KIR AND HLA HAPLOTYPE ANALYSIS IN A FAMILY LACKING THE KIR 2DL1-2DP1 GENES

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ABSTRACT

The killer cell immunoglobulin-like receptor (KIR) gene cluster exhibits extensive allelic and haplotypic diversity that is observed as presence/absence of genes, resulting in expansion and contraction of KIR haplotypes and by allelic variation of individual KIR genes. We report a case of KIR pseudogene 2DP1 and 2DL1 gene absence in members of one family with the children suffering from acute myelogenous leukemia (AML). Killer cell immunoglobulin-like receptor low resolution genotyping was performed by the polymerase chain reaction (PCR)-sequence-specific primers (SSP)/sequence-specific oligonucleotide (SSO) method and haplotype assignment was done by gene content analysis. Both parents and the maternal grandfather, shared the same Cen-B2 KIR haplotype, containing KIR 3DL3, -2DS2, -2DL2 and -3DP1 genes. The second haplotype in the KIR genotype of the mother and grandfather was Tel-A1 with KIR 2DL4 (normal and deleted variant), -3DL1, -22 bp deletion variant of the 2DS4 gene and -3DL2, while the second haplotype in the KIR genotype of the father was Tel-B1 with 2DL4 (normal variant), -3DS1, -2DL5, -2DS5, -2DS1 and 3DL2 genes. Haplotype analysis in all three offsprings revealed that the children inherited the Cen-B2 haplotype with the same gene content but two of the children inherited a deleted variant of the 2DL4 gene, while the third child inherited a normal one. The second haplotype of all three offspring contained KIR 2DL4, -2DL5, -2DS1, -2DS4 (del 22bp variant), -2DS5, -3DL1 and -3DL2 genes, which was the basis of the assumption that there is a hybrid haplotype and that the present 3DL1 gene is a variant of the 3DS1 gene. Due to consanguinity among the ancestors, the results of KIR segregation analysis showed the existence of a very rare KIR genotype in the offspring. The family who is the subject of this case is even more interesting because the father was 10/10 human leukocyte antigen (HLA)-matched to his daughter, all members of the family have the “best” donor KIR-B content and the presence of a rare KIR genotype with KIR 2DP1-2DL1 genes absence.

Keywords: Human leukocyte antigen (HLA) and killer cell immunoglobin-like receptor (KIR) polymorphism; natural killer (NK) cells

INTRODUCTION

Natural killer (NK) cells of innate immunity and CD8+ cytotoxic T cells (CTL) of adaptive immunity are professional killer cells that are necessary for defense against virus-infected or tumor-transformed cells. Natural killer cells recognize and kill target cells with absent or altered expression of human leukocyte antigen (HLA) class I molecules. This missing self-recognition makes NK cells able to eliminate tumoral and virus-infected cells that lack self HLA class I molecules. Furthermore, in the allogeneic context of hematopoietic stem cell transplantation,
NK cells from the donor can be alloreactive against recipient cells. This NK cell alloreactivity is mediated by a balance between activating and inhibitory signals and leads to cytotoxicity and production of proinflammatory cytokines, such as IFN-$\gamma$. Receptors of HLA class I molecules involved in recognition of target cells partly belong to the killer cell Ig-like receptor (KIR) family, encoded by 14 genes and two pseudogenes located on chromosome 19q13.4, within a 100 to 200 kb region of the leukocyte receptor complex (LRC) [1-5]. The number of genes present in KIR haplotypes is variable, however four genes ($KIR\ 3DL3$, $KIR3DP1$, $KIR2DL4$, $KIR3DL2$) are present on virtually all haplotypes, and are thus considered as framework genes. Two major classes of KIR haplotypes (A and B) have been identified, which is unique to the human species and that were shown to have differential associations with disease and reproductive success. The A haplotype contains four genes in addition to the framework genes ($KIR2DL1$, $KIR2DL3$, $KIR3DL1$, $KIR2DS4$), representing a predominantly inhibitory haplotype. There are many B haplotypes, containing various combinations of the activating KIR genes [1-3,6-9]. In addition to the substantial variation in gene content across haplotypes, each KIR gene itself exhibits considerable nucleotide sequence polymorphism explaining why the genomic diversity of the human KIR gene family is its important characteristic attributed to the variability in both gene numbers and allelic polymorphism [7,9-11]. Every KIR haplotype is a combination of a centromeric and a telomeric KIR gene motif. DNA sequencing of single copies of chromosome 19 [8,12] and family segregation analysis [13,14] have defined three common centromeric (Cen-A1, Cen-B1, Cen-B2) and two common telomeric (Tel-A1 and Tel-B1) haplotype structures based on the presence and absence of specific KIR genes. As examples, Cen-A1 is comprised of $KIR3DL3\sim KIR2DL3\sim KIR2DP1\sim KIR2DL1\sim KIR3DP1$ in this gene order, whereas Tel-A1 includes $KIR2DL4\sim KIR3DL1\sim KIR2DS4\sim KIR3DL2$. In this report, we present a detailed HLA and KIR haplotype analysis of a family having rare KIR genotype. All members of the family described here displayed a greater degree of diversity of KIR genetic profiles, that indicates further complexity of underlying haplotypes; in this respect, we described six individuals (grandfather, mother, father and three offspring), who possess an incomplete KIR haplotype with the 3DP1 variant allele (due to lack of 2DP1-2DL1 alleles). The aim of the presented case study was to determine possible explanation of rare KIR genotype present in patient, siblings and parents of the patient, through the segregation KIR analysis in patient’s grandfather and grandmothers.

MATERIAL AND METHODS

Patient History. An 8-year old female patient (M.M.), suffering from acute myelogenous leukemia (AML), her parents [father (M.A.) 37 years old, mother (M.Z.) 32 years old], two siblings (M.M. and M.Z.), aged 14 and 10, maternal grandparents: grandfather (M.M., 67 years old), grandmother (M.M., 56 years old), and paternal grandmother: (M.T., 63 years old), were reported. The paternal grandfather (M.F.), who passed away 2 years ago, was not included in the case study, therefore, we were unable to show both his KIR and HLA genotypes, but only the one inherited by his son, the patient’s father (M.A.).

Family History. There were no known disorders from which direct blood relatives of the patient suffered, with the exception of hypertension, which both of the patient’s grandmothers suffer. There are the data on consanguinity among family members: great-grandmother of the patient’s father (M.A.) and grandfather of the patient’s mother (M.Z.), were siblings. Therefore, the mother and the father of the patient are third-degree cousins.

DNA Extraction and Human Leukocyte Antigen Genotyping. DNA extraction was performed by a silica-based extraction method, using the QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) or the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific Inc., Vilnius, Lithuania) from 200 µL of buffy-coat obtained from 3 mL of blood collected in EDTA-containing vacutainers. The DNA was rehydrated with pure water and the DNA concentration and purity was determined by Eppendorf BioPhotometer (Eppendorf AG, Hamburg, Germany). The HLA and KIR genotyping was performed by a reverse sequence-specific oligonucleotide (SSO) probe method coupled with the multiplexed microsphere-based suspension array platform xMAP technology designed for use with the Luminex® system to identify HLA and KIR alleles encoded by sample
DNA using Lifecodes KIR/HLA SSO Typing kits, Gen-Probe Transplant Diagnostics, Inc. (Stamford, CT, USA). The target DNA was first amplified by polymerase chain reaction (PCR) with biotinylated primers specifically designed for each HLA and KIR locus. The PCR product was denatured and hybridized to complementary oligonucleotide probes immobilized on fluorescently coded microsphere beads. At the same time, the biotinylated PCR product was labeled with phycoerythrin-conjugated streptavidin to allow it to be detected by the Luminex® system. The HLA and KIR alleles were assigned by analysis of the reaction (hybridization) pattern of the target sample using the Quicktype for Lifecodes software. Verification of HLA and KIR genotyping was based on a PCR-SSP (sequence-specific primers) technique using HLA-Ready Gene and KIR-Ready Gene kits (Inno-Train Diagnostik GmbH, Kronberg, Germany). The cycling program involved the initial denaturation at 96 °C for 2 min. followed by 10 cycles of denaturation at 96 °C for 15 seconds, annealing at 65 °C for 60 seconds, and followed by 20 cycles of denaturation at 96 °C for 15 seconds, annealing at 61 °C for 50 seconds, and elongation at 72 °C for 30 seconds. Electrophoresis using 2.0% agarose gel was done. After PCR, created bands in each well were interpreted based on standard protocol available in the kit, using Helmberg SCORE™ software. The genotype of each individual was typed specifically.

RESULTS

The distribution of KIR and HLA genes of the patient’s grandparents (two grandmothers and one available grandfather), is presented in Table 1. The KIR segregation analysis showed that grandfather of the patient (M.M.), appears to be homozygous for the Cen-B2/Tel-A1 KIR haplotype, and that he shares the same KIR haplotype with his wife [grandmother of the patient (M.M.)] with only difference in KIR 2DL4 gene [normal KIR 2DL4 gene variant present in grandfather and deleted KIR 2DL4 gene variant present in grandmother (M.M.)]. The HLA segregation analyses point out that the maternal grandfather (M.M.) and paternal grandmother (M.T.) share the same HLA haplotype: A*32–B*27–C*02–DRB1*16–DQB1*05 (Table 1).

The distribution of KIR and HLA genes of the parents in the family that is under study is given in Table 2. The HLA and KIR haplotype analysis revealed that both parents were lacking the KIR2DP1-2DL1 genes and that they were mutually compatible in eight out of 10 HLA alleles.

Table 3 shows the distribution of KIR and HLA alleles in the offspring, where it is noticeable that all three offspring also lacked the 2DP1-2DL1 genes and that the brothers were mutually 10/10 HLA-matched and both were HLA haploidentical to the patient. Additionally, the patient carries the C1/C2 HLA genotype, as she possess C*07 belonging to

| Table 1. Distribution of KIR and HLA alleles in the paternal and maternal grandparents. |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| **Paternal Grandparents** | **Maternal Grandparents** |
| KIR                  | HLA                  | KIR                  | HLA                  | KIR                  | HLA                  | KIR                  | HLA                  |
| Cen-B2/Tel-B1 & Cen-A1/Tel-A1 | Cen-B2/Tel-B1 (second unknown) | A*11, *32 | 3DL3 | A*02 | 3DL3 | A*02, *24 | 3DL3 |
| 3DS2                | 3SL2                | 2DS2                | B*27, *58 | 2SL2                | 2DS2                | B*08, *41 | 2DS2 |
| 3DLP1               | 3DLP1               | 3DLP1               | C*02, *07 | 3DLP1               | 3DLP1               | C*07, *07 | 2SL2 |
| 2DL4 (normal and deleted) | 2DL4 (normal) | 2DS1               | DRB1*07, *16 | 2DS1               | DRB1*03, *05 | DRB1*03, *03 | 2DL2 |
| 3DS1                | 3DL2                | 3DS1               | DQB1*03, *05 | 3DS1               | DQB1*02 | DQB1*02, *02 | 3DS1 |
| 2DL5                | 2DLP1               | 2DL5                | Xa,Xb  | 3DLP1               | 2DLP1               | 3DLP1 |
| 2DS3                | 2DL4 (normal and deleted) | 2DS3             | 3DLP1 |
| 2DS4 (del 22 bp)    | 3DL2                | 2DS4 (del 22 bp)   | 3DL2 |
| 3DL2                | C*07                | 3DL2 |

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Table 2. Distribution of KIR and HLA alleles in the parents.

| KIR | HLA | KIR | HLA |
|-----|-----|-----|-----|
| Cen-B2/Tel-A1 & Cen-B2/Tel-A1 | A*24, *32 | Cen-B2/Tel-B1 & Cen-B2/Tel-B1 | A*24, *32 |
| 3DL3 | B*08, *27 | 2DL2 | B*08, *07 |
| 2DS2 | C*02, *07 | 3DP1 | DRB1*03, *16 |
| 2DL2 | DQB1*02, *05 | 2DL4 (normal) | 3DL1 |
| 3DL1 | | 2DL4 (normal) | |
| 2DS4 (del 22 bp) | | 2DL5 (null B*004) | |
| 3DL2 | | 2DS5 | |
| | | 2DS1 | |
| | | 2DS4 (del 22 bp) | |
| | | 3DL2 | |

Table 3. Distribution of KIR and HLA alleles in the offspring.

| KIR | HLA | KIR | HLA | KIR | HLA |
|-----|-----|-----|-----|-----|-----|
| Cen-B2/Tel-A1 & Cen-B2/Tel-B1 | A*02, *32 | Cen-B2/Tel-A1 & Cen-B2/Tel-B1 | A*02, *24 | |
| 3DL3 | B*27, *41 | 2DL2 | B*08, *41 | 3DL3 | B*08, *41 |
| 2DS2 | C*02, *07 | 3DP1 | C*07, *07 | 2DL2 | C*07, *07 |
| 2DL2 | DRB1*03, *16 | 2DL4 (normal) | DRB1*03, *03 | 3DL1 | DRB1*03, *03 |
| 3DL1 | DQB1*02, *05 | 2DL5 (null B*004) | DQB1*02, *02 | 2DL4 (deleted) | DQB1*02, *02 |
| 2DS4 (del 22bp) | 3DL2 | 2DS5 | 3DL1 | |
| 2DL5 (null B*004) | | 2DS1 | |
| 2DS5 | | 2DS4 (del 22 bp) | |
| 2DS1 | | 3DL2 | |
| 3DL2 | | | |

The C1 group and C*02, belonging to the C2 group. Also there is one KIR/HLA mismatch between the siblings and both the parents toward the patient for KIR 3DL2 gene, since patient lacks the HLA A*03 and A*11 ligands. Father is 10/10 HLA-matched to the daughter (at a low resolution typing level), while her mother is 8/10 HLA-matched donor.

The analysis of familial HLA haplotypes segregation presented in Figure 1 revealed that all three children inherited the same haplotype from the father (A*02−B*41−C*07−DRB1*03−DQB1*02), two brothers inherited the same HLA haplotype from the mother (A*24−B*08−C*07−DRB1*03−DQB1*02) and the patient inherited the mother’s second haplotype (A*32−B*27−C*02−DRB1*16−DQB1*05), which explains why the brothers were mutually 10/10 HLA-matched and haploidentical compared to the patient.

Figure 2 provides the KIR haplotype analysis showing that all members of the family predominantly possess Cen-B2 motifs in combination with Tel-A1 and Tel-B1, respectively, with the exception of the Cen-A1 motif, present in the paternal grandmother (M.T.). The maternal grandfather (M.M.), parents and offspring in the family carry incomplete KIR haplotypes with the 3DP1 variant allele (due to lack of 2DP1−2DL1 alleles) and that the maternal grandfather, mother and the father are homozygous for the Cen-B2 haplo-
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Also all three offspring inherited the Cen-B2 haplotype with the same gene content but two of the children inherited a deleted variant of the 2DL4 gene, while the third child inherited the normal one. All three offspring inherited the deleted variant of the 2DS4 gene from their mother and they are homozygous for the Cen-B2 haplotype and heterozygous for the Tel-A1/Tel-B1 haplotypes. At the basis of the KIR gene content present in the offspring, we assumed that there is a diversive/hybrid haplotype compared to the father’s KIR haplotype (Tel-B1 containing KIR 3DP1~2DL4~3DS1~2DL5~2DS1~3DL2), since the 3DS1 gene present in the offsprings’ KIR haplotype (KIR 3DP1~2DL4~3DS1~2DL5~2DS1~3DL2), segregated as a 3DL1 gene.

DISCUSSION

The KIR gene content and alleles vary across individual genomes and populations. The KIR genes are variable in gene content and type, which results in different KIR haplotypes, therefore, they can be used to discriminate individuals and populations from different regions or ethnic groups [8,10,13]. Several studies have verified the KIR diversity in different populations [15-24] and analyzed the genetic relationships among populations from different geographical areas and answered questions regarding ethnic origins [25-27]. The KIR diversity is observed as presence/absence of genes, resulting in expansion and contraction of KIR haplotypes [21,28,29], and further diversity is provided by allelic variation of individual KIR genes.

Our results indicate that KIR genotype frequencies described in the parents and offspring of the family have a specific structure: father (M.A.) has Cen-B2/Cen-B2 and Tel-B1/Tel-B1 haplotypes. At the basis of the KIR gene content present in the offspring, we assumed that there is a diversive/hybrid haplotype compared to the father’s KIR haplotype (Tel-B1 containing KIR 3DP1~2DL4~3DS1~2DL5~2DS1~3DL2), since the 3DS1 gene present in the offsprings’ KIR haplotype (KIR 3DP1~2DL4~3DS1~2DL5~2DS1~3DL2), segregated as a 3DL1 gene.

Figure 1. Analysis of the HLA gene segregation revealed that the maternal grandfather (M.M.) and paternal grandmother (M.T.) shared the same HLA haplotype: A*32~B*27~C*02~DRB1*16~DQB1*05, resulting in their transfer to the mother, father and patient as well as to her siblings.
genotypes in several Caucasoid populations. Among their KIR genotypes, the grandfather’s and mother’s genotype (Cen-B2/Tel-A1) is the most frequent (around 6.0% Caucasoids possess it), father’s genotype (Cen-B2/Tel-B1) is present in 2.9% Caucasoids, while the genotypes present in the offspring (Cen-B2/Tel-B1 and Cen-B2/Tel-A1) is very rare, up to 1.0% of Caucasoids possess these rare genotypes. Similar to the previous observation, absence of the 2DP1 and 2DL1 KIR genes is very rare, as the frequencies of these genes range from 87.0% in Belgium to 100.0% in Finland for 2DL1 and from 89.0% in Portugal to 99.0% in Belgium for 2DP1 [31]. Additionally, the KIR gene segregation analysis is interesting in the studied family as all three children should have the 3DS1 gene from the father’s haplotype (Tel-B1 part), but due to occurrence of some of the possible mechanisms: gene duplication, hybridization by asymmetrical recombination or intergenic/lineage recombination during meiosis, we assumed that the present 3DL1 gene is a variant of the 3DS1 gene and 3DS1 allele segregated as 3DL1. As Norman et al.
[32,33] described, the explanation for the existence of 3DL1*009, present in the offspring (according to the results of verification PCR-SSP genotyping), is that this allele is formed by conversion between 3DL1*001 and 3DS1*01301, resulting in a new haplotype with the 3DL1 gene instead of 3DS1. This is the proof that these genes behave as two alleles of the same gene. The possible explanation of the existence of such rare KIR genotypes in grandfather, parents and offspring in the family is consanguinity among their ancestors, which resulted in predominance of the Cen-B2 KIR haplotype in the family, homozygosity of Cen-B2/Tel-A1, present in grandfather (M.M.) and mother (M.Z.), homozygosity of Cen-B2/Tel-B1 present in father (M.A.), presence of the same HLA haplotype, A*32-B*27-C*02-DRB1*16-DQB1*05, in the maternal grandfather (M.M.), paternal grandmother (M.T.), mother (M.Z.), father (M.A.) and the patient (M.M.).

The KIR and HLA segregation analysis of the studied family members was interesting for a several reasons: 1) presence of rare a KIR genotype with KIR 2DP1-2DL1 absence in grandfather and both parents and one Cen-B2 mutually shared haplotype; 2) presence of one diverse KIR haplotype in the offspring and also rare KIR genotype with absence of KIR 2DP1-2DL1 genes; 3) there is the existence of one KIR/HLA missing ligand mismatch for the KIR 3DL2 gene; 4) among all available related donors who all have the “best” B KIR gene content, the father is the most suitable donor due to the 10/10 HLA-match between him and his daughter, which is certainly less frequent than the probability of a match between siblings.

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