Two New Cases of KIR3DP1, KIR2DL4-Negative Genotypes, One of which is also Lacking KIR3DL2

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Abstract The killer immunoglobulin-like receptor (KIR) genes KIR2DL4, KIR3DL2, and KIR3DP1 are present in virtually all humans. KIR2DL4 encodes a receptor present on uterine and decidual natural killer (NK) cells and some peripheral blood NK cells. Its only known ligand is the human leukocyte antigen-G molecule expressed on extravillous trophoblasts, and on tissues in some diseases. KIR3DL2 binds HLA-A*03 and HLA-A*11 as well as HLA-B*27 dimers, and microbial CpG DNA. KIR3DP1 is a pseudogene. During our immunogenetic studies we found two individuals, one from Lower Silesia district in Poland, and another from Western Ukraine, who were reproducibly negative for KIR2DL4 and KIR3DP1 genes, using three different PCR systems. Both individuals displayed very similar genotypes, possessing only KIR3DL3, KIR2DL3, KIR2DP1, KIR2DS1, and probably a rare variant of KIR2DL1. The Pole had also KIR3DL2, which the Ukrainian was apparently lacking. The Lower Silesia has been populated after the Second World War by a remarkable percentage with displaced people from Western Ukraine, which might contribute to genetic similarity of the two individuals described here.

Keywords Killer cell immunoglobulin-like receptor · Framework genes · Deletion

Introduction

Killer cell immunoglobulin-like receptors (KIRs) are present on natural killer (NK) cells and some subpopulations of T lymphocytes. They have either inhibitory (long cytoplasmic tail-receptors KIR2DL and KIR3DL possessing two or three extracellular immunoglobulin-like domains, respectively) or activating (short cytoplasmic tail-receptors KIR2DS or KIR3DS) properties upon binding a ligand. Genes for majority of KIRs are not present in all individuals but only in a fraction of them (haplotypic polymorphism). However, some, so called framework genes, namely KIR3DL3, KIR3DP1, KIR2DL4, and KIR3DL2, are present in virtually all genotypes (Jiang et al. 2012). Among these, KIR2DL4 gene has special properties: it codes for structural features characteristic for both inhibitory and activating KIR; it is expressed in all NK cells whereas other KIRs are rather distributed clonally on some NK cells; and in contrast to other KIRs which function as cell surface receptors, it is localized, in resting NK cells, in endosomes where it may bind its ligand, a soluble HLA-G molecule (Rajagopalan and Long 2012), although...
this view has been recently challenged (Le Page et al. 2014). KIR2DL4 molecule appears as a cell surface receptor on uterine and decidual NK cells and some (mostly activated) peripheral blood NK cells (Goodridge et al. 2009; Hromadnikova et al. 2013; Yan et al. 2007). KIR3DL2 binds HLA-A*03 and HLA-A*11 as well as HLA-B*27 dimers, and also a microbial CpG DNA (Shaw and Kollnberger 2012; Sivori et al. 2010). KIR3DP1 is a pseudogene (Jiang et al. 2012). Here, we describe two cases of KIR2DL4-negative individuals detected in the Polish and Ukrainian populations. Both individuals present similar deletion of not only KIR2DL4 but also neighboring KIR genes, including KIR3DP1. Interestingly, the Ukrainian individual was also lacking another framework gene, KIR3DL2.

Materials and Methods

Study Subjects

The study was approved by the Local Ethical Committee at the Research and Development Centre, Regional Specialised Hospital, Wroclaw, the Bioethics Committee of the Medical University of Poznań, and the Bioethics Committee of the Danylo Halytsky Lviv National Medical University.

No. 89 a 69-year-old Polish male, 82 kg body weight, 171 cm high, body mass index (BMI) of 28.04, with arterial hypertension, morbus ischemicus cordis and aspirin idiosyncrasy but otherwise healthy and without genetic diseases in family.

No. 175 K a 67-year-old Polish male, 90 kg body weight, 177 cm high, BMI of 28.73, with total cholesterol and glucose at upper border of the norm, atherosclerosis, polycystic kidney disease, varices, nosebleed, and osteoporosis.

No. 24 a 22-year-old healthy male from western Ukraine, HIV-, HBV- and HCV-negative blood donor, recruited to a control group for our study on cryptorchidism (Kurpisz et al. 2011).

No. 58 a 19-year-old healthy male from western Ukraine, HIV-, HBV- and HCV-negative blood donor, recruited to a control group for our study on cryptorchidism (Kurpisz et al. 2011).

![Fig. 1](https://example.com) Results of KIR typing using Olerup KIR Genotyping kit™. Interpretation of all results is shown in Table 1 and described in the text. M: marker of the molecular mass; NC: negative control. Polish individual No. 89 K; Ukrainian individual No. 24; Ukrainian individual No. 58.
DNA Isolation

Genomic DNA was isolated from venous blood using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to manufacturer’s recommendations. DNA concentration was measured in NanoDrop 2000 UV–Vis Spectrophotometer (Thermo Scientific, NanoDrop Products, Wilmington, Delaware, USA).

KIR Typing

Three PCR-sequence-specific primers (SSP) systems were used

(a) Olerup SSP KIR Genotyping kit (Olerup GmbH, Vienna, Austria), lot No. 07 N including Taq polymerase. PCR-SSP procedure was performed according to manufacturer’s instruction.

(b) SSP as described by Vilches et al. (2007) that enable to amplify short DNA fragments (96–158 bp), in our modification (Niepieklo-Miniewska et al. 2013)

(c) Multiplex PCR-SSP (four PCR reactions containing primers for 3–4 KIR genes each) as published earlier (Nowak et al. 2011; Sun et al. 2004).

Table 1 Summary of the results of testing individuals No. 89 K, No. 24 and No. 58 with Olerup PCR-SSP kit shown in Fig. 1 and second testing with identical results

| Primer mix | Size of specific PCR product (bp) | Size of control band (bp) | KIR gene | No. 89 K | No. 24 | No. 58 |
|------------|----------------------------------|--------------------------|----------|----------|--------|--------|
| 1          | 145                              | 800                      | 2DL1     | –        | –      | +      |
| 2          | 145                              | 1070                     | 2DL2     | –        | –      | +      |
| 3          | 520                              | 1070                     | 2DL3     | +        | +      | +      |
| 4          | 200                              | 1070                     | 2DL4     | –        | –      | +      |
| 5          | 155                              | 1070                     | 2DL5A, 2DL5B | –    | –      | +      |
| 6          | 1650                             | 430                      | 2DL5A    | –        | –      | –      |
| 7          | 1650                             | 515                      | 2DL5B    | –        | –      | +      |
| 8          | 100                              | 1070                     | 2DS1     | +        | +      | –      |
| 9          | 205                              | 1070                     | 2DS2     | –        | –      | +      |
| 10         | 160                              | 1070                     | 2DS3     | –        | –      | +      |
| 11         | 215                              | 1070                     | 2DS4     | –        | –      | –      |
| 12         | 200                              | 1070                     | 2DS4     | –        | –      | +      |
| 13         | 110                              | 1070                     | 2DS5     | –        | –      | –      |
| 14         | 130                              | 1070                     | 3DL1     | –        | –      | +      |
| 15         | 95                               | 1070                     | 3DL2     | +        | –      | +      |
| 16         | 115                              | 1070                     | 3DL3     | +        | +      | +      |
| 17         | 130                              | 1070                     | 3DS1     | –        | –      | –      |
| 18         | 165                              | 1070                     | 2DP1     | +        | +      | –      |
| 19         | 125                              | 1070                     | 3DP1     | –        | –      | +      |
| 20         | 235                              | 1070                     | 3DP1     | –        | –      | –      |
| 21         | 145                              | 1070                     | 2DS1*001 | –      | –      | –      |
| 22         | 95                               | 1070                     | 2DS1*002-008 | +    | +      | –      |
| 23         | 80                               | 1070                     | 3DL1*00401-00403, 019, 021, 036, 037, 039, 056, 072 | – | – | + |
| 24         | –                                | –                        | Negative control |

Framework genes not detected in described individuals are show in bold.

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.

Results

Upon genomic KIR typing using commercial Olerup kit (see Materials and Methods) in disease association studies on Polish and Ukrainian patients and respective control groups, we noticed a lack of KIR2DL4 gene in one Polish (No. 89 K) and one Ukrainian (No. 24) control individual (Fig. 1). All other samples from both control and patient groups were positive (Fig. 1). The individual No. 24 apparently was lacking another framework gene, KIR3DL2, whereas another Ukrainian control individual, No. 58, possessed both genes (Fig. 1). To confirm this result, we repeated KIR typing using Olerup kit once more, with the same result (data not shown, and see Table 1). Furthermore, we checked the presence of KIR2DL4 and KIR3DL2 genes in these samples using primers published by Vilches et al. (2007) (Fig. 2). Finally, we typed these samples again...
with the use of multiplex PCR-SSP described previously (Sun et al. 2004) (Fig. 3). Our individuals No. 89 K and No. 24 were reproducibly KIR2DL4-negative in all these tests, in contrast to other individuals who were all positive (Fig. 1, 2, 3, and results not shown). They also lacked KIR3DP1 which is present whenever KIR2DL4 is present, with extremely rare exceptions (Jiang et al. 2012). They possessed KIR3DL3, 2DL3, 2DP1, and 2DS1, and No. 89 K had also KIR3DL2, absent from No. 24 genotype. Their genotypes contained KIR2DL3 gene, characteristic for the centromeric segment of the KIR region, but another centromeric gene, KIR2DL1, gave inconsistent results: it was detected by multiplex test (Fig. 3; Table 2) but neither by commercial test (Fig. 1; Table 1) nor by PCR-SSP using primers described by Vilches et al. (2007) (Fig. 2).

**Discussion**

We have detected two KIR3DP1, KIR2DL4-negative individuals, one of which was also negative for the third framework gene, KIR3DL2. Such genotypes are extremely rare in world populations (Djulejic et al. 2010; Ewerton et al. 2007; Gomez-Lozano et al. 2003; Gonzalez-Galarza et al. 2011; Karlsen et al. 2007; Norman et al. 2002; Nowak et al. 2011; Ozturk et al. 2012; Taniguchi and Kawabata 2009; Traherne et al. 2010; Velickovic et al. 2006), and even haplotypes contributing to them, most frequently in heterozygotic configuration, are very rare (Gonzalez-Galarza et al. 2011; Jiang et al. 2012; Pyo et al. 2010; 2013; Traherne et al. 2010). We found in the www. allelefrequencies.net database (Gonzalez-Galarza et al. 2011) only ten KIR2DL4-negative genotypes in addition to two new ones described here (Fig. 4a), three KIR3DL2-negative genotypes plus our Ukrainian (Fig. 4b), and six KIR3DP1-negative genotypes plus two described here (Fig. 4c) out of nearly 13,000 individuals tested worldwide (Gonzalez-Galarza et al. 2011). This speaks in favor of a strong positive selection for all three genes, including KIR3DP1. This pseudogene might be suspected to be preserved simply due to its close proximity to KIR2DL4 which is likely strongly selected for. However, KIR3DP1 is located on centromeric segment of KIR locus, whereas KIR2DL4 is on telomeric segment (Jiang et al. 2012; Pyo et al. 2013) which seems to exclude the close localization as a reason for preservation of KIR3DP1 together with KIR2DL4. Nevertheless, genotypes containing KIR2DL4, but not KIR3DP1, are extremely rare, as only few such genotypes from Cook, Samoa, Tokelau, and Tonga Islands as well as from Turkey have been described so far (Velickovic et al. 2006) (Fig. 4c), and opposite configuration, KIR3DP1+, KIR2DL4+, was found in only single individuals from South Turkey, Brazil, and Bulgaria (Fig. 4a). Homozygous KIR3DL2 deletion is also very infrequent, as only three such cases exist in the database (Gonzalez-Galarza et al. 2011) and our Ukrainian No. 24 is the fourth one (Fig. 4b). Haplotypes with this deletion, but not in homozygous configuration, have been identified in some individuals by gene copy number detection (Pyo et al. 2013). The KIR3DL2 molecule plays an important role in both innate and adaptive immunity. It is expressed on NK cells, as well as on subpopulation(s) of T lymphocytes (Bowness et al. 2011; Wong-Baeza et al. 2013). It binds not only HLA-A*03 and HLA-A*11 heterotrimers as well as HLA-B*27 homodimers, but also microbial CpG DNA (Shaw and Kollnberger 2012; Sivori et al. 2010). It has the highest number of alleles, except for KIR3DL1/S1. This is suggestive of a strong selection for polymorphism (Shaw and Kollnberger 2012).
In some very rare American haplotypes, most of the KIR3DL2 gene (intron 3 to beyond exon 9) was deleted, giving a typing pattern identical to our No. 24. Such a haplotype has also been detected in an American of Ukrainian descent, in addition in homozygous configuration (Traherne et al. 2010).

Interestingly, Lower Silesia was populated in remarkable fraction, after the Second World War, by people resettled from Western Ukraine. Therefore, not only No. 24 but also No. 89 K genotype may be derived from there, whence similarity between these genotypes and with haplotypes described in the USA and mentioned above. It has been established by sequencing that all American KIR3DL2 deletion haplotypes contained exons 1–3 from KIR3DL2*007 allele, suggesting a recent common origin. It would be desirable then to check whether our genotypes No. 89 K and No. 24 bear the same allele that may be derived from the same recombination event.

We had a problem with establishing whether KIR2DL1 gene is present in our genotypes No. 89 K and No. 24. The reagents from both Olerup and Vilches et al. (2007) PCR-SSP gave reproducibly negative results, whereas in the multiplex assay both individuals were clearly positive (Figs. 1, 2, 3; Tables 1, 2). It is likely that both our individuals possess genotypes similar to the haplotypes described earlier in Northern American Caucasian population (Pyo et al. 2013; Traherne et al. 2010) which contained a KIR2DL1/KIR2DS1 hybrid resulting from recombination event which deleted all KIR genes located between these two genes. This may explain why some PCRs detected KIR2DL1 in our samples, but other did not.

In conclusion, we found in our Slavic populations (Polish and Ukrainian), two individuals lacking several framework KIR genes, KIR3DP1, and KIR2DL4, and apparently also KIR2DL1. One of them was also negative for KIR3DL2. Both these individuals were relatively healthy in spite of very limited KIR gene repertoire. However, such genotypes are extremely rare in world populations. Therefore, studies on much larger populations

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**Fig. 3** Results of KIR typing using multiplex PCR-SSP kit (Sun et al. 2004). Samples No. 89 K (lanes 2, 5, 8 and 11), No. 24 (lanes 3, 6, 9 and 12) and framework gene-positive control sample No. 175 K (lanes 4, 7, 10 and 13) were tested for the presence of particular KIR gene groups as shown, together with results, in Table 2. Please note the absence of KIR2DL4 (G1) in samples No. 89 K and 24, and absence of KIR3DL2 (G3) in sample No. 24. KIR2DL1 (G1) gave clearly positive band in samples No. 89 K and 24 in this test, in contrast to negative results of these samples with both Olerup (Fig. 1) and Vilches et al. (2007) (Fig. 2) primers. Summary of these results is given in Table 2.

**Table 2** Interpretation of results of multiplex PCR-SSP KIR typing shown in Fig. 3

| Multiplex group | KIR gene | Amplicon (bp) | 89 K | 24 | 175 K |
|-----------------|----------|--------------|------|----|------|
| 1               | 2DL1     | 437          | +    | +  | +    |
|                 | 2DS3     | 279          | –    | –  | +    |
|                 | **2DL4** | **232**      | –    | –  | +    |
|                 | 2DL2     | 164          | –    | –  | +    |
|                 | 3DL1     | 565          | –    | –  | +    |
|                 | 2DL3     | 334          | +    | +  | –    |
|                 | 2DS2     | 204          | –    | –  | +    |
|                 | 3DS1     | 171          | –    | –  | –    |
| 2               | 2DS1     | 231          | +    | +  | –    |
|                 | **3DL2** | **142**      | +    | –  | +    |
|                 | 2DL5     | 113          | –    | –  | +    |
| 3               | 2DS5     | 194          | –    | –  | –    |
|                 | 3DL3     | 155          | +    | +  | –    |
|                 | 2DS4n    | 130          | –    | –  | –    |
|                 | 2DS4d    | 108          | –    | –  | –    |

Framework genes not detected in described individuals are show in bold.
would be required to determine the effect(s) of these deletions on the health of such individuals.

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