant kidney outcome (Table 3), in which the combination is decreased in AKAR patients compared to SGF recipients and healthy subjects. The KIR3DL1+HLA-Bw4*A allele combination has also been linked with some immunological disorders. Evaluation of the cell lines obtained from human solid tumors showed that there was a direct relationship between a given sufficient level of the HLA-Bw4*A allele expressed by the melanoma targets and the surviving melanoma cells from lysis through KIR3DL1-expressing NK cells and some T lymphocytes.[34] In the stem cell transplantation setting, a reported combinatorial study from Foley et al. showed that KIR3DL1 in combination with certain HLA-A alleles has a protective role in saving target cells from KIR3DL1-dependent NK cells lysis as KIR3DL1-ligand mismatch allows for NK alloreactivity.[35] One study has reported an increased frequency of the KIR3DL1+HLA-A Bw4 combination in patients with acute myeloid leukemia, who received hematopoietic stem-cell transplantation, although the effect was not significant, may be effective in the enhancement of inhibition.[36]

So far, few studies have addressed the effects of NK cell alloreactivity based on the missing-self hypothesis on the outcome of kidney transplantation. Two studies have shown that KIR ligand incompatibility has no effect on kidney allograft survival.[12,23] It has been shown that the presence of KIR2DS3 combined with the presence of the corresponding HLA ligand have an adverse effect on long-term outcomes after transplantation.[37] Furthermore, a significant association between the alloreactivity of NK cells after kidney allograft transplantation and KIR2DL1/HLA-C2 and KIR2DL2,3/HLA-C1 has been reported.[20]

The analysis of peptide-HLA class I tetrameric complexes by Thananchai et al. has indicated that HLA-Bw4*A allele-expressing target cells were resistant to lysis mediated by KIR3DL1-expressing NK

cells. It has been shown that Bw4-bearing HLA-A can serve as inhibitory ligands in a manner similar to that of HLA-B signaling.[38]

We assessed the effect of genetic variation at the KIR/HLA locus using combinatorial analysis on protection/susceptibility toward AKAR. Reduction of the inhibitory compound of KIR-HLA (including KIR3DL1+HLA-Bw4*A) may disturbs the balance of the signal transduction between activating and inhibitory receptors. Furthermore, the ineffective suppression of activating signals results in enhancing the cytotoxic function of NK cells. Together, these findings indicate that the presence of the inhibitory KIR3DL1+HLA-Bw4*A allele combination is a main feature in the SGF group, and might be crucial to improve kidney graft outcome in the long term.

We did not find an association between KIR frequencies or gene content of KIR in the peripheral blood of renal transplant patients and the incidence of acute rejection. We found out that the lack of certain inhibitory KIRs in combination with their HLA ligands might trigger NK cell activation and subsequently leading to susceptibility to AKAR. Using survival analysis, we suggested that the KIR3DL1+HLA-Bw4*A allele could be considered as a protective combination for AKAR. Combinatorial results could help us to select the best possible match for each patient to improve the outcome of allograft kidney transplantation. A comprehensive KIR/HLA combinatorial study has not yet been performed in the context of acute rejection of kidney transplants, and further studies using larger cohorts are still required.

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REFERENCES

1. Kunert K, Seiler M, Mashreghi MF, Klippert K, Schönemann C, Neumann K, et al. KIR/HLA ligand incompatibility in kidney transplantation. Transplantation 2007; 84(11):1527-33.
2. Nankivell BJ, Alexander SI. Rejection of the kidney allograft. N Engl J Med 2010; 363(15):1451-62.
3. Van der Touw W, Bromberg JS. Natural killer cells and the immune response in solid organ transplantation. Am J Transplant 2010; 10(6):1354-8.
4. Vilches C, Parham P. Do NK-cell receptors and alloreactivity affect solid organ transplantation? Transpl Immunol 2006; 17(1):27-30.
5. Kuśnierczyk P. Are killer cell immunoglobulin-like receptor genes important for the prediction of kidney graft rejection? Arch Immunol Ther Exp (Warsz) 2013; 61(4):321-5.
6. Schleinitz N, Vély F, Harlé JR, Vivier E. Natural killer cells in human autoimmune diseases. Immunology 2010. 131(4):451-8.
7. Parham P, Norman PJ, Abi-Rached L, Guethlein LA. Human-specific evolution of killer cell immunoglobulin-like receptor recognition of major histocompatibility complex class I molecules. Philos Trans R Soc Lond B Biol Sci 2012; 367(1590):800-11.
8. Nowak I, Magott-Procelewska M, Kowal A, Miazga M, Wagner M, Niepiekło-Miniewska W, et al. Killer immunoglobulin-like receptor (KIR) and HLA genotypes affect the outcome of allogeneic kidney transplantation. PLoS One 2012; 7(9):e44718.
9. Solgi G, Ghafari H, Ashouri E, Alimoghdam K, Rajalingam R, Amirzargar A. Comparison of KIR gene content profiles revealed a difference between northern and southern Persians in the distribution of KIR2DS5 and its linked loci. Hum Immunol 2011; 72(11):1079-83.
10. Chaisri S, Kitcharoen K, Romphruk AV, Romphruk A, Witt CS, Leelayuwat C. Polymorphisms of killer immunoglobulin-like receptors (KIRs) and HLA ligands in northeastern Thais. Immunogenetic, 2013; 65(9):645-53.
11. Hong HA, Loubser AS, de Assis Rosa D, Naranbhai V, Carr W, Paximadis M, et al. Killer cell immunoglobulin like receptor genotyping and HLA killer-cell immunoglobulin like receptor ligand identification by real time polymerase chain reaction. Tissue antigens 2011; 78(3):185-94.
12. Kreijveld E, van der Meer A, Tijssen HJ, Hilbrands LB, Joosten I. KIR gene and KIR ligand analysis to predict graft rejection after renal transplantation. Transplantation 2007; 84(8):1045-51.
13. Vilches C, Castaño J, Gómez-Lozano N, Estefanía E. Facilitation of KIR genotyping by a PCR-SSP method that amplifies short DNA fragments. Tissue antigens 2007; 70(5):415-22.

14. Wang HD, Zhu BF, Shen CM, Fan AY, Song TN, Liu JL, et al. Diversity distributions of killer cell immunoglobulin-like receptor genes and their ligands in the Chinese Shaanxi Han population. Hum Immunol 2011; 72(9):733-40.
15. Dastmalchi R, Farazmand A, Noshad S, Mozafari M, Mahmoudi M, Esteghamati A, et al. Polymorphism of killer cell immunoglobulin-like receptors (KIR) and their HLA ligands in Graves' disease. Mol Biol Rep 2014; 41(8):5367-74.
16. Hsu KC, Chida S, Geraghty DE, Dupont B. The killer cell immunoglobulin-like receptor (KIR) genomic region: gene order, haplotypes and allelic polymorphism. Immunol Rev 2002; 190(1):40-52.
17. Vidal-Castiñeira JR, López-Vázquez A, Díaz-Peña R, Alonso-Arias R, Martínez-Borra J, Pérez R, et al. Effect of killer immunoglobulin-like receptors in the response to combined treatment in patients with chronic hepatitis C virus infection. J Virol 2010; 84(1):475-81.
18. Tajik N, Shahsavar F, Nasiri M, Radjabzadeh MF. Compound KIR-HLA genotype analyses in the Iranian population by a novel PCR–SSP assay. Int J Immunogenet 2010; 37(3):159-68.
19. Stern M, Ruggeri L, Capanni M, Mancusi A, Velardi A. Human leukocyte antigens A23, A24, and A32 but not A25 are ligands for KIR3DL1. Blood 2008; 112(3):708-10.
20. Van Bergen J, Thompson A, Haasnoot GW, Roodnat JI, de Fijter JW, Claas FH, et al. KIR Ligand Mismatches Are Associated With Reduced Long Term Graft Survival in HLA-Compatible Kidney Transplantation. American Journal of Transplantation 2011; 11(9):1959-64.
21. Legaz I, López-Álvarez MR, Campillo JA, Moya-Quiles MR, Bolarín JM, de la Peña J, et al. KIR gene mismatching and KIR/C ligands in liver transplantation: consequences for short-term liver allograft injury. Transplantation 2013; 95(8):1037-44.
22. Hanvesakul R, Kubal C, Moore J, Neil D, Cook M, Ball S, et al. KIR and HLA-C interactions promote differential dendritic cell maturation and is a major determinant of graft failure following kidney transplantation. PloS one 2011; 6(8):e23631.
23. Tran TH, Unterrainer C, Fiedler G, Döhler B, Scherer S, Ruhenstroth A, et al. No Impact of KIR Ligand Mismatch on Allograft Outcome in HLA-Compatible Kidney Transplantation. Am J Transplant 2013; 13(4):1063-8.
24. Mengel M, Sis B, Haas M, Colvin RB, Halloran PF, Racusen LC, et al. Banff 2011 Meeting report: new concepts in antibody-mediated rejection. Am J Transplant 2012; 12(3):563-70.
25. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16(3):1215.
26. Rajalingam R, Du Z, Meenagh A, Luo L, Kavitha VJ, Pavithra-Arulvani R, et al. Distinct diversity of KIR genes in three southern Indian populations: comparison with world populations revealed a link between KIR gene content and pre-historic human migrations. Immunogenetics 2008; 60(5):207-17.
27. Pyo CW, Guethlein LA, Vu Q, Wang R, Abi-Rached L, Norman PJ, et al. Different patterns of evolution in the centromeric and telomeric regions of group A and B haplotypes of the human killer cell Ig-like receptor locus. PLoS One 2010; 5(12):e15115.
28. Zolfaghari L, Solgi G, Nafar M, Ahmadpour P, Lassanpezeshki M, Ali Amirzargar M, et al. Association of programmed cell death 1 and programmed cell death 1 ligand gene polymorphisms with delayed graft function and acute rejection in kidney allograft recipients. Iran J Kidney Dis 2015; 9(2):138-45.
29. Alausa M, Almagro U, Siddiqi N, Zuiderweg R, Medipalli R, Hariharan S. Refractory acute kidney transplant rejection with CD20 graft infiltrates and successful therapy with rituximab. Clin Transplant 2005; 19(1):137-40.
30. Hollenbach JA, Ladner MB, Saeteurn K, Taylor KD, Mei L, Haritunians T, et al. Susceptibility to Crohn's disease is mediated by KIR2DL2/KIR2DL3 heterozygosity and the HLA-C ligand. Immunogenetics 2009; 61(10):663-71.
31. Tran TH, Mytilineos J, Scherer S, Laux G, Middleton D, Opelz G. Analysis of KIR ligand incompatibility in human renal transplantation. Transplantation 2005; 80(8):1121-3.
32. Vampa ML, Norman PJ, Burnapp L, Vaughan RW, Sacks SH, Wong W. Natural killer-cell activity after human renal transplantation in relation to killer immunoglobulin-like receptors and human leukocyte antigen mismatch1. Transplantation 2003; 76(8):1220-8.
33. Uhrberg M. The KIR gene family: life in the fast lane of evolution. Eur J Immunol 2005; 35(1):10-5.
34. Bakker AB, Phillips JH, Figdor CG, Lanier LL. Killer cell inhibitory receptors for MHC class I molecules regulate lysis of melanoma cells mediated by NK cells, γδ T cells, and antigen-specific CTL. J Immunol 1998; 160(11):5239-45.
35. Foley BA, De Santis D, Van Beelen E, Lathbury LJ, Christiansen FT, Witt CS. The reactivity of Bw4+ HLA-B and HLA-A alleles with KIR3DL1: implications for patient and donor suitability for haploidentical stem cell

transplantations. Blood 200; 112(2):435-43.
36. Shahsavar F[1], Tajik N, Entezami KZ, Fallah Radjabzadeh M, Asadifar B, Alimoghaddam K, et al. KIR2DS3 is associated with protection against acute myeloid leukemia. Iran J Immunol 2010; 7(1):8-17.
37. La Manna G, Corsini S, Iannelli S, Cappuccilli ML, Comai G, Iorio M, et al. Influence of the immunogenetic KIR and HLA systems on long-term renal transplant outcome. Ann Transplant 2013; 18:611-21.
38. Thananchai H, Gillespie G, Martin MP, Bashirova A, Yawata N, Yawata M, et al., Cutting Edge: Allele-specific and peptide-dependent interactions between KIR3DL1 and HLA-A and HLA-B. J Immunol 2007; 178(1):33-7.