sciendo

10.2478/AMB-2019-0023

FAMILY-BASED ASSOCIATION STUDY OF KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTOR GENES WITH LEUKEMIA

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Abstract. *NK cell function is controlled by the cell expression of killer immunoglobulin-like receptors (KIRs) and their ligation with the corresponding HLA ligands. Various malignancies have been associated with certain KIRs surface cell expression and various KIR/HLA ligand combinations. Prior research using case/control study design demonstrates the role of KIR and KIR HLA ligands as genetic factor involved in tumor susceptibility. The objective of this study was to investigate the family-based association of KIRs, HLA class I ligands and KIR/ligand combinations with leukemia diagnosis in families having a leukemia diagnosed child. Sixty-seven families that met the index leukemia case criteria (acute lymphoblastic leukemia, ALL, n = 45; acute myeloid leukemia, AML, n = 13; chronic myeloid leukemia, CML, n = 9; first degree healthy relatives n = 159) were examined. Our study consisted of two phases. In Phase1 case-control study, we primarily compared patients to their healthy siblings to asses if a marker or genotype may be associated with leukemia, excluding the impact of the environment. Phase 2 consisted of a secondary family-based association study. KIR genotyping was performed by PCR-SSP method. KIR HLA ligands were defined by direct method using PCR-SSP method and/or indirect base on high resolution typing of HLA-A, -B, -C alleles. Results of phase 1 showed an increase in the frequency of KIR genotype (with a ratio = 0.57; higher frequency for inhibitory KIRs vs. activating KIRs) among leukemia patients compared to healthy siblings. Results of the phase 2 familial study observed an association between HLA-C1+/B^{Bw4}+/A^{Bw4}+ haplotype (a mediator of inhibitory signals) and leukemia. Also, we concluded that the absence of HLA-A^{Bw4} alleles was related to leukemia development.*

Key words: *KIR, HLA class I ligand, leukemia, family-based study*

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INTRODUCTION

There is potential for transformation of normal cells in tumor cells in each organism. In healthy individuals, tumor transformed cells are destroyed by the self-immune system. NK cells play an important role as first line in self- immune defense. Growing evidence suggests the relevance of NK cell surface receptors members of the immunoglobulin superfamily, particularly KIR and corresponding KIR ligands in tumor development and susceptibility [1]. So far 16 KIR genes and 907 KIR alleles and en-

coding KIRs have been described [1]. HLA class I molecules act as specific ligands for some of these receptors [2-10].

HLA-C1 ligand group (serine and asparagine residues at positions 77 and 80 in the alpha 1 helix of the MHC class I heavy chain) binds specific KIR2DL1, while C2 allotypes (asparagine and lysine residues at the same positions) bind KIR2DL2/2DL3 [2, 3]. HLA-A and B alleles with Bw4 epitope act as ligands for KIR3DL1 [3, 4]. The strength of KIR/ligand binding depends on the amino acid at position 80 in Bw4 molecules. Bw4 alleles with isoleucine ($Bw4^{I80}$) are more potent ligands for KIRs in comparison to Bw4 alleles with threonine ($Bw4^{T80}$) at this position [5]. KIR3DL2 recognizes HLA-A3 and HLA-A11 with selected peptides like EBV-encoded peptide [6]. The activating KIRs: 2DS1, 2DS2 and 3DS1 are thought to share HLA ligand binding specificities with their inhibitory counterparts: KIR2DL1, 2DL2/2DL3 and 3DL1, respectively. KIR2DS1 binds weakly to HLA-C2 allotypes [7], KIR2DS2 may interact weakly to HLA-C1 [7] and KIR3DS1 with HLA-Bw4 molecules [9, 10].

Recently, considerable data from case/controls studies demonstrate the role of KIR and KIR HLA ligands as genetic factor involved in tumor susceptibility [1]. It could be implied that genetically predetermined KIR/HLA combinations in terms of stronger activation or lower inhibitory potential influence NK antitumor activity and malignancy susceptibility [11]. As such, KIR-mediated activation due to activating receptors KIR3DS1, KIR2DS3 and KIR2DS1 was associated with protection from hematology malignancies [12-14]. Similarly, genotypes with inhibitory potential are presented as risk factors for tumor development as KIR2DL2/2DL3/HLA-C1 in leukemia patients [15]. However, additional data contradicts the hypothesis of KIR2DS1 [16-18], KIR2DS3 [17, 18], KIR2DS2 and KIR2DS5's as predisposing mediating activation effect for leukemia [18]. Nevertheless, none of the prior studies considered the environmental factors, which also influence tumor development. We found only two prior studies evaluating KIR/HLA polymorphisms in the context of oncohematological diseases [13, 19]. Besson et al's work [13] demonstrates the activating receptors KIR3DS1 and KIR2DS1 as protective factors in a familial study of Hodgkin's lymphoma. Sugioka et al. [19] report KIR2DL2, KIR2DL5, KIR2DS1, KIR2DS2 and KIR2DS3 as being protective in a group of patients with various hematological diseases compared to healthy family members.

Thus, we hypothesized that a family-based study will determine whether there are genetic differences that act as predetermined predisposing factors for leukemia development, assuming that leukemia affected and non-affected siblings were exposed to similar environmental factors.

MATERIAL AND METHODS

Study population

Sixty-seven families were included that met the index leukemia case. Patients study sample consisted of ALL (n = 45, 30 males and 15 females; average age 19 ± 10.1 years), AML (n = 13, 7 males and 6 females; average age 35 ± 13.5 years) and CML (n = 9, 7 males and 2 females; average age 38.4 ± 12.1 years). First degree healthy relatives (97 parents and 62 siblings) were included in the study after an interview in order to elicit their medical history. Written and informed consent was obtained from parents and written and informed assent was obtained from children participants. The study was approved by the local ethics committee and IRB boards.

Methods

Measures

DNA was extracted from peripheral blood using iPrep PureLink® gDNA™ Blood kit (Invitrogen, USA) and iPrep™ Purification instrument (Invitrogen, USA).

KIR genotyping was performed by PCR-SSP method with commercially available Olerup SSP® KIR typing kit (Olerup SSP AB, Sweden). Briefly 24 locus specific primer sets in KIR genotyping kit allow detection of 16 KIR genes and pseudogenes and discrimination of *KIR2DL5A, KIR2DL5B, KIR3DL1*004* alleles and two groups *KIR2DS4* alleles (group 1 – *KIR2DS4*001* from group 2 – *KIR2DS4*003/004/006/007*). The results obtained were interpreted using the worksheet provided by the manufacturer.

KIR HLA Ligands were determined using direct genotyping by commercially available Olerup SSP® KIR HLA ligand typing kit (Olerup SSP AB, Sweden) and/or indirect using KIR ligand calculator [20] base on high resolution typing of HLA-A, -B, -C alleles (AlleleSEQR HLA-A, -B and -C PCR/Sequencer Kit, Atria Genetics, USA) as previously described [21]. KIR HLA ligand typing kit use 5 primers sets to distinguish HLA-C1, -C2, $-Bw4^{I80}$, $-Bw4^{T80}$ and $-A^{Bw4}$+ ligand group. The results obtained were interpreted using the worksheet provided by the manufacturer. HLA-A3 and HLA-A11 was determined based on individual HLA-A genotype, which was examined by Olerup SSP® "HLA-A low resolution" (Olerup SSP AB, Sweden) or by AlleleSEQR HLA-A PCR/Sequencer Kit (Atria Genetics, USA) according to the manufacture's instruction.

KIR gene, HLA class I ligand group and KIR/HLA ligand combination frequencies were estimated by direct counting. Individual KIR haplotype and KIR genotype were defined according to the allele frequency net database (AFND) [1, 22].

Data Analysis

In the present study we used two models for comparative analysis.

Phase 1: In the case/control study we compared carrier frequencies, in terms of the presence or absence of KIR genes, their HLA ligands and KIR/HLA ligand combinations between patients and their healthy siblings using Pearson's chi-squared test and Fisher exact test. Also, KIR haplotype and KIR genotype frequencies were included in the analysis. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for variables with statistical significance. Statistical analysis was performed using SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL).

Phase 2: In the family-base study, a 2nd analysis with "transmission disequilibrium test" (TDT) was conducted [23]. Measures of pairwise linkage disequilibrium (LD) between KIR genes in, such as D' and r2 [24], were estimated using Haploview (http://www.broad.mit.edu/personl/jcbarret/haplo/) according to the model of Besson et al [13]. |D'| and r2 varied between 0 and 1. The TDT analysis was used to evaluate if there is distortion in the trasmmission of alleles of interest (KIR and KIR HLA class I ligands) from parents to affected offspring. TDT was performed after genotypic reconstruction for each KIR gene, using all first-degree members of the families. In cases were one parent was missing (18/67 in our sample) families can be analysed either by method of Knapp M (RC-TDT) [25] or by method of Spielman RS (Sib-TDT) [26]. The family-based association study was conducted by FBAT program [27], in which these three described methods for genotype reconstruction are implicated (TDT, RC-TDT, and Sib-TDT).

RESULTS

I. Phase 1. Case-control association study

1. KIR gene, haplotype and genotype frequencies

The presence of the 16 KIR genes (14 genes and 2 pseudogenes) in patients and their healthy siblings are presented in Table 1.

The frequency of the KIR genotypes (presence/absence of KIR gene) in each of the two groups is showed in Table 2.

In both patients and non-affected brothers/sisters KIR genotype ID1 was the most frequent, which corresponds to homozygous carrier of two KIRA haplotypes (KIRAA). No other KIRAA haplotype combination was found, so the frequency of KIRAA in each group was relevant to KIR genotype ID1 rate (21.4% and 29.0 % respectively). All other individuals were KIRBx (KIRBB or KIRAB) (Table 2).

Although no significant difference in frequency of KIR genes, genotypes and haplotype combinations were found when patients and healthy siblings were compared, we analysed the frequency of genotypes with respect to the ratio of the number of activating to inhibitory KIR genes calculated for all individuals according to the model of Karabon et al. [12]. In this approach KIR2DL4 was included in activating KIR group (the transmembrane arginine near extracellular domain has predominantly activating function [28, 29]).

Table 1. Frequencies of KIR genes in leukemia patients (n = 56) and their healthy siblings (n = 62)

	Patients n (%)	Healthy siblings n (%)	p
KIR2DL1	54 (96.4)	61 (98.4)	ns
KIR2DL2	40 (71.4)	39 (62.9)	ns
KIR2DL3	46 (82.1)	53 (85.5)	ns
KIR2DL5	36 (64.3)	36 (58.1)	ns
KIR3DL1	52 (92.9)	59 (95.2)	ns
KIR2DS1	24 (42.9)	23 (37.1)	ns
KIR2DS2	40 (71.4)	33 (62.9)	ns
KIR2DS3	27 (48.2)	29 (46.8)	ns
KIR2DS4	53 (94.6)	59 (95.1)	ns
KIR2DS5	24 (42.9)	19 (30.7)	ns
KIR3DS1	24 (42.9)	24 (38.7)	ns
KIR3DL2/KIR3DL3/KIR2DL4	56 (100)	62 (100)	ns

Table 2. Frequency of KIR genotypes in leukemia patients (n = 56) and their healthy siblings (n = 62)

Patients n (%)	Healthy sibs n (%)	ID*	KIR haplotype combination	3DL1	2DL1	2DL3	2DS4	2DL2	2DL5	3DS1	2DS1	2DS2	2DS3	2DS5	2DL4	2DP1	3DP1 3DL2, 3DL3
12 (21.4)	**18 (29.0)**	1	AA	■	■	■	■	□	□	□	□	□	□	□	■	■	■
7 (12.5)	**8 (12.9)**	5	Bx	■	■	■	■	■	■	□	□	■	■	□	■	■	■
6 (10.7)	5 (8.2)	4	Bx	■	■	■	■	■	□	□	□	■	□	□	■	■	■
2 (3.6)	3 (4.8)	2	Bx	■	■	■	■	□	■	■	■	□	□	■	■	■	■
8 (14.2)	**8 (12.9)**	6	Bx	■	■	■	■	■	■	■	■	■	■	■	■	■	■
4 (7.1)	4 (6.5)	3	Bx	■	■	■	■	■	■	■	■	■	□	■	■	■	■
3 (5.4)	4 (6.5)	71	Bx	■	■	□	■	■	■	□	□	■	■	□	■	■	■
4 (7.1)	2 (3.2)	73	Bx	■	■	□	■	■	■	■	■	■	■	■	■	■	■
1 (1.8)	1 (1.6)	69	Bx	□	■	■	□	□	■	■	■	□	□	■	■	■	■
2 (3.6)	1 (1.6)	72	Bx	■	□	□	■	■	□	□	□	■	□	□	■	□	■
1 (1.8)	1 (1.6)	81	Bx	□	■	□	□	■	■	■	■	■	■	■	■	■	■
2 (3.6)	0	9	Bx	■	■	■	■	■	■	□	■	■	□	■	■	■	■
2 (3.6)	0	13	Bx	■	■	■	■	■	■	■	□	■	■	□	■	■	■
1 (1.8)	0	150	Bx	□	■	■	■	□	■	■	■	□	■	■	■	■	■
1 (1.8)	0	70	Bx	□	■	■	□	■	■	■	■	■	■	■	■	■	■
0	3 (4.8)	7	Bx	■	■	■	■	■	■	■	■	■	■	□	■	■	■
0	1 (1.6)	22	Bx	■	■	■	■	■	■	■	□	■	■	■	■	■	■
0	1 (1.6)	62	Bx	■	■	■	■	■	□	□	□	■	■	□	■	■	■
0	1 (1.6)	90	Bx	■	■	□	■	■	■	■	■	■	■	□	■	■	■
0	1 (1.6)	104	Bx	■	□	□	■	■	□	□	□	■	□	□	■	■	■

The filled squares correspond to the presence of the KIR gene and the empty squares – to lack of the corresponding KIR gene; * Identification number defined according to http://www.allelefrequencies.net/kir6001a.asp

Only Group 1 KIR2DS4 alleles (2DS4*001) positive individuals accounted as positive for this activating KIR gene. Group 2 KIR2DS4 alleles (included the deleted 2DS4*003, *004) were not functional. Ratios ranged from 0.2 (prevalence of inhibitory KIR genes) to 1.20 (overrepresentation of activating KIR genes). KIR genotype corresponding to ratio 0.57 (seven inhibitory-KIR genes and four activating-KIR genes) was found to have a tendency for higher frequency among patients than among their healthy brothers/sisters (14.3% vs 4.8%, p = 0.078, OR = 2.41 [95%CI 0.73-16.6]) (Table 3).

Table 3. KIR genotypes ration in leukemia patients (n = 56) and their healthy siblings (n = 62)

Ration Act. KIRs / Inh. KIRs	0.2	0.33	0.4	0.43	0.5	0.57	0.66	0.71	0.75	0.8	0.83	0.86	1.0	1.2
Healthy siblings n (%)	12 (19.4)	3 (4.8)	7 (11.3)	5 (8.1)	6 (9.7)	3 (4.8)	5 (8.1)	4 (6.5)	0	1 (1.6)	0	8 (12.9)	7 (11.3)	1 (1.6)
Patients n (%)	6 (10.7)	5 (8.9)	6 (10.7)	2 (3.6)	5 (8.9)	8 (14.3)	2 (3.6)	1 (1.8)	1 (1.8)	1 (1.8)	4 (7.1)	11 (19.6)	2 (3.6)	2 (3.6)
p	ns	ns	ns	ns	ns	0.078	ns	ns	ns	ns	nd	ns	ns	ns
OR [95% CI]						2.41 [0.73-16.6]								
Inhibitory KIR profile ← Inh KIR = Act. KIR → Activating KIR profile														

Abbr.: ns – not significant, nd – not defined, act. KIR – activating KIR; inh. KIR – inhibitory KIR; [95% CI] confidencial interval

2. Frequencies of KIR ligands and KIR/ligand combinations

Not significant associations were observed comparing leukemia patients and their healthy siblings according to the frequencies of KIR HLA class I ligands and HLA class I ligand genotypes (Table 4).

Table 4. Frequencies of KIR ligands and KIR/Ligands combinations in leukemia patients (n = 56) and their healthy siblings (n = 62)

	Patients n (%)	Healthy siblings n (%)	p
HLA-C1	43 (76.8)	45 (72.5)	ns
HLA-C2	38 (65.9)	40 (64.5)	ns
HLA-C1C1	18 (32.1)	22 (35.5)	ns
HLA-C2C2	13 (23.2)	17 (27.4)	ns
HLA-C1C2	25 (44.6)	23 (37.1)	ns
HLA-Bw6	43 (76.8)	50 (80.6)	ns
HLA-Bw4	38 (67.9)	51 (66.1)	ns
HLA- $Bw4^{I80}$	30 (53.6)	34 (54.8)	ns
HLA-$Bw4^{T80}$	11 (19.6)	10 (16.1)	ns
HLA- $Bw4^{I80}$ $Bw4^{T80}$	2 (3.6)	3 (4.8)	ns
HLA-$Bw4^{I80}$ $Bw4^{I80}$	7 (12.5)	7 (11.3)	ns
HLA- $Bw4^{T80}$ $Bw4^{T80}$	4 (7.1)	2 (3.2)	ns
HLA- A^{Bw4}+	25 (44.6)	26 (42.0)	ns
HLA- A3/11	13 (23.2)	18 (29.1)	ns

The frequencies of inhibitory KIR/ligand combinations and the activating counterpart as well as KIR inhibitory/KIR activating countrpart/appropriate ligand between the two groups are presented in Table 4. There were no statistically significant differences in the frequencies of KIR/ligand genotypes between the compared groups.

II. Phase 2: Family-based study

1. Genotype reconstruction in families

Genotypic reconstruction in families was carried out according to the Mendelian laws of inheritance and the model described by Besson et al. [13]. The procedure consisted of two steps. In the first step the following rules were followed:

1. Individuals missing particular KIR were determined as negative for the two alleles of this gene, for example KIR2DS5-/KIR2DS5-.

2. KIR2DL2/2DL3 and KIR3DL1/3DS1 segregate as alleles.

3. The used KIR genotyping kit could discriminate two allele groups of KIR2DL5 (2DL5A and 2DL5B) and KIR2DS4 (group 1 and group 2), which helped in the segregation analysis.

4. The framework KIR genes were excluded.

5. Like the framework KIR genes, KIR2DL1 was ubiquitous distributed (96%) and also was excluded.

This step allowed us to determine 100% of KIR2DL2/2DL3 and KIR3DL1/3DS1 alleles and more than 50% of the remaining KIR genotypes (number of KIR alleles) in the analysis families. In the next step we used the known KIR genotypes for estimation of pairwise LD between KIRs based on the genotypes determined in this first reconstruction step (Figure 1).

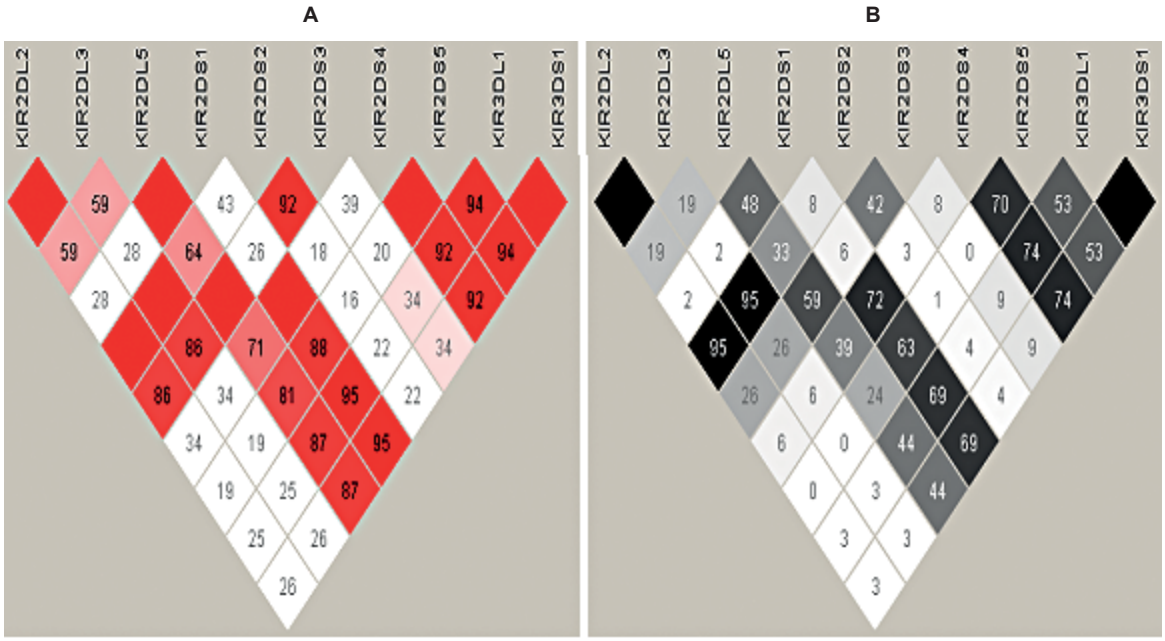

Fig. 1. Pairwise linkage disequilibrium between KIR genes in the family sample. Each square represents the magnitude of pairwise LD between KIR genes, as measured by D' (panel A) or r2 (panel B). Red squares represent a |D'| and black squares represent r2 value of 1.0, corresponding to complete and perfect LD, respectively. All other colours in both figure indicate |D'| or R2 values < 0.8

KIR region was in strong LD, and three pairs of KIRs were found to be in perfect LD (r2 = 1). KIR2DL2 and KIR2DS2 were in perfect positive LD thus KIR2DL2+/KIRDS2+ and KIR2DL2-/KIRDS2- were the only haplotypes observed. By analogy KIR2DL5 can participate in the following haplotypes with KIR2DS1 and KIR2DS3: *KIR2DL5+/KIR2DS1+* or *KIR2DL5-/KIR2DS1-* and *KIR2DL5+/KIR2DS3+* or *KIR2DL5-/KIR2DS3-*. These results made possible the complete genotypic determination of these four KIRs. KIR2DS4 and KIR2DS5, whose number of gene copies were not fully defined in all families, presented complete LD (|D'| = 1) with several other KIRs (Figure 1). Based on previously reported haplotypes [13], where KIR2DS4 is observed in complete negative LD (the presence of one gene in a given haplotype excluding the presence of the other) with KIR2DS1 and KIR2DS5 [13], we accepted that KIR2DS1 and KIR2DS4 were always inherited on different chromosomes: 2DS1+/2DS4- or 2DS1-/2DS4+. The same goes for KIR2DS4 and KIR2DS5 with only two haplotypes: 2DS4+/2DS5- or 2DS4-/2DS5+.

After these two reconstruction steps, 225 members of 67 families (patients and first degree relatives) had known genotypes for eight KIR gene systems – 2DS2, 2DL2, 2DL3, 3DS1, 3DL1, 2DL5, 2DS3, 2DS5, 2DS1, and 2DS4 – which were used for the family-based association study.

2. Family-based leukemia's association study

14 different KR haplotypes were observed, which were combined in 28 KIR genotypes, 16 occurred among parents and their generation, 8 were observed only in parents' group, one was unique for leukemia patients and the remaining three genotypes were seen only in the healthy offspring. The results of the phase 2-family-based association study of KIR and KIR HLA class I ligands showed significant association between the absence of HLA-A^{Bw4} alleles and leukemia ($p = 0.0455$). With respect to HLA class I haplotype, one haplotype HLA-C1 pos. C2 neg. Bw6 neg. Bw4 pos. A^{Bw4} pos. A3/11 neg. was found with p value 0.038, demonstrating its potential influence in the leukemia development.

DISCUSSION

Previous disease association studies of KIRs used solely case/control design. Family-base studies of NK receptors are carried out almost exclusively for the detailed examination of organization of the KIR region. To date, only two studies employing family-based association study design of KIRs and their ligands were conducted: one with hematology malignancies and one with Hodgkin disease [13, 19]. Advantages of using a family-based association model incorporate obtaining of full KIR genetic information (number of gene copies, alleles), more accurate determination of KIR haplotypes and the inherited KIR/HLA genotypes. Hence, in our study we included families meeting the leukemia case index. Using all available information from KIR and HLA class I ligand genotyping we were able to determine KIR profiles and the number of gene copies in 225 participants from 67 families.

Our results show an association between leukemia development and KIR/HLA class I gene systems. Although our proposed model for family-based KIR gene reconstruction was guided by the model of Besson et al. [13], it was also unique for our population, as it was constructed based on the established KIR pairwise LD adapted for Bulgarians.

In Phase 1 we primarily compared the distribution of individual KIRs, KIR genotypes, KIRA/B haplotype combinations, HLA class I ligands and KIR/ligand combinations between leukemia patients and their healthy brothers/sisters. The aim of patient/healthy sibling study was to define whether there are differences that act as genetic genetic differences that act as predetermined predisposing factors for leukemia development, assuming that leukemia affected and non-affected siblings were exposed to similar environmental factors.The comparative analysis did not show any differences in any of tested markers. However, this was not surprising, since according to Mendelian laws of inheritance probability of a pair offspring to be genetically identical is 25%. Nevertheless, KIR genotype with activating/inhibitory KIR ratio of 0.57 was more frequent in the leukemia patients group than in the healthy siblings group. In this genotype, there was a higher prevalence of inhibitory KIR genes vs. activating KIR genes. Therefore, it contributes to higher leukemia susceptibility due to more inhibitory NK cell activation potential.

Phase 2: The second purpose of our research was to conduct a family-based leukemia association study to compare alleles transmittion distribution in leukemia affected offspring compared with the expected distribution of alleles among healthy offspring. For this task we developed and implemented a model for KIR gene reconstruction in families that met the leukemia case index. From the TDT analysis we found that the absence of HLA-A^{Bw4} ligand and the carriage of HLA-C1+/B^{Bw4}+/A^{Bw4}+ haplotype was in association with leukemia disease ($p < 0.05$). Some peculiarity should be noted in the context of HLA-C1+/B^{Bw4}+/A^{Bw4}+ haplotype. All studied individuals possessed at least one inhibitory KIR (KIR2DL2 or KIR2DL3) for

HLA-C1 group ligand, which means that this haplotype participates in a functional inhibitory KIR/ligand interaction. At the same time, simultaneous presence of both alleles from HLA locus binding KIR3DL1 (HLA-B^{Bw4}+ и A^{Bw4}+) defines more powerful inhibitory potency. On the other side the same HLA class I ligands also could interact with activating KIRs (KIR2DS2 and KIR3DS1 respectively). As a whole the frequencies of activating KIRs were lower compared to the inhibitory counterpart and we accepted that HLA-C1+/B^{Bw4}+/A^{Bw4}+ haplotype should be considered as a mediator of inhibitory signals. The observed difference in HLA-A^{Bw4} allele distribution could not be compared with the available literature data due to the lack of such association in case/control or family-based study. Furthermore, the role of HLA-A^{Bw4} molecule as ligand for KIR3DL1/S1 is not fully understood and our data did not allow us to interpret the absence of HLA-A^{Bw4} and its role in the inhibition. Rather we can only suggest its role as a risk factor for leukemia, particularly when family history is positive for malignancy.

CONCLUSION

Although a small number of differences were reported in our study – case/control (phase 1) and family-based (phase 2), we found significant inhibitory potency variations in leukemia patients' group. The results were in accordance with our hypothesis for the role of certain KIRs and/or KIR/ligand combinations, acting in a direction of increased NK cell inhibition or decreased activation, but more as a risk factor in leukemia susceptibility. We also considered the possible disguise of KIR and KIR/ligand genotypes effects from other polymorphic genes.

Conflict of interests: *The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.*

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Received: *January, 2019 –* ***Revised:*** *February, 2019*
Accepted: *April, 2019*