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Distribution of KIR genes in the Croatian population

Marija Kamenaric Burek [a,*], Zorana Grubic [a], Katarina Stingl [a], Renata Zunec [a]

[a] Clinical Units for Tissue Typing, Clinical Department for Transfusion Medicine and Transplantation Biology, University Hospital Centre Zagreb, Zagreb HR-10000, Croatia

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ABSTRACT

The KIR locus with genes involved in immune processes is among the most polymorphic and structurally diverse human loci. KIR genes encode activating and inhibitory receptors that differ in specificity for HLA class I ligands and signaling potential. These receptors are expressed principally by natural killer (NK) cells and subpopulations of T cells. This study represents the first report of the distribution of KIR genes, KIR genotypes and KIR/HLA pairs in 121 unrelated healthy Croatian individuals. Twenty-three different genotypes were observed in the Croatian population and all 16 KIR genes known to date were found. The most frequent KIR genotype was the AA genotype. All individuals had at least one inhibitory KIR/HLA pair with the majority of individuals with three inhibitory KIR/HLA pairs. The most frequent KIR/HLA pair was the KIR2DL3/C1 group. Our results demonstrated the similarity of the Croatian population's KIR repertoire with other Caucasian populations reported so far.

1. Introduction

Killer cell immunoglobulin-like receptors (KIRs) are glycoproteins principally found on the surface of natural killer (NK) cells, one of the most important protagonists of the early immune response to virus infection. KIRs can also be found on some subsets of T-lymphocytes, which represent the basis of the adaptive immune response to infection [1]. The KIR gene cluster, containing polymorphic and highly homologous genes, encompasses a segment of about 150 kb within the leukocyte receptor complex (LRC) on chromosome 19 (19q13.4). The KIR gene family currently numbers 14 genes (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1, KIR3DL2, KIR3DL3, KIR3DS1) and 2 pseudogenes (KIR2DP1, KIR3DP1). The diversity of the KIR region is achieved by polymorphism at the individual KIR gene locus as well as by variable gene content. Three KIR genes, KIR2DL4, KIR3DL2 and KIR3DL3, or the so-called framework genes, are present in nearly all individuals. Among 11 additional KIR genes which have been characterized, five encode inhibitory receptors (KIR3DL1, KIR2DL1-3 and KIR2DL5) while the remaining six genes carry the code for the activating receptors (KIR2DS1-5, KIR3DS1). Finally, the KIR gene cluster contains two pseudogenes, KIR2DP1 and KIR3DP1, with KIR3DP1 also being a framework gene [2].

Interactions between KIRs and their appropriate ligands on target cells result in the production of positive or negative signals, which regulate the NK cell function. Specific HLA class I molecules are known to be the ligands for some of the KIRs. In accordance with the simple rule, HLA-C molecules with asparagine at position 80 (group C1) provide the ligand for inhibitory KIR2DL2 and KIR2DL3, whereas HLA-C molecules with lysine at position 80 (group C2) provide the ligand for KIR2DL1 and, with reduced avidity, for KIR2DS1. Nevertheless, KIR2DL2 and KIR2DL3 violated the simple rule through interactions with several C2 allotypes, notably C*05:01 and C*02:02, and two HLA-B allotypes (B*46:01 and B*73:01) that share polymorphisms with HLA-C [3]. The C1 and C2 are non-overlapping subsets of HLA-C allotypes and individuals can be either C1/C1 homozygous, C2/C2 homozygous or C1/C2 heterozygous. Subset of HLA-A and HLA-B molecules (with the exception of HLA-A25 and HLA-B13) that carry a Bw4 epitope are ligands for the KIR3DL1 receptor [4–7]. Specificity of molecules with a Bw4 epitope differs from specificity of molecules with a Bw6 epitope by the content of amino acids at positions 77–83. Amino acid at position 80 critically determines the binding to KIR3DL1. Molecules with the Bw6 epitope have asparagine (N) at this position and do not bind to KIR3DL1, while molecules with Bw4 epitope have either isoleucine (I) or threonine (T) at this position and are ligands for KIR3DL1 [7]. The KIR3DL1 allotype correlated with the non-binding phenotype is 3DL1*004 allele whose protein is not expressed on the NK cell surface [8].

Abbreviations: HLA, human leukocyte antigen; NK, natural killer cell; KIR, killer cell immunoglobulin-like receptors; PCR, polymerase chain reaction; LRC, leukocyte receptor complex; SSP, sequence-specific primer.

* Corresponding author. Address: Clinical Units for Tissue Typing, Clinical Department for Transfusion Medicine and Transplantation Biology, University Hospital Centre Zagreb, Kispaticeva 12, Zagreb HR-10000, Croatia. Fax: +385 1 23 67 337.

E-mail addresses: mburek@kbc-zagreb.hr (M.K. Burek), zgrubic@kbc-zagreb.hr (Z. Grubic), katarinastingl@gmail.com (K. Stingl), rzunec@kbc-zagreb.hr (R. Zunec).

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KIR3DL2 has HLA-A3 and HLA-A11 molecules as its ligand, but only when certain virally derived peptides are loaded. The only known ligand of KIR2DL4 is HLA-G, but the physiological relevance of this is still unclear [9]. The oldest and the most prevalent activating receptor is KIR2DS4, which can be found in two forms: "full-length" and "deleted" forms. The "full-length" 2DS4 (2DS4*001) binds two HLA-A allotypes (A*11:01, A*11:02) and six HLA-C allotypes, three carrying the C1 epitope (C*01:02, C*14:02, C*16:01) and three carrying the C2 epitope (C*02:02, C*04:01, C*05:01). The "deleted" 2DS4 (2DS4*003-009) is not functional [10]. Ligands for KIR2DL5, KIR2DS2, KIR2DS3, KIR2DS5, KIR3DS1 and KIR3DL3 have not yet been identified. In the case of KIR2DS2, it is likely that at one time it did bind to HLA class I, but was then actively selected to lose that function. KIR2DS2 has similar Ig-like domains to inhibitory KIR2DL2, but has no clearly detectable avidity for HLA-C molecules with C1 epitope [4].

Human KIR haplotypes vary in the number and type of genes present, having between seven and fifteen genes. The order of the KIR genes along the chromosome has been determined for two distinct haplotypes, termed A haplotype and B haplotype. A major difference between the two haplotypes is that the A haplotype is enriched with KIRs that bind HLA class I, whereas the B haplotype is enriched with KIRs that have either lost that function or have never had it [11].

The A haplotype is generally non-variable in its gene organization and consists of five genes coding inhibitory receptors (KIR2DL1, KIR2DL3, KIR3DL1, KIR3DL2, KIR3DL3), one gene coding an activating KIR which is always KIR2DS4, and finally, the KIR2DL4 gene. KIR2DL4 is an unusual KIR family member in terms of its signaling properties. Although it has a long cytoplasmic tail that is typical of inhibitory KIR, the engagement of this receptor results in the activation of NK cells, not for cytotoxicity, but for cytokine and chemokine secretion [9]. The B haplotypes are more variable and are characterized by the presence of more than one activating KIR gene [1,12]. All human populations have both A and B haplotypes, but their distribution varies considerably across distinct populations.

As KIRs have a profound impact on the haematopoietic stem cell transplantation outcome, placental development and the outcome of pregnancy and autoimmune diseases development, it is important to determine the distribution of KIR genes and KIR–HLA ligand interaction in a given human population. The aim of this study was to analyze the KIR gene and genotype frequencies in the Croatian population, evaluate the relationship between KIR receptors and their HLA ligands and compare the obtained data with the neighboring populations and other populations described in literature.

2. Materials and methods

The study included 121 unrelated healthy individuals representing the Croatian population. All subjects are residents of the capital city, Zagreb, but they originate from different geographic regions of Croatia. This group consisted of individuals which have already been analyzed as the control group for HLA-based studies, and thus already have defined HLA genes [13].

Genomic DNA was prepared from whole blood with the NucleoSpin®Blood commercial kit using the corresponding silica membrane columns (Macherey–Nagel, Düren, Germany).

KIR genotyping was performed using the commercially available Olerup SSP™ KIR Genotyping kit (Qiagen Vertriebs GmbH, Vienna, Austria).

Amplification products were analyzed on ethidium bromide (EtBr) stained 2% agarose gel. The amplification was checked on a UV transilluminator and photographed. The results were interpreted using a worksheet for specific amplification patterns. The

Table 1
Frequencies of killer cell immunoglobulin-like receptor (KIR) genes in the Croatian population and the comparison of the observed data with some other Caucasian and non-Caucasian populations reported to date.

	2DL1	2DL2	2DL3	2DL4	2DL5	3DL1	3DL2	3DL3	2DS1	2DS2	2DS3	2DS4	2DS5	3DS1	2DP1	3DP1
Croatia *N* = 121 (%)	95	55	95	100	49	96	100	100	32	55	34	97	21	31	96	100
Macedonia[a]	95	59	89	100	42	94	100	100	48	56	36	95	30	39	98	99
Italy[b]	95	53	88	nt	30	96	nt	nt	36	53	33	89	28	35	nt	nt
Germany[c]	93	56	87	nt	44	96	nt	nt	37	62	28	98	31	nt	nt	nt
Czech Republic[d]	95	59	86	nt	35	94	nt	nt	43	57	36	74	26	38	nt	nt
Poland[e]	93	55	91	nt	nt	93	nt	nt	44	54	28	78	30	36	nt	nt
France[f]	97	50	91	100	47	96	100	100	36	51	31	96	27	44	97	97
Greece[g]	89	50	88	100	nt	90	100	100	43	54	37	88	21	46	nt	nt
Japan[h]	99	15	100	100	48	95	99	100	45	15	17	87	32	46	100	100
Chinese Han[i]	99	17	99	100	35	94	100	100	34	17	12	94	23	33	99	100
Africa[j]	79	100	85	100	52	98	100	nt	23	45	19	97	24	13	nt	nt
Australian Aborigines[k]	73	79	67	nt	nt	55	100	nt	82	85	82	51	0	78	nt	nt
Brazil Parana[l]	97	47	89	100	52	94	100	100	40	47	26	93	34	39	96	100
Amazonian Amerindian[m]	93	65	80	100	nt	65	98	100	88	58	10	78	90	70	nt	nt
Persia[n]	94	57	85	100	60	92	100	100	42	50	31	94	41	41	94	100
North Indians[o]	88	79	65	100	79	88	100	100	54	62	43	81	47	39	nt	nt

nt = not typed.
[a] Djulejic et al. *N* = 214 (%).
[b] Bontadini et al. *N* = 217 (%).
[c] Uhrber et al. *N* = 120 (%).
[d] Pavlova et al. *N* = 125 (%).
[e] Majorczyk et al. *N* = 363 (%).
[f] Denis et al. *N* = 108.
[g] Niokou et al. *N* = 233 (%).
[h] Yawata et al. *N* = 41 (%).
[i] Jiang et al. *N* = 104.
[j] Norman et al. *N* = 62 (%).
[k] Toneva et al. *N* = 67 (%).
[l] Rudnick et al. *N* = 289 (%).
[m] Ewerton et al. *N* = 40 (%).
[n] Solgi et al. *N* = 100(%).
[o] Rajalingam et al. *N* = 72 (%).

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presence of each KIR gene was determined by the presence of the DNA band of expected size.

The observed frequencies of KIR genes and KIR/HLA pairs in individuals were determined by direct counting. The frequency of AA and Bx genotypes was deduced in the following manner: the genotype was taken as having a B haplotype if any of the KIR2DL2, KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5 or KIR3DS1 genes were present; if only variable KIR2DL1-2DL3-3DL1-2DS4 genes were present, the individual was considered as having an A haplotype.

3. Results and discussion

3.1. KIR gene frequencies

The presence of 16 KIR genes and their observed frequency in the Croatian population is shown in Table 1. The framework gene KIR2DL4, KIR3DL2, KIR3DL3 and the pseudogene KIR3DP1 were found in all subjects. Data from other analyzed populations revealed that these genes are present in all individuals, with very few exceptions; the only published exceptions were the absence of KIR2DL4 in one CEPH family member, one individual from the Bubi population on Bioko Island Equatorial Guinea, two individuals from South Asia and two family members in Northern Ireland [2,14]. Regarding the genes encoding inhibitory KIRs, the population reports worldwide state that the presence of these genes in a given population is nearly always at a frequency greater than 90%. The exceptions are KIR2DL2 and KIR2DL5 that are generally present in 30–59% of individuals, with a higher frequency found only among Australian Aborigines at 79% [15], Amazonian Amerindian at 65% [16] and North Indians at 79% [17]. In our study, the frequency of inhibitory KIR genes is around 95% for KIR2DL1, KIR2DL3 and KIR3DL1, while KIR2DL2 and KIR2DL5 are present in much smaller groups of subjects (55% and 49%, respectively). Activating KIRs show a significantly greater variation in their presence within a population. The most striking deviation of a given population concerning the activating KIR genes is the exceedingly high frequency of genes KIR2DS1, KIR2DS2, KIR2DS3 and KIR3DS1 among Australian Aborigines (>80%), while in the remaining populations these genes are present in around 35–55% of individuals. Also, the gene KIR2DS5, whose average frequency among the majority of populations is 20–30%, reaches 90% in Amazonian Amerindian, 41% in Persians [18] and 47% in North Indians [17]. The comparison of the data obtained in this study for the Croatian population, with various other populations reported to date is shown in Table 1. Not surprisingly, gene frequencies very similar to those found among Croatians were reported for neighboring or geographically close populations [19–24]. On the other hand, the biggest differences were observed comparing our results with distant populations from East Asia, Australian Aborigines and Brasil. For example, the gene KIR2DS3, with a frequency of 34% in our population, is found much less frequently in Japanese (17%), Chinese Han (12%) and Amazonian Amerindian (10%) populations [25,26]. The same observation was found for genes KIR2DL2 and KIR2DS2 which are present with a 3–5-fold higher frequency among Croatians than individuals belonging to Japanese or Chinese Han populations (55% vs. 15–17%).

The PCR-SSP method used for KIR genotyping in this study allowed us to distinguish groups of alleles at KIR2DS4 and at KIR3DL1. In this way, for KIR2DS4, our study group could be divided into four subgroups: subjects who carried only the KIR2DS4*001-002 group of alleles (13.22%), individuals positive for the KIR2DS4*003-009 group of alleles (57.02%), individuals exhibiting both groups of KIR2DS4 alleles (26.44%) and finally KIR2DS4-negative individuals (3.30%). The ratio of deleted to non-deleted versions of KIR2DS4 is approximately 2:1, similar to other Caucasoid populations. In the case of KIR3DL1 gene, we can also distinguish KIR3DL1 expressed alleles (3DL1*001-002, *005-009, *015-044, *056, *057) within 65% of the study group and KIR3DL1 non-expressed alleles (3DL1*004) among 35% of individuals. The KIR3DL1*004 allele exist at a frequency of ~20% in the Caucasian

Table 2
The distribution of KIR gene profiles observed in the Croatian population ($N = 121$).

KIR 3DL1	KIR 2DL1	KIR 2DL3	KIR 2DS4	KIR 2DL2	KIR 2DL5	KIR 3DS1	KIR 2DS1	KIR 2DS2	KIR 2DS3	KIR 2DS5	KIR 2DL4	KIR 3DL2	KIR 3DL3	KIR 2DP1	KIR 3DP1	Genotype group	Genotype ID*	Number of individuals	Frequency %
■	■	■	■								■	■	■	■	■	AA	1	40	33.05
■	■	■	■		■	■	■			■	■	■	■	■	■	AB	2	9	7.43
■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	AB	3	6	4.95
■	■	■	■	■				■			■	■	■	■	■	AB	4	16	13.22
■	■	■	■	■	■			■	■		■	■	■	■	■	AB	5	18	14.87
■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	AB	6	6	4.95
■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	AB	7	8	6.61
■	■	■	■		■	■	■		■		■	■	■	■	■	AB	8	2	1.65
■	■	■	■	■	■	■		■	■		■	■	■	■	■	AB	13	1	0.82
■	■	■	■			■					■	■	■	■	■	AB	14	1	0.82
■	■	■	■				■				■	■	■	■	■	AB	15	1	0.82
■	■	■	■		■		■			■	■	■	■	■	■	AB	35	1	0.82
■	■	■	■		■			■	■		■	■	■	■	■	AB	36	1	0.82
	■	■		■	■	■	■	■		■	■	■	■	■	■	BB	68	1	0.82
	■	■		■	■	■	■	■	■	■	■	■	■	■	■	BB	70	1	0.82
■	■		■	■	■			■	■		■	■	■	■	■	BB	71	2	1.65
■			■	■				■			■	■	■		■	BB	72	2	1.65
				■	■	■	■	■		■	■	■	■		■	BB	74	1	0.82
■			■	■	■	■	■	■		■	■	■	■		■	BB	76	1	0.82
	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	BB	87	1	0.82
	■	■		■	■	■	■	■	■		■	■	■	■	■	BB	159	1	0.82
■		■	■	■				■			■	■	■		■	BB	240	1	0.82
■		■	■	■			■	■			■	■	■	■	■	BB	245	1	0.82

*According to Middleton [27]; ■ Gene present in the haplotype; □ gene absent in the haplotype.

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Table 3
The coexistence of KIR genes and their HLA ligands in the same individual ($N = 121$).

KIR/HLA	3DL2 A*03/A*11	3DL1 expressed Bw4	2DL2 C1 + C2(C*02:02; C*05:01)	2DL3 C1 + C2(C*02:02; C*05:01)	2DL1 C2	2DS1 C2	2DS4 full length A*11; C1 (C*01,*14,*16); C2 (C*02, *04, *05)
+/−	86 (71.1%)	18 (14.8%)	5 (4.1%)	10 (8.3%)	36 (29.8%)	15 (12.4%)	19 (15.7%)
+/+	**35 (28.9%)**	**58 (47.9%)**	**62 (51.23%)**	**105 (86.7%)**	**79 (65.3%)**	**24 (19.8%)**	**29 (23.9%)**
−/−	0 (0%)	7 (5.8%)	10 (8.3%)	1 (0.8%)	4 (3.3%)	24 (19.8%)	27 (22.3%)
−/+	0 (0%)	38 (31.4%)	44 (36.4%)	5 (4.1%)	2 (1.6%)	58 (47.9%)	47 (38.8%)

population [8]. This allelic polymorphism is important for further calculations of KIR-ligand pairs.

3.2. KIR genotypes

The distribution of KIR genotypes among Croatians, including the information about KIR groups and genotype ID (according to Middleton [27]), is given in Table 2. Twenty-three different genotypes were observed in our population. The distribution of genotypes in terms of the combination of haplotypes is as follows: AA 33.05%, AB 57.02% and BB 9.91%. Following the Middleton classification, the most frequent genotype found in 40 individuals (33.05%) was KIR2DL1, 2DL3, 2DL4, 2DS4, 3DL1, 3DL2, 3DL3, 2DP1, 3DP1, which corresponds to genotype group AA1. Other frequent genotypes were AB5, found in 18 individuals (14.87%), AB4, present in 16 individuals (13.22%) and AB2, for which 9 individuals were positive (7.43%). Out of thirteen genotypes which occurred only once, 5 belong to the AB group and 8 are a part of the BB group. Six individuals possessed all 16 KIR genes known today, termed as genotype AB6.

This distribution is similar to those found in other Caucasian population studies performed in Europe. Population studies to date revealed 398 genotypes that differ in their KIR gene content, and each population carries a distinct gene content profile [27]. Individuals carrying both A and B haplotypes are more common among Caucasians and Africans, while individuals carrying homozygous A haplotypes are more frequently found among Asians (Chinese, Japanese, Koreans), where AA genotypes are present in around 60% of cases [2,25,26,28]. B haplotypes have been shown to be more prevalent in Australian Aborigines and Asian Indians, which is in correlation with the higher frequency of activating KIR genes in those populations.

3.3. KIR-ligand relation

The correlation between the presence/absence of inhibitory/activating KIR gene and their associated HLA ligands (HLA-Bw4, HLA-A3/A11, C1 and C2 group) is illustrated in Table 3. Based on the HLA-C genotyping, each individual in this study was defined as C1/C1, C1/C2 or C2/C2. The majority of individuals were C1/C2 heterozygous (52.89%) while the others presented as C1/C1 homozygous (33.05%) or C2/C2 homozygous (14.04%). The KIR gene which had its HLA ligand present in most cases was KIR2DL3 (C1, 86.7%). On the other hand, individuals positive for KIR3DL2 gene also carried its ligand, HLA-A3/A11, in only 28.9% of cases. These frequencies are the outcome of KIR and HLA genes being located on different chromosomes and their independent inheritance, as well as the disparity in the occurrence of a given KIR and HLA. For example, the frequency of KIR3DL2 in our population is 100% since this is the framework gene, but only 12.41% of Croatians carry HLA-A3 and 6.91% carry HLA-A11. Variable KIR and HLA gene families segregate independently, yielding many individuals who express KIR receptors for which they lack HLA class I ligands, and vice versa, thus creating human diversity in the number and type of KIR–HLA combinations [4].

Table 4
The number and type of inhibitory KIR receptor/HLA ligand pair combinations present in the Croatian population ($N = 121$).

Number of pairs	KIR receptor + HLA ligand	Number of individuals
1	2DL1 + C2 2DL3 + C1 2DL2/3 + C1	6 (4.95%)
2	2DL1 + C2 3DL1 + Bw4 2DL2/3 + C1 3DL1 + Bw4 2DL1 + C2 2DL2/3 + C1 2DL3 + C1 3DL1 + Bw4 2DL2/3 + C1 3DL2 + A3/11 2DL2 + C1 3DL1 + Bw4 2DL1 + C2 2DL3 + C1 2DL3 + C1 3DL2 + A3/11 2DL2 + C1 3DL2 + A3/11 2DL1 + C2 3DL2 + A3/11	49 (40.49%)
3	2DL1 + C2 2DL2/3 + C1 3DL1 + Bw4 2DL1 + C2 2DL3 + C1 3DL1 + Bw4 2DL3 + C1 3DL1 + Bw4 3DL2 + A3/11 2DL1 + C2 3DL1 + Bw4 3DL2 + A3/11 2DL1 + C2 2DL3 + C1 3DL2 + A3/11 2DL2/3 + C1 3DL1 + Bw4 3DL2 + A3/11 2DL1 + C2 2DL2 + C1 3DL2 + A3/11 2DL1 + C2 2DL3 + some C2 3DL1 + Bw4	54 (44.62%)
4	2DL1 + C2 2DL3 + C1 3DL1 + Bw4 3DL2 + A3/11 2DL1 + C2 2DL2/3 + C1 3DL1 + Bw4 3DL2 + A3/11 2DL1 + C2 2DL3 + some C2 3DL1 + Bw4 3DL2 + A3/11 2DL1 + C2 2DL2/3 + some C2 3DL1 + Bw4 3DL2 + A3/11	12 (9.91%)

The number and type of inhibitory KIR receptor/HLA ligand pair combinations found among our study subjects are presented in Table 4. All individuals had at least one inhibitory KIR/HLA pair. The largest proportion of our study group had three KIR/HLA pairs (44.62%), while only a small number of individuals carried only one or four such pairs ($N = 6$ and $N = 12$, respectively). This is in concordance with reported data about the majority of Caucasians, Hispanics and African Americans, which carry either two or three inhibitory KIR/HLA combinations [4]. Some population studies reported results that described individuals not presenting any of the inhibitory KIR/HLA pairs [24,29]. NK cells that do not interact with self-MHC class I are hypo-responsive to many stimuli and fail to reject MHC class I-deficient cells. The presence of the inhibition pair is important to avoid self-aggression, but there are also other receptors on the surface of NK cells that can be responsible for the inhibition of cellular activation [4,24].

In summary, we have demonstrated that the Croatian population possesses the general features of the KIR gene locus reported by previous studies of Caucasoid populations. By providing this data about the distribution of KIR genes, KIR genotypes and KIR/HLA combinations, the present study may serve as a reference for further investigations into the biological implications of the individual KIR repertoire variety, as well as in gaining insight into the ethnic distribution of different KIR locus profiles and their functional significance.

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