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REVIEW

Are Killer Cell Immunoglobulin-Like Receptor Genes Important for the Prediction of Kidney Graft Rejection?

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Abstract Killer cell immunoglobulin-like receptors (KIRs) are expressed on natural killer cells and minor subpopulations of thymus-derived (T) lymphocytes. KIRs may have a long cytoplasmic tail and inhibit cell activation upon ligand (HLA class I) binding, or they may have a short cytoplasmic tail and activate a cell after ligand binding. They are encoded by up to 14 genes present in different individuals in different combinations, whence their associations with several human diseases. KIR involvement in the fate of kidney allograft has not been extensively studied; nevertheless some associations had already been noticed. Their results are not concordant: some authors found no effect of *KIR* genotype, whereas others detected protective effect of *KIR2DL2/KIR2DS2* or *KIR–KIR* ligand mismatch. We found an association of *KIR2DS4* gene with acute rejection and a protective effect of *KIR2DS5* gene. Interestingly, in patients, whose end-stage renal disease was caused by glomerulonephritis, the effect of *KIR2DS4* was stronger than *HLA* mismatch, whereas opposite was true for recipients with other causes of renal failure.

Keywords Killer immunoglobulin-like receptor · HLA · Genetics · Kidney graft acute rejection · Glomerulonephritis

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Introduction: Killer Cell Immunoglobulin-Like Receptors

Killer cell immunoglobulin-like receptor (*KIR*) genes encode cell surface glycoproteins present on natural killer (NK) cells and subpopulations of thymus-derived (T) lymphocytes. They serve to detect changes in human leukocyte antigen (HLA) class I expression, induced on the surface of cells by infection with viruses or by malignant transformation. Inhibitory KIRs bind HLA class I molecules on normal cells of the body, which protects these cells from lysis by NK cells. Infected or transformed cells frequently loose or diminish amount of HLA class I on their surface, which exposes them to NK cell attack. Some activating KIRs also bind HLA class I molecules, albeit with lower affinity, and their physiological ligands are not known (Parham et al. 2012). KIR molecules may possess two (KIR2D) or three (KIR3D) immunoglobulin-like extracellular domains. Inhibitory KIRs have a long intracellular region possessing inhibitory motifs (KIR2DL, KIR3DL), whereas activating KIRs have short intracellular tail (KIR2DS, KIR3DS) and need to be in a complex with adapter molecule to transduce activating signal to the cell (see Fig. 1).

Although KIRs are not as specific as T cell receptor, they display some level of ligand selectivity (see Table 1). Several two-domain KIRs bind HLA-C molecules, which fall into two groups depending on amino acid residue in position 80: KIR2DL1 and KIR2DS1 bind C2 allomorphs (lysine 80) whereas KIR2DL2 and KIR2DL3 bind C1 (asparagine 80). It turned out recently that KIR2DL2 may bind also some C2 molecules in addition to C1 (Parham et al. 2012).

KIR molecules are encoded by 14 genes (plus two pseudogenes, not coding for any expressed product) clustered on chromosome 19 (region 19q13.4). A peculiarity of

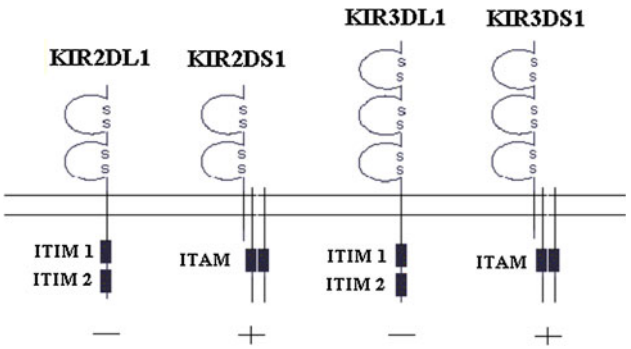

Fig. 1 Schematic presentation of structure of KIR (killer cell immunoglobulin-like receptor) molecules (from Kuśnierczyk 2006, with permission, modified). *KIR2DL1* two immunoglobulin-like-domain, long cytoplasmic tail, receptor 1; *KIR3DS1* three immunoglobulin-like domain, short cytoplasmic tail, receptor 1; *ITIM* immunoreceptor tyrosine-based inhibitory motif, *ITAM* immunoreceptor tyrosine-based activating motif, + activation, − inhibition

Table 1 Known ligands of KIR molecules (based on Kuśnierczyk 2013, and references therein, modified)

KIR	Ligand
2DL1	C2
2DL2	C1 and some C2
2DL3	C1
2DL4	HLA-G
2DS1	C2
2DS4	HLA-A*11, some C1, C2, and non-identified melanoma antigen
3DL1	HLA-Bw4
3DL2	HLA-A*03, A*11, and microbial CpG DNA
3DS1	Unknown (HLA-Bw4?)

No ligands have so far been detected for remaining KIR molecules (KIR2DL5, KIR2DS2, KIR2DS3, KIR2DS5, and KIR3DL3; see Parham et al. 2012)

C1 all HLA-C alleles possessing asparagine residue in position 80, *C2* all HLA-C alleles possessing lysine residue in this position

KIR genetics is their haplotypic polymorphism: humans, even in the same population, differ in the number and kind (inhibitory versus activating) of *KIR* genes (see www.allelefrequencies.net database, Gonzalez-Galarza et al. 2011; Hollenbach et al. 2013; Fig. 2). Therefore, individuals may possess as few as 8 or as many as 13 *KIR* genes (plus one or two pseudogenes). A given genotype may include mostly inhibitory *KIRs* (so called *AA* genotypes, consisting of two *A* haplotypes; Fig. 2), or either few or many activating *KIRs* in addition to inhibitory ones (*Bx* genotypes; [1] Fig. 2). The number of *KIR* genotypes in a population is high, and each genotype is present with a relatively low frequency (see Fig. 2 for twenty most frequent *KIR* genotypes in Poles). Thus, depending on genotype, the immune system of an individual may be more or less prone to activation of NK and T lymphocytes. This is reflected in many *KIR*-disease associations described so far, caused by too little or too much activation of the immune system (Boyton and Altmann 2007; Campbell and Purdy 2011; Kuśnierczyk 2006, 2013; Parham 2005).

KIR and *HLA* genes are located on separate chromosomes (19th and 6th, respectively), and therefore are inherited independently. As a result, a given individual may possess KIRs, for which he/she has no ligand. For example, a person with *AA KIR* genotype will have KIR2DL1 receptor, so if he/she does not have C2 group HLA-C allele, then this KIR2DL1 would not meet its ligand in the body. To make things even more complicated, not all *KIRs* present in the genotype of a given individual are expressed on all NK cell clones (Parham 2005). In the process called NK cell "education" or "licensing", immature, hyporeactive NK cell becomes mature and reactive only if its inhibitory KIR encountered its ligand (Björkström et al. 2010; Elliott and Yokoyama 2011; Schönberg et al. 2011). So, in the example mentioned above, i.e., in the C1/C1 individual, these clones of NK cells which express only KIR2DL1 inhibitory receptor, but do not express other inhibitory KIR molecules reacting with self HLA class I, would not be "licensed" to mature. If they matured, they could be autoaggressive because they would not be inhibited by HLA class I molecules present on normal, healthy cells in the body.

KIR Gene Associations with Kidney Graft Rejection

Acute rejection of allogeneic kidney transplant is mediated by alloreactive T lymphocytes recognizing both HLA alloantigens (most importantly, HLA-A, -B and -DR) and minor histocompatibility antigens (Nankivell and Alexander 2010; Sun et al. 2011; Womer and Kaplan 2009). However, NK cells are also infiltrating kidney allografts (Sun et al. 2011; Totterman et al. 1989). Recipient NK cells were found to exhibit an increased cytotoxicity ex vivo against donor cells three days after transplantation, and this seemed to depend on the number of activating *KIR* genes in the recipient encoding receptors potentially recognizing donor HLA (Vampa et al. 2003). Moreover, peripheral blood of recipients acutely rejecting kidney allograft contained higher numbers of NK cells than blood of non-rejectors (Cooksey et al. 1984). Therefore, several investigators studied possible associations of *KIR* and KIR ligand genes with renal transplant rejection (Table 2). Thus, an extensive study of compatibility versus incompatibility of

[1] Without family studies, it is difficult to differentiate between *AB* and *BB* genotypes, because each *KIR* gene characteristic for *A* haplotype may be also present in some *B* haplotype (Parham 2005). Therefore, non-*AA* genotypes are frequently denoted as *Bx*.

Genotype		*KIR* gene												Frequency
No.	Haplo.	*2DL1*	*2DL2*	*2DL3*	*2DS1*	*2DS2*	*2DS3*	*2DS4fl*	*2DS4del*	*2DS5*	*3DL1*	*3DS1*	*2DL5*	N=117
1	AA	■		■					■		■			26.0
2	Bx	■	■	■	■	■	■		■	■	■	■	■	6.8
3	Bx	■		■	■				■	■	■	■	■	6.8
4	Bx	■	■	■	■	■			■	■	■	■	■	5.1
5	Bx	■	■	■		■	■		■		■		■	5.1
6	Bx	■	■	■		■	■	■	■		■		■	5.1
7	Bx	■	■	■		■		■	■		■			5.1
8	Bx	■		■				■	■		■			5.1
9	Bx	■		■	■		■		■		■	■	■	3.4
10	Bx	■	■	■		■			■		■			2.6
11	Bx	■	■	■	■	■	■	■		■	■	■	■	1.7
12	Bx	■	■	■	■	■	■		■		■	■	■	1.7
13	Bx	■	■	■	■	■		■		■	■		■	1.7
14	Bx	■	■	■	■	■	■	■			■	■	■	1.7
15	AA	■		■				■			■			1.7
16	Bx		■			■		■	■		■			1.7
17	Bx	■	■	■	■	■	■			■		■	■	1.7
18	Bx	■	■			■	■		■		■		■	1.7
19	Bx	■	■		■	■	■		■		■	■	■	1.7
20	Bx	■	■		■	■	■			■		■	■	1.7

Fig. 2 Twenty most frequent *KIR* genotypes in the Polish population ($n = 117$). Framework genes (*KIR3DL3*, *KIR3DP1*, *KIR2DL4*, and *KIR3DL2*), present in all individuals, are omitted. *KIR2DS4fl* and *KIR2DS4del* denominations of *KIR2DS4* full-length gene and its variant with 22 bp deletion, respectively. *Filled and blank fields* gene present and absent, respectively

KIR ligand genes between 2,757 donors and recipients from all continents did not detect any effect (Tran et al. 2005). Similarly, negative result was obtained by Kreijveld et al. (2007) who typed not only for KIR ligand but also for *KIR* genes themselves. However, two studies found protective effect of recipient *KIR2DL2/KIR2DS2*-positive genotype, particularly when KIR2DL2 ligand (*C1*) was present in the donor (Cirocco et al. 2007; Kunert et al. 2007). On the other hand, an association of recipient (not donor!) *C2* with longer graft and patient survival was observed in English patients (Hanvesakul et al. 2011). Interestingly, the effect of *KIR*–KIR ligand mismatch in *HLA*-identical recipient–donor pairs was as strong as that of *HLA-A*, *-B* mismatch in pairs matched for *HLA-DR* only (van Bergen et al. 2011).

We typed 283 kidney graft recipients for *KIR* genes and compared their *KIR* gene frequencies with those of 690 healthy Polish volunteers. No significant differences were found, indicating that end-stage renal failure is not related to *KIR* genotype. Among our patients, ninety-three exhibited symptoms of acute graft rejection ("rejectors"), and 190 had stable graft function ("non-rejectors"). When *KIR* gene frequencies in these two groups were compared, several differences were noted. First, *KIR2DS5* gene was two times less abundant in rejectors than in non-rejectors (Nowak et al. 2010). Second, the presence of *KIR2DS4* full-length gene (*KIR2DS4fl*) and 22 base-pair deletion variant (*KIR2DS4del*) was increasing a probability of acute rejection at least twofold. Last but not least, Kaplan–Meier analysis revealed that the two variants of *KIR2DS4* gene had opposite effects on the protection exerted by *KIR2DS5* gene. Namely, *KIR2DS5* seemed to protect the graft stronger in the presence of *KIR2DS4fl* than in its absence. However, the effect of *KIR2DS5* was stronger in the absence than in the presence of *KIR2DS4del* (Nowak et al. 2012). The explanation of these findings is not clear. Normal-length KIR2DS4 receptor binds HLA-A*11 and some HLA-C (from both C1 and C2 group) molecules (Graef et al. 2009; Table 1), whereas a ligand for KIR2DS5 is not known. Circumstantial evidence suggests, however, that KIR2DS5 may interact with HLA-C, at least in some instances (van der Meer et al. 2008). If KIR2DS4 and KIR2DS5 were expressed on cytotoxic effector cells (NK or T) which could kill antigen-presenting cells (Hanvesakul et al. 2011), or on T regulatory cells like KIR3DL1 homologue in mice (Qin et al. 2011), then they both might contribute to the inhibition of anti-transplant response. On the other hand, if the defective *KIR2DS4* alleles (*KIR2DS4del*) produced a functional soluble protein

Table 2 KIR and KIR ligand gene associations with acute kidney graft rejection

Genes examined	Ethnicity	Recipient–donor pair number	Effect	References
KIR ligands compatibility	European, North American, Latinoamerican, Australian, African, Middle East, Asian	2,757	None	Tran et al. (2005)
KIRs and KIR ligands	German	224	2DL2/S2 protective if C1 present	Kunert et al. (2007)
KIRs and KIR ligands	Dutch Caucasian	69	None[a]	Kreijveld et al. (2007)
KIRs in HLA-matched transplantation	North American	12	2DL2/S2 protective	Cirocco et al. (2007)
KIRs and KIR ligands	Dutch	397	KIR–KIR ligand mismatch decreased long-term graft survival in HLA-matched recipients	van Bergen et al. (2011)
KIRs and KIR ligands	English	760	Donor and recipient HLA-Bw4 and KIR, none; recipient C2 associated with longer graft and patient survival	Hanvesakul et al. (2011)
KIRs	Polish	283	2DS4fl and 2DS4del increased a chance of rejection; 2DS5 protected if 2DS4fl present but 2DS4del absent	Nowak et al. (2012)

[a] Patients with steroid-resistant acute rejection episode were excluded

(Maxwell et al. 2002; Middleton et al. 2007), this molecule might block the interaction of KIR2DS5 with its ligand and abolish its effect.

Both *KIR* genes mentioned above and *HLA-B*, *-DR* (but not *HLA-A*) matching influenced a chance of acute graft rejection episode. However, multivariate analysis revealed an interesting difference between patients, whose end-stage renal disease was caused by glomerulonephritis, and those with other nephropathies. In the non-glomerulonephritis group, *HLA-B*, *-DR* matching seemed to be much more important for rejection than the presence or absence of *KIR2DS4* gene variants. In contrast, opposite was true for patients with glomerulonephritis: here, differences between *HLA*-matched and mismatched donor–recipient pairs were small, whereas odds ratios for acute graft rejection in individuals possessing one *KIR2DS4* variant (full-length or defective) was much higher than in those negative for both, and simultaneous presence of both *KIR2DS4* variants even much stronger increased the probability of rejection (Nowak et al. 2012).

Conclusions

In patients, whose end-stage renal disease was caused by glomerulonephritis, *KIR2DS4* gene presence seemed to affect kidney graft rejection stronger than *HLA* incompatibility, whereas *HLA* matching exerted a major effect on graft fate in recipients with other renal diseases.

Therefore, our results suggest that typing of kidney graft recipients with end-stage renal disease caused by glomerulonephritis for *KIR2DS4* and *KIR2DS5* genes, in addition to *HLA* typing, may help to predict the outcome of renal transplantation.

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