

Increased Frequency of KIR2DS4 del/del among AA Genotype Carriers with B-Cell Acute Lymphoblastic Leukemia in Southwestern Iranian Population

Sepide Namdari¹, Mohammad Reza Bordbar², Omid Reza Zekavat², Shirin Farjadian^{1*}

¹Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran; ²Department of Pediatrics, Shiraz University of Medical Sciences, Shiraz, Iran

ABSTRACT

Acute Lymphoblastic Leukemia (ALL) is known to be the most common malignancy among children. The early onset of ALL suggests a key role of genetic factors in its development. Killer Cell Immunoglobulin-like Receptor (KIR) gene complex that encodes a group of key receptors expressed at the surface of natural killer cells has been of great interest as a possible genetic factor affecting the susceptibility to ALL. In this case-control study, we aimed to verify whether the inheritance of specific KIR genes or genotypes were associated with susceptibility to childhood B-ALL in southwestern Iranian population. To this end, KIR genotyping was carried out for 120 patients with childhood B-ALL and 170 unrelated healthy controls using PCR-SSP method. The frequencies of 11 KIR genes and KIR2DS4 variants were investigated among patients and healthy controls. We found no association between KIR genes and genotypes, centromeric and telomeric genotypes as well as the number of activating or inhibitory KIRs and susceptibility to childhood B-ALL. Moreover, no association between childhood B-ALL and KIR B-content scores was detected. However, AA genotype carriers homozygous for KIR2DS4 deleted variant were found to be at a higher risk of developing childhood B-ALL compared with those who inherited at least one KIR2DS4 full-length variant. It seems that the inheritance of different KIR genes does not affect the risk of childhood B-ALL in southwestern Iranian population. However, among individuals with AA genotype, homozygosity for KIR2DS4 deleted allele seems to increase the risk of childhood B-ALL.

Keywords: Acute lymphoblastic leukemia; Killer cell immunoglobulin-like receptor; AA genotype; Malignancy

INTRODUCTION

Acute Lymphoblastic Leukemia (ALL) is caused by transformation and excessive proliferation of lymphoid progenitor cells [1]. ALL is the most common malignancy among children and childhood ALL accounts for about 80% of all ALL patients [1,2]. ALL can arise from both B cell and T cell precursors. B-ALL comprises about 88% of all of ALL patients. In B-ALL, patient age and WBC count at diagnosis are independent prognostic factors. Patients diagnosed between the ages of 1 and 10 have a better outcome compared with those <1 or ≥ 10 years old [3]. Patients with WBC count ≥ 50,000/mm³ at diagnosis have worse outcome than those with WBC count <50,000/mm³. According to National Cancer Institute/Rome categorization patients between 1-10 years with a WBC count <50,000/mm³ are considered 'standard risk' while

the remainder are termed 'high risk' [4]. Unlike patients with B-ALL, age and WBC count at diagnosis are not considered as determining prognostic factors in patients with T-ALL.

The etiology of this leukemia is unknown. Due to the early onset of this malignancy, it is likely that there is a critical role for genetic factors in increasing the risk of ALL. A notable genomic region that has been questioned to have an association with ALL is Killer Cell Immunoglobulin-like Receptor (KIR) gene complex. Many studies have been accomplished in order to investigate this association in different populations [5-10]. KIR gene loci, located on chromosome 19q13.4, contain a family of highly homologous genes including 6 activating genes, 8 inhibitory genes and 2 pseudogenes [11]. Due to the polygenic and multi-allelic nature of this genomic region, different individuals may vary in both the number and allele of

Correspondence to: Farjadian S, Department of Immunology, Shiraz University of Medical Sciences, Zand St, 71348-45794 Shiraz, Iran, Tel: +98 71 32351575; Email: farjadsh@sums.ac.ir

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KIR genes [12]. The diversity in KIR gene repertoire is also reported among different populations [13,14].

KIR genes encode one of the most important receptors expressed at the surface of Natural Killer (NK) cells and some T cells. KIRs have a key role in the function and development of (NK) cells [15]. The ligands for most of KIR receptors are Human Leukocyte Antigen (HLA) class I molecules. Viral infection and transformation of cells lead to the down-regulation of MHC class I molecules at the cell surface which can be sensed by NK cells through KIRs, leading to spontaneous destruction of the target cells [16].

There are two major KIR haplotypes, termed A and B. The genes 2DL4, 3DP1, 3DL2, and 3DL3, termed framework loci, are present in all haplotypes. A haplotypes have a fixed gene content consisting of seven genes and two pseudo genes; 2DL1, 2DL3, 2DL4, 2DS4, 3DL1, 3DL2, 3DL3, 2DP1 and 3DP1. Unlike A haplotypes, B haplotypes vary in gene content but must include at least one of the B-specific genes: 2DS1, 2DS2, 2DS3, 2DS5, 2DL2 and 2DL5 [17]. Considering KIR haplotype zygosity, all individuals have either AA genotype or Bx genotype (having one or two B haplotypes).

There are two distinct KIR gene clusters due to linkage disequilibrium. The C4 gene cluster in the centromeric half consists of 2DL2, 2DL5, 2DS2 and 2DS3, and the T4 gene cluster in the telomeric half comprises 2DL5, 3DS1, 2DS1 and 2DS5. According to the presence or absence of C4 and T4 clusters, Bx genotypes are classified as C4T4, C4Tx, CxT4 and CxTx. C4T4 denotes the presence of both C4 and T4 while CxTx represents the absence of both C4 and T4. Further, C4Tx indicates the presence of C4 and the absence of T4; and CxT4 represents the absence of C4 and the presence of T4 [18].

The single activating KIR gene in A haplotype, 2DS4, has a null allele with a 22 base pair deletion in its coding region. Due to the high population frequency of this null allele, it is probable that individuals who are homozygous for A haplotype do not express any activating KIRs.

Although the association between KIR genes and childhood ALL has been studied, due to KIR gene diversity among different populations, we aim to investigate this association in Iranian population.

MATERIALS AND METHODS

Study population

We studied 120 children with well-documented B-ALL who were diagnosed based on the standards for ALL diagnosis in the 2008 WHO classification of lymphoid neoplasms [19], along with 170 healthy individuals as controls. The study protocol was approved by our university Ethics Committee (approval number: IR.SUMS.REC.1398.1116). Written informed consent was obtained from all patients' parents or legal guardians prior to their inclusion in the study. Patients were recruited from Amir Oncology Hospital affiliated with Shiraz University of Medical Sciences (SUMS). We received the remaining blood samples containing EDTA anticoagulant of each patient which were collected for the routine laboratory tests. Genomic DNA was extracted from whole blood using a salting out method [20]. The demographic and laboratory information was extracted from patients' medical files. DNA samples of 170 unrelated healthy adults with no history of

autoimmune diseases and malignancies were obtained from SUMS Immunogenetics Bio bank and used as the control group.

KIR Genotyping

KIR genotyping was performed by polymerase chain reaction using sequence specific primers (PCR-SSP) for 2DL1, 2DL2, 2DL3, 2DL5, 3DL1, 2DS1, 2DS2, 2DS3, 2DS5, 3DS1 genes. Primer sequences were based on those designed by Vilches et al. [21]. In order to determine KIR2DS4 gene variants (Full-Length (fl) and deleted (del)), an alternative pair of primers were used based on sequences reported by Ashouri et al. [22]. An additional pair of primers specific for non-polymorphic sequences of the HLA-DRA gene was used in every reaction as internal positive control. DNA samples with known KIR patterns obtained from Institute for Cancer Research, SUMS were used as positive controls. PCR mixture without template DNA was used as negative control. Total PCR volume for each reaction was 10 μ l containing 5 μ l of 2x master mix, 5 pmol of each specific primer, 0.5 pmol of each internal control primer, and 30 ng of genomic DNA. DNA amplification was carried out using following condition: initial heating at 95°C for 3 min, then 10 cycles of 20 s at 94°C, 30 s at 65°C and 30 s at 72°C followed by 20 cycles of 20 s at 94°C, 30 s at 61°C and 30 s at 72°C continued with 72°C for 5 min for further extension. PCR products of different KIR genes were verified by agarose gel electrophoresis (2%) containing DNA safe stain. To differentiate between 2DS4 fl and 2DS4 del alleles, electrophoresis was done on 3.5% agarose gel. In order to allow PCR product size identification, 50 bp DNA ladder was loaded in every gel electrophoresis. DNA bands were detected using a gel documentation system.

KIR genotypes were then determined for each sample based on allele frequency database. Centromeric and telomeric KIR genotypes were determined according to the study by Cooley et al. [23]. We also used the KIR B-content score (which can vary from 0 to 4) as the number of centromeric and telomeric gene-content motifs containing B haplotype-defining genes [23].

Statistical analysis

To compare KIR gene and genotype frequencies in patient and control groups, two-tailed chi-square with Yates' correction was applied. Odds Ratio (OR) and 95% Confidence Interval (CI) were calculated for every significant difference. All data were analyzed using Graph Pad Prism version 6 and a P-value < 0.05 was considered statistically significant.

RESULTS

Frequency of KIR genes and genotypes in patients and controls

In order to examine whether there is a relationship between KIR genes and childhood B-ALL, we performed KIR genotyping for 120 patients with B-ALL and 170 healthy controls. KIR gene profiles of patients with childhood B-ALL and controls are depicted in Table 1. As presented in Table 2, no significant difference was observed in the frequency of KIR genes between patients and controls. There was also no significant difference in the distribution of either AA and Bx genotypes or centromeric and telomeric KIR genotypes between patients and controls (Table 3). No significant difference was detected in B-content scores as well as Bx genotype subsets between patients and controls (Tables 4 and 5).

Table 1: Frequency of KIR genes in patients with B-ALL compared to the healthy controls.

KIR genes	B-ALL (n=120)	Standard-risk B-ALL (n=77)	High-risk B-ALL (n=43)	Controls (n=170)	B-ALL vs. Controls	Standard-risk vs. Controls	High-risk vs. Controls	Standard-risk vs. High-risk
	N %				P-value			
3DL1	116 96.60%	73 94.80%	43 100%	163 95.90%	0.97	0.96	0.38	0.32
2DL1	120 100%	77 100%	43 100%	170 100%	>0.9999	>0.9999	>0.9999	>0.9999
2DL3	112 93.30%	71 92.20%	41 95.30%	149 87.60%	0.16	0.4	0.24	0.78
2DS4	116 96.60%	73 94.80%	43 100%	165 97.10%	0.88	0.61	0.57	0.32
2DS1	34 28.30%	24 31.10%	10 23.20%	65 38.20%	0.1	0.35	0.1	0.48
2DS2	54 45%	35 45.40%	19 44.10%	93 54.70%	0.13	0.23	0.29	>0.9999
2DS3	42 35%	27 35.00%	15 34.80%	64 37.60%	0.74	0.81	0.87	>0.9999
	24 20%	16 20.80%	8 18.60%	51 30.00%	0.08	0.18	0.19	0.96
	38 31.60%	24 31.10%	14 32.50%	66 38.80%	0.26	0.31	0.56	>0.9999
	54 45%	35 45.40%	19 44.10%	92 54.10%	0.15	0.26	0.32	>0.9999
	58 48.30%	37 48.00%	21 48.80%	94 55.30%	0.29	0.36	0.56	>0.9999

Note: P-value was calculated for presence versus absence of each KIR gene based on a 2 × 2 contingency table.

Table 2: Distribution of KIR genotypes in patients with standard-risk or high-risk B-ALL and healthy controls.

KIR genotype	B-ALL (n=120)	Standard-risk B-ALL (n=77)	High-risk B-ALL (n=43)	Controls (n=170)	B-ALL vs. Controls	Standard-risk vs. Controls	High-risk vs. Controls	Standard-risk vs. High-risk
	N %				P-value			
Cent-KIR genotypes								
AA	66 55%	42 54.50%	24 55.80%	76 44.70%	0.13	0.3	0.24	0.8
AB	46 38.30%	29 37.60%	17 39.50%	73 42.90%				
BB	8 6.60%	6 7.80%	2 4.60%	21 12.40%				
Tel-KIR genotypes								
AA	80 66.60%	53 68.80%	27 62.70%	101 59.40%	0.45	0.27	0.4	0.17
AB	36 30%	20 25.90%	16 37.20%	62 36.50%				
BB	4 3.30%	4 5.20%	0 0%	7 4.10%				
KIR genotypes								
AA	46 38.30%	30 38.90%	16 37.20%	52 30.60%	0.21	0.25	0.52	>0.9999
Bx	74 61.60%	47 61.00%	27 62.70%	118 69.40%				

Abbreviations: Cent: Centromeric, Tel: Telomeric.

Table 3: B-content scores in patients with standard-risk or high-risk B-ALL and healthy controls.

B content score	B-ALL (n=120)	Standard-risk B-ALL (n=77)	High-risk B-ALL (n=43)	Controls (n=170)	B-ALL vs. Controls	Standard-risk vs. Controls	High-risk vs. Controls	Standard-risk vs. High-risk
	N %				P-value			
0	46 38.30%	30 38.90%	16 37.20%	52 30.60%	0.16	0.19	0.2	0.16
1	48 40%	31 40.20%	17 39.50%	61 35.90%				
2	20 16.60%	10 12.90%	10 23.20%	41 24.10%				
3	6 5%	6 7.80%	0 0%	16 9.40%				
4	0 0%	0 0%	0 0%	0 0%				

Table 4: Distribution of Bx genotype subsets in patients with B-ALL and healthy controls.

Bx genotype subsets	B-ALL (n=74)	Controls (n=170)	P-value
	N %		
C4T4	8 10.80%	18 15.30%	0.56
C4Tx	30 40.50%	38 32.20%	
CxT4	14 18.90%	28 23.70%	
CxTx	22 29.70%	34 28.80%	

Distribution of KIR genes and genotypes in B-ALL subgroups

Of 120 patients with B-ALL, 77 patients were classified as “standard-risk group” and 43 patients as “high-risk group”. Our results revealed no significant difference in the frequency of KIR genes between patients in the standard-risk or the high-risk group and controls, as well as between patients with standard-risk and those with high-risk B-ALL (Table 2).

Among 77 patients in the standard-risk group, 30 patients (39%) were found to be carriers of AA genotypes and 47 (61.0%) carriers of Bx genotypes. No significant difference was observed in the distribution of AA and Bx genotypes between standard-risk group patients and controls. Moreover, no significant difference was found in the distribution of centromeric and telomeric KIR genotypes between standard-risk group patients and controls.

Of 43 patients in the high-risk group, 16 (37.2%) were AA and

27 (62.7%) were Bx genotype carriers. No significant difference was found in the distribution of AA and Bx genotypes between high-risk group patients and controls. There was also no significant difference in the distribution of centromeric and telomeric KIR genotypes between high-risk group patients and controls. Furthermore, we found no significant difference in the distribution of AA, Bx, centromeric and telomeric KIR genotypes between these two groups (Table 3).

As presented in Table 4, we observed no significant difference in the distribution of B-content scores between either group of patients and controls as well as between the two groups of patients. Patients in standard-risk or high-risk group were compared with controls and also with each other in terms of the number of aKIR versus iKIR genes. As shown in Table 6, no significant difference was observed.

Table 5: Comparison of the number of activating KIR (aKIR) genes versus inhibitory KIR (iKIR) genes between patients with B-ALL and healthy controls.

Number of aKIRs vs. iKIRs	B-ALL	Standard-risk B-ALL	High-risk B-ALL	Controls	B-ALL vs. Controls	Standard-risk vs. Controls	High-risk vs. Controls	Standard-risk vs. High-risk
	(n=120)	(n=77)	(n=43)	(n=170)				
			N %					P-value
iKIR>aKIR	88 73.30%	53 68.80%	35 81.40%	110 64.70%				
aKIR>iKIR	10 8.30%	8 10.40%	2 4.70%	26 15.30%	0.16	0.58	0.08	0.3
iKIR=aKIR	22 18.30%	16 20.80%	6 13.90%	34 20%				

KIR2DS4 variants in patients with B-ALL and controls

Our data revealed no significant difference in the distribution of KIR2DS4 variants in patients with B-ALL and controls (Table 7). However, when we compared B-ALL patients and controls with AA genotypes in terms of KIR2DS4 variants, we found a significant difference (P=0.03). Further analysis revealed that KIR2DS4 del/del genotype was significantly more frequent among AA genotype carriers in the patient group than those in the control group (P=0.023, OR 4.26, 95% CI: 1.28-12.46).

DISCUSSION

NK cells are important components of the innate immunity against tumor or virally infected cells with decreased expression of HLA class I molecules. Leukemic cells have been reported to have down regulated expression of HLA class I molecules [24-27]. The reason why NK cells are not capable of destroying these leukemic cells and eradicating them is of question. One possibility is that NK cell failure to destroy leukemic cells with aberrant expression of HLA class I molecules may be due to the

NK receptors present in these patients. KIRs are key NK receptors most of which recognize HLA class I molecules as their ligands [28]. Moreover, the polygenic and multi-allelic nature of KIRs results in a vast diversity of KIR genotypes [29]. Considering these two facts, KIRs are very likely to be the NK receptors responsible for the dysfunctionality of NK cells in the destruction of leukemic cells.

Several studies have been carried out in order to investigate whether there is a relationship between KIR genes and the susceptibility to hematological malignancies [30-32], however, controversial results were obtained. We performed a case-control study to find out whether this relationship exists in patients with childhood B-ALL. Our results revealed no association between none of the activating and inhibitory KIR genes and susceptibility to childhood B-ALL. We also found no relationship between AA and Bx genotypes, as well as centromeric and telomeric genotypes and this disease. Moreover, the number of centromeric and telomeric gene-content motifs that contained B haplotype-specific genes was not found to be associated with B-ALL development.

Table 6: Distribution of KIR2DS4 variants in patients with B-ALL and healthy controls.

KIR2DS4	B-ALL	Standard-risk B-ALL	High-risk B-ALL	Controls	B-ALL vs. Controls	Standard-risk vs. Controls	High-risk vs. Controls	Standard-risk vs. High-risk	B-ALL with AA genotype	Controls with AA genotype	AA B-ALL vs. AA controls
	(n=116)	(n=73)	(n=43)	(n=165)					(n=46)	(n=52)	
Alleles		N %								N %	P-value
fl	40 17.20%	27 18.50%	13 15.10%	68 20.60%	0.37	0.68	0.32	0.51	4 4.30%	19 18.30%	0.005
del	192 82.80%	119 81.50%	73 84.90%	262 79.40%					88 95.60%	85 81.70%	
Genotypes		N %								N %	P-value
fl/fl	8 6.90%	4 5.50%	4 9.30%	21 12.70%					0 0%	4 7.70%	
del/del		50 68.50%	34 79.10%	118 71.50%	0.2	0.07	0.61	0.15	42 91.30%	37 71.20%	0.03fi
fl/del	24 20.70%	19 26.00%	5 11.60%	26 15.80%					4 8.70%	11 21.20%	

Note: fl: Full-length variant, del: Deleted variant

¶ Comparison of the distribution of KIR2DS4 variants in AA genotype carriers between patients with B-ALL and healthy controls based on a 2 × 2 contingency table: del/del versus other genotypes (P=0.023, OR 4.26, 95% CI: 1.28-12.46); fl/fl versus other genotypes (P=0.16); fl/del versus other.

Table 7: KIR gene profiles of patients with childhood B-ALL and healthy controls (Gray and white cells denote the presence and the absence of the related genes, respectively).

Genotype ID	Genotype	Centromeric genotype	Telomeric genotype	Bx subset	A haplotype-associated KIRs					B haplotype-associated KIRs					Framework KIRs			KIR pseudogenes		Frequency		
					3DL1	2DL1	2DL3	2DS4	2DL2	2DL5	3DS1	2DS1	2DS2	2DS3	2DS5	2DL4	3DL2	3DL3	2DP1	3DP1	B-ALL patients	Controls
1	AA	A/A	A/A	~	Gray	Gray	Gray	Gray	White	White	White	White	White	White	White	White	White	White	White	White	46	52
2	AB	A/A	A/B	CxT4	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	8	14
3	AB	A/B	A/B	CxT4	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	4	8
4	AB	A/B	A/A	CxTx	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	10	19
5	AB	A/B	A/A	C4Tx	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	20	18
6	AB	A/B	A/B	C4T4	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	4	8
7	AB	A/B	A/B	C4Tx	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	4	6
8	AB	A/A	A/B	CxTx	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	4	1
9	AB	A/B	A/B	CxTx	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	2	3
10	AB	A/B	A/A	CxTx	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	0	1
11	AB	A/B	A/B	C4Tx	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	0	1
14	AB	A/B	A/B	C4Tx	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	6	0
27	AB	A/A	A/B	CxTx	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	0	4
28	AB	A/A	A/B	CxT4	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	0	1
62	AB	A/B	A/A	CxTx	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	0	2
68	AB	A/B	B/B	CxT4	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	0	1
69	AB	A/A	B/B	CxT4	White	Gray	White	White	Gray	White	White	White	White	White	White	White	White	White	White	White	2	2
70	AB	A/B	B/B	C4T4	White	Gray	White	White	Gray	White	White	White	White	White	White	White	White	White	White	White	2	1
71	AB	B/B	A/A	C4Tx	White	Gray	White	White	Gray	White	White	White	White	White	White	White	White	White	White	White	4	8
73	AB	B/B	A/B	C4T4	Gray	Gray	White	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	2	9
86	AB	A/B	B/B	CxT4	Gray	Gray	White	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	0	1
90	AB	B/B	A/B	C4Tx	White	Gray	White	White	Gray	White	White	White	White	White	White	White	White	White	White	White	2	3
94	AB	B/B	A/B	C4Tx	Gray	Gray	White	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	0	1
154	AB	A/A	B/B	CxT4	Gray	Gray	White	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	0	1
159	AB	A/B	B/B	C4Tx	White	Gray	White	White	Gray	White	White	White	White	White	White	White	White	White	White	White	0	1
202	AB	A/A	A/B	CxTx	Gray	Gray	White	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	0	2
381	AB	A/B	A/B	CxTx	Gray	Gray	Gray	Gray	White	Gray	White	White	White	White	White	White	White	White	White	White	0	2
Total																					120	170

Our data were consistent with those of Babor et al. which were extracted from the analysis of 185 childhood B-ALL and 33 childhood T-ALL patients along with ethnically matched controls [7]. Similarly, Shahsavari et al. they did not find any associations between KIR genes or genotypes and ALL in a study of 38 patients with ALL and 200 healthy controls with Iranian origin [32]. In a family-based association study including 67 families of ALL, AML and CML patients, Varbanova et al. aimed to find genetic differences that predisposed the affected siblings to leukemia, considering that siblings were exposed to identical environmental factors [10]. None of KIR genes and genotypes was recognized as predisposing factors for leukemia, which is consistent with our data on childhood B-ALL.

Our results were also in line with the data were reported by Oevermann et al. who did not find any relationships between KIR genes, genotypes, and KIR B-content scores and susceptibility to ALL in 328 patients with European origin [33]. Conversely, Almalte et al. reported that all aKIR genes were associated with resistance to childhood B-ALL [6]. They studied the six known aKIR genes in Canadian patients of white origin. However, they failed to study iKIR genes in these patients. The conflict between the results from this study and ours maybe due to the diversity of KIR gene repertoire among different populations. It would have been of interest if the frequencies of iKIR genes had been determined in this study as well; because of the linkage disequilibrium that strongly exists between some of the aKIR and iKIR genes. Investigating the frequency of iKIR genes would have also helped to learn how KIR genotypes were related to the disease.

KIR2DS3 was reported to be significantly less frequent in ALL patients compared with healthy controls in a study by Zhang et al. [5]. However, the sample size was small in this study ($n=61$).

Our results were in stark contrast of those of Misra et al. who reported all six aKIR genes (2DS1, 2DS2, 2DS3, 2DS4, 2DS5 and 3DS1) to be significantly more frequent among patients with childhood ALL compared with controls [9].

When considering the number of both aKIRs and iKIRs, we observed that $aKIRs > iKIRs$, $iKIRs > aKIRs$, and $aKIRs = iKIRs$ were distributed similarly in B-ALL patients and controls, which agrees with the results from the study by Shahsavari et al. [32].

Moreover, we compared AA genotype carriers in the two groups of patients and controls in terms of KIR2DS4 variants. Our results revealed that the frequency of del/del genotype was significantly higher among patients than controls. It implies that individuals with AA genotypes who have no aKIRs are at a higher risk of developing ALL than those who carry at least one aKIR. This may be due to the lack of enough activating signals for NK cells to become activated and carry out effector functions, in AA genotype carriers homozygous for KIR2DS4 del variant.

Our results are consistent with those of Giebel et al. who found a higher frequency of the KIR2DS4 del allele in patients with CML compared with healthy controls [31]. Moreover, KIR2DS4 del allele was found to be associated with increased risk of Hodgkin's lymphoma in a study by Besson et al. [30].

CONCLUSION

However, in a previous study by Mezger et al. it was concluded that neither KIR2DS4 del variant nor KIR2DS4 del/del genotype were associated with increased susceptibility to childhood B-ALL [34]. In this study, the frequency of different KIR2DS4 genotypes including del/del, fl/fl, and fl/del as well as 2DS4^{-/-} were compared between patients with childhood B-ALL and healthy controls and no significant difference was obtained.

Since KIR molecules interact with MHC class I molecules as their

ligands to regulate the function and development of NK cells, it would have been beneficial if MHC class I typing had been carried out in our study as well. Moreover, the product of different KIR alleles may differ in the affinity for their cognate MHC I ligands, the expression level at the cell surface and their signaling strength. Hence the lack of KIR gene polymorphism investigation is another potential limitation of our study. In addition to KIRs, other NK cell receptors take part in NK cell activation and effector function and need to be taken into account when studying NK cell activity. Finally, it would have been of great benefit if we had conducted our study on B-ALL patients with a larger sample size.

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