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Killer Cell Immunoglobulin-Like Receptors in SFS – Marrow Donor Registry (MK-SFSMDR): Feasibility in Identifying Better Donors

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Abstract

BACKGROUND: Different Killer Immunoglobulin-Like Receptor (KIR) genes are relevant for each donor-recipient pair based on the degree of human leukocyte antigen matching and the specific leukemia subtype. The inhibitory or non-inhibitory effects of haplotype B among unrelated or sibling transplants are a perfect example for inconsistent results. Donors possessing KIR B haplotype show a correlation with decreased relapse risks and enhanced disease-free survival following unrelated donor transplants for acute myeloid leukemia.

AIM: The aim of this study was to investigate the KIR gene polymorphism in Scientific Foundation SPIROSKI-Marrow Donor Registry (MK-SFSMDR).

MATERIALS AND METHODS: The study sample consisted of 516 healthy unrelated individuals, aged 18–55 years. All individuals were registered donors of the MK-SFSMDR, having signed a Marrow Donor Registration form and provided buccal swabs for analysis. KIR gene content typing was performed by HistoGenetics Lab (Ossining, NY 10562, USA) on Illumina platform. Data analysis was performed using the population genetics analysis package Arlequin.

RESULTS: We found that all 16 KIR genes were observed in the MK-SFSMDR donors and framework genes *KIR3DP1, KIR2DL4, KIR3DL2, and KIR3DL3* were present in all individuals. The observed frequencies of other KIR genes were as follows: *KIR2DP1* (0.971), *KIR2DL1* (0.971), *KIR2DL2* (0.562), *KIR2DL3* (0.882), *KIR2DL5* (0.523), *KIR3DL1* (0.942), *KIR2DS1* (0.0401), *KIR2DS2* (0.564), *KIR2DS3* (0.312), *KIR2DS4* (0.942), *KIR2DS5* (0.308), and *KIR3DS1* (0.374). We defined 35 genotypes from which one was AA group (genotype ID 1 = 27.13%) and the rest were Bx groups (34 genotypes = 72.87%).

CONCLUSIONS: We found that all 16 KIR genes were observed in the MK-SFSMDR donors (n = 516) and framework genes *KIR3DP1, KIR2DL4, KIR3DL2, and KIR3DL3* were present in all individuals. The observed and estimated frequencies of other KIR genes were presented. We defined 35 genotypes from which one was AA group (genotype ID 1 = 27.13%) and the rest were Bx groups (34 genotypes = 72.87%). Inclusion of KIR genotypes in molecular screening of bone marrow donors may increase efficacy for stem cell transplantation.

Introduction

In the Republic of North Macedonia, in addition to the government-funded registry, an additional non-governmental registry was established, Scientific Foundation SPIROSKI-Marrow Donor Registry (MK-SFSMDR) [1]. This registry will contribute to the increasing of the number of newly registered donors in the Republic of North Macedonia and will be a great benefit for the patients in need of a bone marrow transplantation.

Recruitment of marrow donors started on January 15, 2022, and through a collection of buccal swabs and written consents, the realization of the Marrow Donors Recruitment Program in the non-governmental sector of our country has begun. Two main routes were defined: (1) online submissions through the newly created website (https://www.sfsmdr.mk/user-registration/) [2] for the Marrow Donor Registry and (2) Marrow Donor Recruitment Actions conducted in the field. All online submissions, buccal swab sets, and written consents were sent by post. Marrow donors were instructed to use four sticks of buccal swabs, fulfill and sign the Marrow Donor Consent form, as well as the written request for molecular testing outside the country and return back the materials by post [1].

Samples from the buccal swabs obtained through both online submissions and Marrow Donor Recruitment Actions were anonymized using a GRID number, labeled with barcodes, and sent for molecular analysis at HistoGenetics, USA (cytomegalovirus, ABO, rhesus, human leukocyte antigen [HLA], and killer immunoglobulin-like receptor [KIR]). The obtained molecular analysis results and the marrow donor's personal data were stored in the Prometheus Light software for Marrow Donor Registries (Steiner, s.r.o) [1].

Different KIR genes are valid for each donor-recipient pair based on the degree of HLA matching and the type of leukemia. The inhibitory or non-inhibitory effects of haplotype B among unrelated or sibling transplants are a perfect example for inconsistent results. KIR B haplotype donors are associated with reduced relapse risks and improved disease-free survival after unrelated donor transplants for acute myeloid leukemia (AML).

Previously, we investigated 63 patients diagnosed with various hematological malignancies. Among them, acute myeloblastic leukemia was predominant, accounting for 40 cases (63.5%), followed by chronic myeloid leukemia with 11 cases (17.5%), acute lymphoblastic leukemia with 8 cases (12.7%), non-Hodgkin lymphoma with 2 cases (3.17%), aplastic anemia with 1 case (1.59%), and chronic lymphocytic leukemia with 1 case (1.59%). Our findings revealed a statistically significant difference for KIR3DL2 (F = 1 in the control group, and 0.95 in the patient's group, p = 0.001). In addition, we noted another statistically significant difference for the frequency of Bx3 and Bx439 genotypes both found more often in patients' group (p = 0.017 and p = 0.009, respectively) [3].

Furthermore, in our study performed on 24 patients and their HLA-matched sibling donors, we found that comparison of KIR gene frequencies between the total 24 donors and healthy Macedonians reveals statistically significant difference for KIR2DS1 (F = 0.481 in the controls group, and 0.76 in the patients group, p = 0.004). This significance is even higher when the frequency of KIR2DS1 in controls is compared with the frequency in donors from pairs with graft-versus-host disease (F = 0.923, p = 0.002) [4].

It appears that different KIR parameters are valid for each donor-recipient pair based on the degree of HLA matching, T-cell depletion intensity, and the specific type of leukemia. The protective or opposite effects of haplotype B among unrelated or sibling transplants are a perfect example for inconsistent results [5].

This investigation was partially presented at 14th Balkan Congress of Human Genetics and 9th Rare Disease SEE Meeting 2023, Skopje, October 05–07, 2023. In addition, the findings were published in abstract form [6].

The aim of this study was to analyze KIR gene polymorphism within the MK-SFSMDR.

Materials and Methods

The studied sample consists of 516 healthy unrelated individuals, aged 18–55 years. All individuals are donors of the MK-SFSMDR which signed Marrow Donor Registration and took buccal swabs. *KIR* gene content typing was performed by HistoGenetics (Ossining, NY 10562, USA) on Illumina platform. The analysis protocol classified genotypes as "B" if any of the genes *2DL2*, *2DL5*, *3DS1*, *2DS1*, *2DS2*, *2DS3*, or *2DS5* were present. If none of these genes were detected, the genotype was categorized as "AA." Differentiating between AB and BB genotypes was not attempted, and any combinations of these were labeled as "Bx." [7]. Population genetics analysis package, Arlequin, was used for analysis of the data [8].

Results

We found that all 16 KIR genes were observed in the MK-SFSMDR donors and framework genes *KIR3DP1, KIR2DL4, KIR3DL2, and KIR3DL3* were present in all individuals. The observed frequencies (OFs) of other KIR genes were as follows: *KIR2DP1* (0.971), *KIR2DL1* (0.971), *KIR2DL2* (0.562), *KIR2DL3* (0.882), *KIR2DL5* (0.523), *KIR3DL1* (0.942), *KIR2DS1* (0.0401), *KIR2DS2* (0.564), *KIR2DS3* (0.312), *KIR2DS4* (0.942), *KIR2DS5* (0.308), and *KIR3DS1* (0.374). Estimated frequencies (EFs) were as follows: *KIR2DP1* (0.830), *KIR2DL1* (0.830), *KIR2DL2* (0.338), *KIR2DL3* (0.656), *KIR2DL5* (0.309), *KIR3DL1* (0.759), *KIR2DS1* (0.226), *KIR2DS2* (0.340), *KIR2DS3* (0.170), *KIR2DS4* (0.759), *KIR2DS5* (0.168), and *KIR3DS1* (0.209) (Table 1).

We found positive and negative statistically significant linkage disequilibrium (p < 0.05) between most of the *KIR* genes, except for *KIR2DL4, KIR3DL2, KIR3DL3*, and *KIR3DP1* (Table 2).

We defined 35 genotypes from which one was AA group (genotype ID 1 = 27.13%) and the rest were Bx groups (34 genotypes = 72.87%) (Figure 1).

Table 1: Observed and estimated KIR gene frequencies in SFS-MDR, year 2023 (n = 516)

Frequency	Frequencies for KIR genes in SFS-MDR, year 2023 (n = 516)															
	Pseudogenes		Inhibitory KIR								Non inhibitory KIR					
	KIR 2DP1	KIR 3DP1	KIR 2DL1	KIR 2DL2	KIR 2DL3	KIR 2DL4	KIR 2DL5	KIR 3DL1	KIR 3DL2	KIR 3DL3	KIR 2DS1	KIR 2DS2	KIR 2DS3	KIR 2DS4	KIR 2DS5	KIR 3DS1
OF	0.971	1.000	0.971	0.562	0.882	1.000	0.523	0.942	1.000	1.000	0.401	0.564	0.312	0.942	0.308	0.374
S.D. of OF	0.007	0.000	0.007	0.022	0.014	0.000	0.022	0.010	0.000	0.000	0.022	0.022	0.020	0.010	0.020	0.021
EF	0.830	1.000	0.830	0.338	0.656	1.000	0.309	0.759	1.000	1.000	0.226	0.340	0.170	0.759	0.168	0.209

OF: Observed frequency was obtained by direct counting; OF = S.D. of OF: Standard deviation of observed frequency; EF: Estimated frequencies were calculated using the formula EF = 1−√(1-OF). KIR: Killer immunoglobulin-like receptor, SFS-MDR: Scientific Foundation SPIROSKI - Marrow Donor Registry

Table 2: KIR genes showing statistically significant LD (p < 0.05) in SFS-MDR, year 2023 (n = 516)

KIR gene	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
KIR3DL1		−	+	+	−	+	+	+	+	+	+	&	&	&	−	&
KIR2DL1	−		+	−	+	−	−	−	+	−	+	&	&	&	+	&
KIR2DL3	+	+		+	+	+	−	+	+	+	−	&	&	&	+	&
KIR2DS4	+	−	+		−	+	+	+	+	+	+	&	&	&	−	&
KIR2DL2	−	+	+	−		+	−	−	+	+	−	&	&	&	+	&
KIR2DL5	+	−	+	+	+		+	+	+	+	+	&	&	&	−	&
KIR3DS1	+	−	−	+	−	+		+	−	+	+	&	&	&	−	&
KIR2DS1	+	−	+	+	−	+	+		+	+	+	&	&	&	−	&
KIR2DS2	+	+	+	+	+	+	−	+		+	−	&	&	&	+	&
KIR2DS3	+	−	+	+	+	+	+	+	+		−	&	&	&	+	&
KIR2DS5	+	+	−	+	−	+	+	+	−	−		&	&	&	−	&
KIR2DL4	&	&	&	&	&	&	&	&	&	&	&		&	&	&	&
KIR3DL2	&	&	&	&	&	&	&	&	&	&	&	&		&	&	&
KIR3DL3	&	&	&	&	&	&	&	&	&	&	&	&	&		&	&
KIR2DP1	−	+	+	−	+	−	−	−	+	+	−	&	&	&		&
KIR3DP1	&	&	&	&	&	&	&	&	&	&	&	&	&	&	&	

+: Significant positive LD, −: Significant negative LD, &: LD not calculated, LD: Linkage disequilibrium. SFS-MDR: Scientific Foundation SPIROSKI - Marrow Donor Registry

Discussion

We presented for the 1st time OF and EFs of KIR receptors within the MK-SFSMDR, as well as KIR genotype groups and 35 genotype IDs determined with sequencing on Illumina platform. Previously published results of KIR genotyping with commercially available PEL-FREEZ KIR genotyping SSP kit (Dynal Biotech, Brown Deer, WI) have shown 56 different KIR genotypes in Macedonian population [9], 24 different KIR genotypes in Macedonian Roma population [10], and 45 KIR genotypes in Macedonian Albanian population [11]. Differences in KIR genotypes between our and previously published populations from Republic of North Macedonia could be explained mainly by different methods of investigation and different composition of populations.

Presearch high-throughput donor KIR genotyping seems to be more attractive compared to KIR genotyping of individual donors during ongoing donor searches [12]. More than 3 million KIR-genotyped donors are currently available through the abovementioned channels [13]. Cost issues related to presearch donor KIR genotyping should be manageable, owing mainly to NGS-related cost savings; however, it is acknowledged the existence of several competing strategies for donor classification, which require validation in independent cohorts to propagate their use [14]. Previously, it has been observed that AML transplants using KIR B haplotype Better or Best (≥2 B activating gene loci ± Cen B/B) unrelated donors result in decreased relapse rates and enhanced survival outcomes [15]. Published findings suggest that inhibitory combinations in Iranian patients have a protective role against AML by developing potent Natural Killer (NK) cells during education. These results underscore the promising potential for utilizing KIR/HLA-I combination studies in the selection of



Figure 1: KIR locus profiles observed (KIR genotypes) and the number of individuals displaying each profile. KIR: Killer cell immunoglobulin-like

donors for allogeneic NK cell therapy in hematological malignancies [16]. A multi-center retrospective study was conducted in China, analyzing and comparing 2519 patients with hematopathy (mainly, acute lymphoblastic leukemia, AML, aplastic anemia, and myelodysplastic syndrome) against 18,108 individuals without known pathology. The study identified four genes, namely, *KIR2DL5*, *2DS1*, *2DS3*, and *3DS1*, as protective factors significantly reducing the risk of aplastic anemia [17].

Therefore, the World Marrow Donor Association has expanded the standards for the deposition of donor data within donor registries. This includes information on donor identity, donor status, HLA typing, infectious disease markers, donor KIR genes, donor MIC genes, donor transfusions, pregnancies, and donations. Moreover, our comprehensive KIR genotyping of all donors will increase the feasibility of identifying optimal donors for transplantation.

Conclusions

We found that all 16 KIR genes were observed in the MK-SFSMDR donors (n = 516) and framework genes *KIR3DP1, KIR2DL4, KIR3DL2, and KIR3DL3* were present in all individuals. The OF and EFs of other KIR genes were presented. We defined 35 genotypes from which one was AA group (genotype ID 1 = 27.13%) and the rest were Bx groups (34 genotypes = 72.87%).

Inclusion of KIR genotypes in molecular screening of bone marrow donors may increase efficacy for stem cell transplantation.

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