Killer Immunoglobulin-like Receptor (KIR) and HLA Genotypes Affect the Outcome of Allogeneic Kidney Transplantation

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Abstract

Background: Recipient NK cells may detect the lack of recipient’s (i.e., self) HLA antigens on donor renal tissue by means of their killer cell immunoglobulin-like receptors (KIRs). KIR genes are differently distributed in individuals, possibly contributing to differences in response to allogeneic graft.

Methodology/Principal Findings: We compared frequencies of 10 KIR genes by PCR-SSP in 93 kidney graft recipients rejecting allogeneic renal transplants with those in 190 recipients accepting grafts and 690 healthy control individuals. HLA matching results were drawn from medical records. We observed associations of both a full-length KIR2DS4 gene and its variant with 22-bp deletion with kidney graft rejection. This effect was modulated by the HLA-B,-DR matching, particularly in recipients who did not have glomerulonephritis but had both forms of KIR2DS4 gene. In contrast, in recipients with glomerulonephritis, HLA compatibility seemed to be much less important for graft rejection than the presence of KIR2DS4 gene. Simultaneous presence of both KIR2DS4 variants strongly increased the probability of rejection. Interestingly, KIR2DS5 seemed to protect the graft in the presence of KIR2DS4del but in the absence of KIR2DS4fl.

Conclusions/Significance: Our results suggest a protective role of KIR2DS5 in graft rejection and an association of KIR2DS4 with kidney rejection, particularly in recipients with glomerulonephritis.

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Introduction

Acute or chronic rejection of solid organ grafts such as kidney is mediated by alloreactive T lymphocytes recognizing major (HLA) and minor histocompatibility antigens by means of antigen-specific T cell receptors (TCR) [1,2]. However, a contribution of natural killer (NK) cells has also been postulated. Thus, infiltration of renal tissue by NK cells suggests not only their role as a direct cause of allograft rejection but also their potential role in the reaction in opposite direction in hematopoietic cell transplantation [9–11]. In addition, KIRs are expressed also on some T lymphocytes, particularly on special subpopulation of CD4+CD28− cytotoxic T cells involved in autoimmune vasculitis [12–14], potentially influencing their activity in graft rejection.
Human KIRs are encoded by genes located in the chromosomal region 19q14. KIR genetics is characterized by both allelic (up to more than 50 alleles for some KIRs) and haplotypic (i.e., different numbers of inhibitory and activating genes on individual chromosomes) polymorphism [8,15]. As a result, above 97% of unrelated persons differ by their KIR genotype [16–17]. Two categories of KIR haplotypes were described: A-type haplotypes containing mostly inhibitory KIRs, and only KIR2DS4 and KIR2DL4 as activating ones, and B-type haplotypes, characterized by one or more of other activating KIRs in addition to inhibitory ones. For this reason, people may differ substantially in their NK and T cell responses, depending on KIR genotype. We have recently published results showing a contribution of KIR2DS5 gene to a tolerance of kidney graft as well as to other clinical situations [18]. Here, we focused on kidney graft rejection and compared frequencies of 10 KIR genes in recipients rejecting the allogeneic renal transplant with those in recipients accepting such a graft. Our study is the first report on different HLA and KIR genetic associations of kidney graft acute rejection in recipients whose pre-transplant renal failure resulted from glomerulonephritis versus those whose renal failure was a result of other disease.

**Materials and Methods**

**Kidney graft recipients and controls**

All individuals, including kidney graft recipients, donors, and healthy controls, were Polish Caucasians. Two hundred eighty-three kidney patients (clinical data presented in Table 1) underwent first transplantation and received deceased donor kidney between 1989 and 2008 (166 patients after 2000). All patients were treated with triple-therapy (Figure 1) as initial immunosuppression that incorporated cyclosporine (n = 219) or tacrolimus (n = 64, beginning in 2000) in combination with azathioprine (n = 129) or mycofenolate mofetil (n = 154, since 1998) (Figure 2) and steroids. No induction with antibodies was used. During the follow up (mean time was 7 years) there were 246 (87%) patients who were treated with the same calcineurin inhibitor. Among 29 patients who changed the type of calcineurin inhibitor, 20 patients were converted from CsA to tacrolimus after an episode of rejection treated with methylprednisolone. Calcineurin inhibitor was withdrawn in 8 individuals. There were 233 patients who received the same type of purine metabolism inhibitor during follow up; azathioprine (n = 84) and mycofenolate mofetil (n = 149). Azathioprine was replaced by mycofenolate mofetil in 33 patients (in 20 patients after an episode of acute rejection) or stopped in 12 patients. The frequency of a change in treatment regimen was almost 2-fold higher in patients who suffered a rejection (refers to 38% and 21% of patients with and without rejection, respectively, p = 0.0063).

93 recipients exhibited symptoms of acute graft rejection based on clinical criteria (an increase in serum creatinine level of at least 20% above the baseline measurements not attributable to another cause) confirmed by histopathological examination according to Banff criteria [19]. Apart from three patients, all had a biopsy-proven acute rejection episode. Remaining 190 recipients experienced stable graft function during long-term follow-up. 31 (33%) patients who suffered acute rejection subsequently lost their grafts in comparison to 22 (12%) patients without an episode of rejection. During the follow-up, 5 out of 93 (5.4%) patients with AGR and 13 out of 190 (6.8%) patients without AGR died for different reasons.

Six hundred and ninety unrelated healthy volunteers, constituting a basic control group in KIR studies performed in our laboratory, were recruited in the years 2001–2008 by the Regional Center of Blood Transfusion, Wroclaw, as well as by clinics of the Wroclaw Medical University, the Medical University of Warsaw, and the Pomeranian Medical University, Szczecin.

The same cohorts of patients and controls have already been used to describe a protective effect of KIR2DS5 gene on kidney graft rejection and some other clinical situations [10]. The Bioethics Committee of the Wroclaw Medical University specifically approved this study. Signed written informed consent was given by all participants.

**DNA isolation and KIR typing**

DNA was isolated from venal blood as described [20,21]. The presence or absence of KIR genes was detected by either individual [20–22] or multiplex [23] polymerase chain reactions (PCR) which, when tested on the same samples, gave virtually identical results. Our KIR typing has been validated three times per year by the International KIR Exchange program organized by the Immunogenetics Center of the University of California at Los Angeles.

**HLA-A, -B, and -DR typing of donors and recipients has been routinely done before transplantation either in Non-Public Tissue Typing Facility at our Institute or in other transplant centers in Poland, and it was drawn from the clinical histories of the patients.**

**Tissue samples were available for only 42 donors, therefore their HLA-C typing was possible only in these instances, had statistically insufficient power, and therefore its results are not presented here.**

Recipient HLA-C variants encoding C1 and C2 epitopes were described and discussed earlier [18].

**Statistical analysis**

General linear model (GLM) with binomial errors was used to investigate relationship between clinical and genetic variables and probability of rejecting the transplanted kidney (Table 2). Frequencies of KIR genes in recipients and HLA matching between donors and recipients were explanatory variables. Clinical characteristics (age, sex, creatinine, course of transplantation and time of observation) was concomitant variables. Akaike’s information criterion (AIC) was used as a measure of fit of models. Bootstrap approach was employed to estimate model’s coefficients and 95% confidence intervals. Chi-squared test with Yates’ continuity correction was used to test hypothesis that rejection and type of genotype were independent. To test differences in KIR’s distribution among patients and control, a group permutation test was employed. This procedure was based on Mahalanobis distance (D_M) between two groups and test performed in 10 000 permutations. Odds ratio (OR) was computed as a measure of effect size. Probability that graft is not rejected at a given time, S(t), was computed according to the Kaplan-Meier method, comparing KIR genotypes. Haplotype frequencies (HF) among two KIR: 2DS4 (full-length or deletion variant) and 2DS5 were estimated with maximum likelihood function [24].

Measures for the estimation of linkage disequilibrium (LD) were the correlation of two alleles frequencies, r, global squared correlation between two loci, R², and Kulback-Leiber divergence two loci from LE [24,25]. For two loci 2DS4 and 2DS5, r and R² obtained as: r = D_{ij}/\sqrt{D_{ii}D_{jj}}, where p_i and q_j are the population allele frequencies of the ith allele on locus 2DS4 and the jth allele on locus 2DS5, D_{ij} = x_{ij} - p_iq_j, and x_{ij} is the frequency of the haplotype with alleles i and j on loci 2DS4 and 2DS5, respectively.

R² = Σ_i Σ_j D_{ij}² / p_iq_j, Kulback-Leiber divergence [26,27], D_{KL}, is a measure of distance between the observed haplotype distribution
and the expected distribution assuming LE: $D_{KL} = \sum_{ij}^{n_{ij}} x_{ij} \log \frac{x_{ij}}{p_{ij}}$. Chi-square statistic was calculated to test that all of the $D_{ij}$'s between 2DS4 and 2DS5 are zeros: $\chi^2 = \sum_{ij}^{n_{ij}} \frac{n_{ij}^2 - \pi_{ij}}{\pi_{ij}}$. Likelihood ratio statistic, LRS, was used to test for differences in haplotype frequencies between rejectors, non-rejectors and controls.

$$\text{LRS}_{df} = 2(\text{LL}_{\text{Rejectors}} + \text{LL}_{\text{Non-rejectors}} - \text{LL}_{\text{Combined}}),$$

where log likelihoods were produced based on haplotype frequencies and LRS is approximately a $\chi^2$. Results were regarded as statistically significant at $p < 0.05$. All data were analyzed using R version 2.2.1.

Results

**HLA and KIR2DS4 gene effects on acute kidney graft rejection**

Frequencies of KIR genes were not different between patients and controls (Table 3). However, there were some differences between patients with acute graft rejection (AGR) (determined by Banff criteria) and patients without AGR. First, the frequency of the KIR2DS5 gene in patients with AGR was two times lower than in control individuals ($p = 0.0056$). This protective effect of KIR2DS5 gene on kidney graft rejection has already been published recently on the same cohorts of patients and controls [18]. Multivariate analysis indicated significant protective effect of HLA-B,-DR matching (Table 2) but HLA-A did not affect graft fate (data not shown). We also observed that the presence of both KIR2DS4 full-length (KIR2DS4fl) and 22-base pair deletion variant (KIR2DS4del) gene was increasing a probability of rejection at least twofold (Table 2). This effect was amplified to a great extent by the HLA-B,-DR mismatching (matching = 0, Figure 3).

Effects of KIR2DS4 gene variants and KIR2DS5 gene on the probability of graft rejection

Probability that graft was not rejected at a given time, $S(t)$, was computed for the presence or absence of KIR2DS4fl, KIR2DS4del or both (Figure 4). Simultaneous presence of both gene variants strongly increased the probability of graft rejection, whereas the presence of only KIR2DS4fl, only KIR2DS4del, or none of them gave much lower probability of rejection. Interestingly, the two variants of KIR2DS4 gene had opposite influence on the effect of KIR2DS5 gene: KIR2DS5 seemed to protect the graft stronger in the presence of KIR2DS4fl than in its absence (Figure 5), but stronger in the absence than presence of KIR2DS4del (Figure 6).

Combinations of KIR genes gave 133 different genotypes present in patients and/or controls (data not shown). These genotypes were divided into AA and BX genotypes, containing two A haplotypes or at least one B haplotype, respectively (for a definition of A and B haplotypes, see Introduction). Two individual AA genotypes were distributed significantly differently between patient subgroups: among patients with the genotype No. 1 acute graft rejection (AGR) was nearly four times less frequent than lack of AGR (21.9% vs 78.1%), whereas in those with the genotype No. 2 the ratio of AGR and non-AGR was 1:1 (Table 4). Interestingly, these two genotypes differed only by the absence of full-length KIR2DS4 gene in the genotype No. 1 and its presence in the genotype No. 2, and both were devoid of KIR2DS5 by definition, as AA genotypes. An additional AA genotype containing KIR2DS4fl but no KIR2DS4del gene was extremely rare.
Figure 2. Patient disposition according to purine metabolism inhibitor use. AGR, acute graft rejection; Aza, azathioprine; MMF, mycophenolate mofetil.
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Table 1. Clinical characteristics of patients.

|                  | Patients with AGR | Patients without AGR | p     | OR   | 95%CI          |
|------------------|-------------------|----------------------|-------|------|----------------|
| **Age**          |                   |                      |       |      |                |
| Mean +/- SD      | 43.65±11.31       | 43.36±11.46          | 0.8   |      |                |
| min-max          | 15–67             | 16–72                |       |      |                |
| **Sex**          |                   |                      |       |      |                |
| Females/Males    | 32/61             | 92/98                | 0.03  | 0.56 | 0.33–0.93      |
| % of Females     | 34.4%             | 48.4%                |       |      |                |
| N (%)            | N (%)             |                      |       |      |                |
| **Cause of renal failure** |                 |                      |       |      |                |
| Glomerulonephritis | 58 (62.4)        | 74 (39.0)            | 0.0002| 2.60 | 1.56–4.33      |
| Interstitial nephritis | 10 (10.7)      | 27 (14.2)            | 0.46  | 0.73 | 0.34–1.58      |
| Cystic kidney    | 10 (10.7)         | 24 (12.6)            | 0.7   | 0.83 | 0.38–1.82      |
| Hypertensive nephropathy | 3 (3.2)       | 13 (6.8)             | 0.28  | 0.45 | 0.13–1.63      |
| Diabetic nephropathy | 5 (5.4)        | 7 (3.7)              | 0.54  | 1.49 | 0.46–4.81      |
| Other            | 7 (7.6)           | 45 (23.7)            | 0.0009| 0.26 | 0.11–0.61      |

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(one individual among patients and 8 in controls), therefore it could not play any role in rejection and was omitted from our calculations. Within BX (i.e., non-AA) genotype group, fraction of patients with AGR was nearly two times less frequent than those without AGR. These differences between genotype groups were not accidental ($\chi^2 = 6.675$, df = 2, $p = 0.035$).

\textbf{KIR2DS4} and \textbf{KIR2DS5} are in negative linkage disequilibrium (LD) in all populations tested so far [28]. Table 5 shows that they are in negative LD also in our population. \textbf{KIR2DS4/KIR2DS5} haplotype frequencies for non-rejectors and controls were very similar, and these two groups were combined in the following calculations. Likelihood ratio statistics (LRS) for patients with AGR versus combined group of patients without AGR and controls was high, which suggests that haplotype frequencies in the former were different from those in the latter ($p = 0.05$). We see, for example, that a haplotype \textit{KIR2DS4-}/\textit{KIR2DS5+} (−/2DS5) was three times less frequent in patients with AGR than in other groups. This seems to confirm a protective role of \textit{KIR2DS5} in graft rejection shown above.

\textbf{Difference between patients with glomerulonephritis and those with other kidney diseases in association of acute graft rejection with \textit{KIR2DS4} and HLA genotype}

Multivariate analysis revealed also a difference between patients whose end stage renal failure was caused by glomerulonephritis and those with other nephropathies. Namely, in the non-glomerulonephritis group, \textit{HLA-B,-DR} matching seemed to be much more important for acute graft rejection than the presence or absence of \textit{KIR2DS4} gene variants [Figure 7, right panel]. Thus, in the case of perfect \textit{HLA-B,-DR} matching (matching $= 4$), the presence or absence of \textit{KIR2DS4fl} and \textit{KIR2DS4del} genes only very weakly influenced graft fate. Recipients of completely \textit{HLA-B,-DR} incompatible grafts (matching $= 0$) possessing both forms of \textit{KIR2DS4} gene had only 6 times higher chance of kidney rejection than recipients of similarly \textit{HLA-B,-DR}-incompatible grafts negative for \textit{KIR2DS4} [Figure 7, right panel].

In contrast, in recipient group with glomerulonephritis, \textit{HLA} incompatibility seemed to be much less important than \textit{KIR2DS4} for graft rejection. For example, completely \textit{HLA}-mismatched \textit{KIR2DS4}-negative recipients had only about 1.4 times higher chance of acute rejection than perfectly \textit{HLA}-matched \textit{KIR2DS4}-negative recipients [Figure 7, left panel]. On the other hand, the presence of both forms of \textit{KIR2DS4} gene had strong effect on graft rejection in glomerulonephritis group, as even perfect HLA matching did not reduce a chance of rejection below odds ratio of 85 [Figure 7, left panel]. Individuals from the glomerulonephritis group possessing both variants of \textit{KIR2DS4} and perfect \textit{HLA-B,-DR} matching had about 15 times higher chance of rejection than analogous persons from the non-glomerulonephritis group (see Figure 7, both panels).

\begin{table}[h]
\centering
\caption{Variables significantly associated with probability of graft rejection.}
\begin{tabular}{|c|c|c|c|}
\hline
Variable & OR & 95% CI & $P$ \\
\hline
\textit{HLA-B,-DR} match & 0.46 & 0.30 & 0.70 \quad 0.0003 \\
\textit{KIR2DS4fl} & 2.02 & 1.05 & 3.90 \quad 0.03 \\
\textit{KIR2DS4del} & 2.61 & 1.19 & 5.75 \quad 0.02 \\
\textit{HLA-B,-DR} match $\times$ GN & 2.01 & 1.46 & 2.76 \quad 0.0000 \\
\hline
\end{tabular}
\end{table}

Abbreviations: CI, confidence interval; GN, glomerulonephritis; HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptor; \textit{KIR2DS4fl}, \textit{KIR2DS4} 22-base pair deletion variant of the \textit{KIR2DS4} gene; OR, odds ratio.

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\textbf{Figure 3. Dependence of odds ratio for kidney graft rejection on \textit{HLA-B,-DR} matching, \textit{KIR2DS4} full length (\textit{KIR2DS4fl}) and/or \textit{KIR2DS4} deletion variant (\textit{KIR2DS4del}) gene presence.} For odds ratio calculations, the recipient group with complete (n = 4) \textit{HLA-B,-DR} match with the donor and a lack of any \textit{KIR2DS4} variant (\textit{KIR2DS4fl} and \textit{KIR2DS4del} negative: fl-/del-) was taken as 1.

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Discussion

We compared the distribution of KIR genes in patients rejecting and non-rejecting kidney graft as well as in healthy controls. Among individual KIR genes, only KIR2DS4 (both full-length and deletion variants) was remarkably more frequent in patients with AGR than in patients with stable graft function and controls. Moreover, this effect was particularly strong in the absence of KIR2DS5 gene which exerted opposite effect, i.e., its presence decreased the chance of graft rejection as published already on the same cohorts of patients and controls [18].

It is interesting in this context that KIR2DS4 molecule was expressed on remarkable proportion of CD4+CD28- T cell clones isolated from an acute coronary syndrome patient [29]. Also, dialyzed patients exhibited an increased number of circulating CD4+CD28- T cells [30]. In addition, CMV positivity is universal in our transplant population (data not shown), and the association of CMV infection and the presence of CD4+CD28- cells is well documented (ref.30 and references therein). CD4+CD28- T cells, virtually absent from peripheral blood of healthy individuals but present in acute coronary syndrome and rheumatoid vasculitis [29], multiple sclerosis [14] and, most important here, in end-stage renal disease [30,31], were found to be resistant to immunoregulation [14] and therefore postulated to play a role in autoimmune diseases and aging [12]. KIR2DS4fl encodes an activating receptor which might possibly be involved in stimulation of effector cells (e.g., CD4+CD28- T cells) contributing to transplant rejection, whereas KIR2DS4del potentially codes for a soluble molecule [32]. It might be that, in kidney graft recipients, this soluble KIR2DS4 molecule is masking a ligand for membrane-bound KIR2DS4 or another receptor of some regulatory cells (T, NK, or other). The ligand for KIR2DS5 receptor is not known, however it has been observed that the simultaneous presence of KIR2DS5 gene and HLA-C-encoded epitopes for both KIR2DL1 and KIR2DL2/3 receptors significantly decreased leukemia-free survival of hematopoietic stem cell-transplanted patients [33], which could suggest KIR2DS5 interaction with HLA-C. Thus, in the case of renal transplant recipients, soluble KIR2DS4 might block interaction of KIR2DS5 with its ligand which otherwise would protect a graft from rejection. In recipients negative for KIR2DS4del gene the presence of KIR2DS5 seems to favor the

| Table 3. KIR gene frequencies in controls and patients. |

| KIR | Group | 2DL1 | 2DL2 | 2DL3 | 2DS1 | 2DS2 | 2DS3 | 2DS4fl | 2DS4del | 2DS5 | 3DL1 | 3DS1 |
|-----|-------|-----|-----|-----|-----|-----|-----|-------|-------|-----|-----|-----|
| Patients Present | 268 | 142 | 257 | 105 | 143 | 81 | 82 | 236 | 65 | 256 | 93 |
| N = 283 % | 94.70 | 50.18 | 90.81 | 37.10 | 50.53 | 28.62 | 28.97 | 83.39 | 22.97 | 90.46 | 32.86 |
| Control Present | 665 | 374 | 623 | 299 | 369 | 214 | 194 | 561 | 205 | 642 | 264 |
| N = 690 % | 96.38 | 54.20 | 90.29 | 43.33 | 53.48 | 31.01 | 28.12 | 81.30 | 29.71 | 93.04 | 38.26 |
| ρ | 0.3 | 0.3 | 0.9 | 0.4 | 0.4 | 0.5 | 0.8 | 0.5 | 0.03 | 0.2 | 0.1 |
| 95% CI | 0.35–1.29 | 0.65–1.12 | 0.66–1.71 | 0.58–1.03 | 0.67–1.17 | 0.66–1.21 | 0.77–1.42 | 0.80–1.67 | 0.51–0.97 | 0.43–1.16 | 0.59–1.06 |

Abbreviations: CI, confidence interval; KIR, killer immunoglobulin-like receptor; KIR2DS4fl, KIR2DS4 full length gene; KIR2DS4del, KIR2DS4 22-base pair deletion variant of the KIR2DS4 gene; OR, odds ratio.

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Figure 4. Effects of full-length KIR2DS4 gene (fl) and its deletion variant (del) on the outcome of renal transplantation. Kaplan Meier estimations of probability that graft is not rejected at a given time, S(t). Left panel: KIR2DS4fl present, KIR2DS4del present or absent; right panel: KIR2DS4fl absent, KIR2DS4del present or absent.

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acceptance of the graft, particularly in the presence of KIR2DS4fl gene. The reason for the latter effect is not clear; either both KIR2DS5 and KIR2DS4fl act in the same direction, e.g. expressed on the same or on two different regulatory cells, or, alternatively, the presence of KIR2DS4fl gene, by excluding the presence of its allele, KIR2DS4del, from the same chromosome, decreases its frequency in KIR2DS4fl-positive patient population.

KIR2DS4 molecule differs from KIR2DS1 and KIR2DS2/3 by weaker interaction with HLA-C and by binding to HLA-A*11 [34]. As mentioned in Material and Methods section, blood, lymphoid tissue or DNA samples of majority of donors were not available for our study, and their HLA-C typing was not possible here. However, in a recent study, Hanvesakul and coworkers [35] have not detected any association between donor HLA-C-encoded KIR ligand and acute rejection of kidney allograft. On the other hand, these authors have reported an effect of recipient HLA-C on the kidney graft rejection, i.e., a protective effect of C2 on allograft survival. They observed donor-derived NK cells in the allograft at the time of transplantation. These NK cells could stimulate maturation of dendritic cells which would then be capable of indirect stimulation of adaptive immune system for alloreactivity. In vitro studies of Hanvesakul et al. [35] have shown that donor-derived, interleukin-15-activated NK cells promoted efficiently the maturation of recipient dendritic cells only when these were C2-negative. They propose that, after kidney transplantation, donor NK cells interacting with recipient C2-positive dendritic cells do not stimulate them for maturation as efficiently as in C2-negative recipients, and this is beneficial for graft survival. In our earlier study, we have not observed such an effect of recipient C2 on graft survival; on the contrary, we have rather seen some, albeit weak and non-significant, association of C2 with kidney rejection [18]. The reason for this discrepancy may lie in numbers: our sample (283 patients) was 2.7 times less numerous than that of the British group (760 individuals). Alternatively, the
British and our patients might have differed in a cause of renal failure: we have found a substantial genetic difference between recipients with glomerulonephritis and those with other conditions, whereas Hanvesakul et al. [35] did not show a clinical status of their patients before transplantation.

*HLA-A*11 allele (a *KIR2DS4* ligand, see above) frequency in the Polish population is 6.239%, as established by Schmidt and colleagues [36]; therefore, its effect on kidney graft survival, if any, could not be strong. Nevertheless, we have noticed that a majority of *HLA-A*11-positive graft-rejecting recipients (4 out of 5) possessed *KIR2DS4fl* gene, whereas only one fifth of All-positive patients with stable graft function (4 out of 20) typed positive for *KIR2DS4fl* gene. However, the numbers were far too small for any conclusion. No difference between distribution of *KIR2DS4del* variant in All-positive patients with and without AGR was seen (data not shown).

So far, only few publications dealt with *KIR* gene associations with kidney graft rejection. Tran et al. [37] studied an effect of *KIR* ligand (i.e., C1, C2, and Bw4) matching on graft survival in 1416 recipients of cadaver kidney (both first grafts and regrafts) from all inhabited continents, but did not detect any effect. Similarly negative result was obtained by Kreijveld et al. [38] who tested not only *KIR* ligands, but also *KIR* genes themselves as well as combinations of both in Dutch population. On the other hand, upon testing a *KIR* polymorphism in *HLA*-identical recipient-donor pairs from U.S.A., Ciocco et al. [39] obtained a result suggesting protective effect of *KIR2DL2* and *KIR2DS2* genes; however, their study was based on extremely low number of individuals (only 12 recipient-donor pairs). Nevertheless, this finding was confirmed by Kunert et al. [40] on 105 graft recipients and 119 controls in Germany. Finally, van Bergen et al. [41] observed an effect of *KIR*-KIR ligand mismatch between recipient and donor in Dutch population, but only in *HLA-A, B, DR*-compatible donor-recipient pairs, i.e., when *HLA*-identical partners differed in their *KIR* gene repertoire, resulting in lack of a ligand in donor for a *KIR* present in recipient. Notably, the effect of *KIRs* in *HLA*-matched pairs was as strong as that of *HLA-A, B* mismatch in pairs matched only for *HLA-DR* [41,42]. In our study, we observed a strong effect of *KIR2DS4* variants in patients with glomerulonephritis, where *HLA-B, DR* mismatch exerted much weaker influence on the graft fate, whereas in recipients without glomerulonephritis the effect of *HLA*-mismatch was predominant. The striking finding of this study was the stronger association of *KIR2DS4* polymorphism than *HLA* incompatibility in GN patients. We can speculate that engagement of KIRs in inflammatory pathway activation defined by their polymorphism may represent a common link between autoimmune and alloimmune response. The possibility that *KIR*-ligand interaction may aggravate both the natural history of glomerulonephritis sustaining immune injury leading to end-stage renal disease and influence alloimmune response.
response causing acute rejection cannot be ruled out. In this setting, \textit{KIR2DS4} polymorphism can provoke the immune response as it can modulate autoimmunity. We can hypothesize that GN and non-GN patients may differ in KIR2D receptor expression on NK and T cells, particularly in those rejecting vs. non-rejecting the graft, as it has recently been shown in liver transplantation \cite{43}.

In summary, our results suggest that typing of the recipient for \textit{KIR2DS4} and \textit{KIR2DS5} genes may help to predict the outcome of renal transplantation. We show for the first time, that the effect of \textit{KIR} genotype on the fate of kidney graft in recipients with glomerulonephritis seems to be stronger than that of \textit{HLA} matching, whereas opposite is true for patients with other causes of end-stage renal disease. The lack of strong association of graft rejection with \textit{HLA-B,-DR} mismatching in recipients with glomerulonephritis could not have been observed in earlier studies done without stratification for the presence or absence of \textit{KIR2DS4} gene. However, a small number of data that was collected over a long time period is a limitation to the reliability of our findings. Therefore, more definitive studies would require data input from much higher number of patients and, preferably, from more than one institution.

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\textbf{Author Contributions}

Conceived and designed the experiments: PK IN MM-P. Performed the experiments: IN EM AW WŁ MM AK MW WN-M M. Kaminska. Analyzed the data: PK IN MM-P. Contributed reagents/materials/analysis tools: MM-P AP RP EB M. Klinger DS. Wrote the paper: PK IN MM-P.

\textbf{References}

1. Womer KL, Kaplan B (2009) Recent developments in kidney transplantation – a critical assessment. Am J Transplant 9: 1265–1271.
2. Nankivell BJ, Alexander MJ (2010) Rejection of the kidney allograft. N Engl J Med 363: 1451–1462.
3. Andersen CB, Ladeboed SD, Larsen S (1994) Acute kidney graft rejection. A morphological and immunohistochemical study on ‘zero-hour’ and follow-up biopsies with special emphasis on cellular infiltrates and adhesion molecules. APMIS 102: 23–37.
4. Hancock WW, Gee D, De Moerloose P, Rickles FR, Ewan VA, et al. (1985) Immunohistological analysis of serial biopsies taken during human renal allograft rejection. Changing profile of infiltrating cells and activation of the coagulation system. Transplantation 39: 430–438.
5. Totterman TH, Hanas E, Bergstrom R, Larsson E, Tufveson G (1989) Immunologic diagnosis of kidney rejection using FACS analysis of graft-infiltrating functional and activated T and NK cell subsets. Transplantation 47: 817–823.
6. Cooksey G, Robins RA, Blamey RW (1984) Natural killer cells in renal allograft rejection. Br J Surg 71: 874–877.
7. Vampa ML, Norman PJ, Burnapp L, Vaughan RW, Sacks SH, et al. (2003) Natural killer-cell activity after human renal transplantation in relation to killer immunoglobulin-like receptors and human leukocyte antigen mismatch. Transplantation 76: 1220–1228.
8. Parham P (2005) MHC class I molecules and KIRs in human history, health and survival. Nature Rev Immunol 5: 201–214.
9. Moretta L, Locatelli F, Pende D, Marcenaro E, Mingari MC, et al. (2011) Killer Ig-like receptor-mediated control of natural killer cell alloreactivity in haploidentical hematopoietic stem cell transplantation. Blood 117: 525–530.
10. Velardi A, Ruggeri L, Mancusi A, Aversa F, Christiansen FT (2009) Natural killer cell allorecognition of missing self in allogeneic hematopoietic transplantation: a tool for immunotherapy of leukemia. Curr Opin Immunol 21: 525–530.
11. Pegram HJ, Ritchie DS, Smyth MJ, Wiernik A, Prince HM, et al. (2011) Alloreactive natural killer cells in hematopoietic stem cell transplantation. Leuk Res 35: 14–21.

\begin{figure}
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\caption{Effect of glomerulonephritis on the dependence of odds ratio for kidney graft rejection on \textit{HLA-B,-DR} matching, \textit{KIR2DS4} full length (\textit{KIR2DS4}fl) and/or \textit{KIR2DS4} deletion variant (\textit{KIR2DS4}del) gene presence. For odds ratio calculations, the non-GN group with complete (n = 4) \textit{HLA-B,-DR} match and lack of any \textit{KIR2DS4} variant (\textit{KIR2DS4}fl and \textit{KIR2DS4}del negative: fl-/del) was taken as 1. doi:10.1371/journal.pone.0044718.g007}
\end{figure}
12. Weng NP, Akbar AN, Goronzy J (2009) CD28null + T cells: their role in the age-associated decline of immune function. Trends Immunol 30: 306–312.
13. Snyder MR, Nakajima T, Leibson PJ, Weyand CM, Goronzy JJ (2004) Stimulatory killer Ig-like receptors modulate T cell activation through DAP12-dependent and DAP12-independent mechanisms. J Immunol 173: 3725–3731.
14. Thewissen M, Somers V, Hellings N, Fraussen J, Danneels J, et al. (2007) CD4+CD28null T cells in autoimmune disease: Pathogenic features and decreased susceptibility to immunoregulation. J Immunol 179: 6511–6523.
15. Middleton D, Menegh A, Moscoso J, Arnáiz-Villena A (2008) Killer immunoglobulin-like receptor gene and allele frequencies in Caucasoid, Oriental and Black populations from different continents. Tissue Antigens 71: 105–113.
16. Middleton D, Gonzalez A (2009) The extensive polymorphism of KIR genes. Immunology 129: 8–19.
17. Shilling HG, Young N, Guethlein LA, Cheng NW, Gardiner CM, et al. (2002) Genetic control of human NK repertoire. J Immunol 169: 239–247.
18. Nowak I, Majerczyk E, Winiarski A, Pawlik A, Magott-Procelewska M, et al. (2010) Does the KIR2DS5 gene protect from some human diseases? PLoS ONE 5: e12381.
19. Racusen LC, Solec K, Colvin RB, Castro MC, et al. (1999) The Banff'97 working classification of renal allograft pathology. Kidney International 55: 713–723.
20. Majerczyk E, Pawlik A, Laszczew W, Nowak I, Winiarski A, et al. (2007) Associations of killer cell immunoglobulin-like receptor genes with complications of rheumatoid arthritis. Genes Immun 8: 670–683.
21. Laszczew W, Maticzak M, Cislo M, Nockowski P, Winiarski A, et al. (2004) Gene for the activating natural killer cell receptor, KIR2DS1, is associated with susceptibility to psoriasis vulgaris. Hum Immunol 65: 756–766.
22. Václav C, Castano J, Gómez-Lozano N, Estefanía E (2007) Facilitation of KIR genotyping by a PCR-SSP method that amplifies short DNA fragments. Tissue Antigens 70: 415–422.
23. Sun JY, Gashul L, Miller MM, Goto RM, Rodríguez R, et al. (2004) Development of a multiplex PCR-SSP method for killer cell immunoglobulin-like receptor genotyping. Tissue Antigens 64: 462–468.
24. Excoffier L, Slatkin M (1995) Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 12: 921–927.
25. Abdallah JM, Godfieren B, Cerceo-lysilou C, Perez-Escar C (2003) Linkage disequilibrium fine mapping of quantitative trait loci: a simulation study. Genet Sel Evol 35: 513–532.
26. Bhasi K, Zhang L, Brazeau D, Zhang A, Ramanathan M (2006) Information-theoretic identification of predictive SNPs and supervised visualization of genome-wide association studies. Nuclear Acids Res 34: e101.
27. Liu Z, Lin S (2005) Multilocus LD measure and tagging SNP selection with generalized mutual information. Genet Epidemiol 29: 333–364.
28. Singleton RM, Martin MP, Meyer D, Gao X, Carrington M (2008) Methods for assessing gene content diversity of KIR with examples from a global set of populations. Immunogenetics 60: 711–725.
29. Yen JH, Moore BE, Nakajima T, Scholl D, Schaid DJ, et al. (2001) Major histocompatibility complex class I-recognizing receptors are disease risk genes in rheumatoid arthritis. J Exp Med 193: 1159–1167.
30. Yadav AK, Jha V (2011) CD4+CD28null+ cells are expanded and exhibit a cytolytic profile in end-stage renal disease patients on peritoneal dialysis. Nephrol Dial Transplant 26: 1698–1699.
31. Betjes MGH, Huisman M, Weimar W, Lijens NHR (2008) Expansion of cytolytic CD4+CD28null T cells in end-stage renal disease. Kidney International 74: 760–767.
32. Middleton D, Gonzalez A, Gilmore PM (2007) Studies on the expression of the deleted KIR2DS4*003 gene product and distribution of KIR2DS4 deleted and nondeleted versions in different populations. Hum Immunol 68: 128–134.
33. van der Meer A, Schaap NPM, Schattenberg AVMB, van Cranenbroek B, Tijsen HJ, Joosten I (2008) KIR2DS4 is associated with leukemia free survival after HLA identical stem cell transplantation in chronic myeloid leukemia patients. Molec Immunol 45: 3631–3638.
34. Graef T, Moesta AK, Norman PJ, Ah-Rached L, Vago L, et al. (2009) KIR2DS4 is a product of gene conversion with KIR3DL2 that introduced specificity for HLA-A*11 while diminishing avidity for HLA-C. J Exp Med 206: 2537–2572.
35. Hanvesakul R, Kubal C, Moore J, Neil D, Cook M, Ball S, et al. (2011) KIR and HLA-C interactions promote differential dendritic cell maturation and is a major determinant of graft failure following kidney transplantation. PLoS ONE 6: e23631.
36. Schmidt AH, Solloch UV, Pingel J, Baier D, Bohme I, et al. (2011) High resolution human leukocyte antigen allele and haplotype frequencies of the Polish population based on 20,653 stem cell donors. Hum Immunol 72: 558–565.
37. Trau TH, Mytilineos J, Scherrer S, Laux G, Middleton D, et al. (2005) Analysis of KIR ligand incompatibility in human renal transplantation. Transplantation 80: 1121–1123.
38. Kreijvik E, van der Meer A, Tijsen HJ, Hilbrands LB, Joosten I (2007) KIR gene and KIR ligand analysis to predict graft rejection after renal transplantation. Transplantation 84: 1045–1051.
39. Ciocco RC, Mathew JM, Burke GW 3rd, Esquenazi V, Miller J (2007) Killer cell immunoglobulin-like receptor polymorphisms in HLA-identical kidney transplant recipients: lack of 2DL2 and 2DS2 may be associated with poor graft function. Tissue Antigens 69 (Suppl 1): S123–S124.
40. Kunert K, Seiler M, Mashreghi MF, Klippert K, Schönenmann C, et al. (2007) KIR/HLA ligand incompatibility in kidney transplantation. Transplantation 84: 1527–1533.
41. van Bergen J, Thompson A, Haasoot GB, Roorda J, de Fijter JW, et al. (2011) KIR-ligand mismatches are associated with reduced long-term graft survival in HLA-compatible kidney transplantation. Am J Transplant 11: 1959–1964.
42. Raijänmaa R, Gebel HM (2011) KIR/HLA mismatching in human renal allotransplantation: Emergence of a new concept. Am J Transplant 11: 1771–1772.
43. López-Alvarez MR, Campillo JA, Legaz I, Blanco-García RM, Salgado-Cecilia G, et al. (2011) Divergences in KIR/2DS+ natural killer and KIR/2DS+ CD8+ T-cell reconstitution following liver transplantation. Hum Immunol 72: 229–237.