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**RESEARCH**

# Analysis of killer cell immunoglobulin-like receptor genotypes in acute and chronic myeloid leukemia

## Akut ve kronik miyeloid lösemilerde killer cell immunoglobulin-like reseptör genotip analizi

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**Abstract**

**Purpose:** Interactions between natural killer (NK)-expressed killer-cell immunoglobulin-like receptors (KIRs) and Major Histocompatibility Complex (MHC) class I molecules determine immune responses against tumour cells. Previous studies have shown that alloreactive potential of NK cells provide a promising treatment option for patients with hematological malignancies. The aim of this study was to investigate the KIR genes and genotypes in patients with acute myeloid leukemia (AML) and chronic myeloid leukemias (CML).

**Materials and Methods:** We investigated the frequency of KIR in AML (n=34) and CML (n=56) patients from Southern (Mediterranean) region of Türkiye and compared the results with 100 healthy controls. Polymerase chain reaction (PCR) and sequence-specific oligonucleotide (SSO) methods were used to identify KIR genes in peripheral blood samples.

**Results:** We observed an increased frequency of inhibitory KIR2DL3 in myeloid leukemia patients (91.3%) compared to controls (78%). Additionally, the frequency of KIR2DL3 was significantly higher in CML patients compared to controls (93.5% vs. 78%,). Notably, there was also a decreased incidence of inhibitory KIR2DL2 and its activating counterpart KIR2DS2 in CML patients compared to controls (34.8% vs. 52%, both, respectively). Additionally, there was a tendency for inhibitory KIR genes to predominate in patients with myeloid leukemia.

**Conclusion:** Our findings suggest a potential role for the KIR genes in the pathogenesis of myeloid leukemia. Further studies are needed to investigate the relationships between KIR gene polymorphisms and human leukocyte antigen (HLA) ligands in leukemia and the impact of NK cell responses on clinical outcomes.

**Keywords:**. Leukemia, KIR, myeloid, NK cell, genotype

**Öz**

**Amaç:** Doğal öldürücü (NK) tarafından ifade edilen killer-cell immunoglobulin-like receptors (KIR'lar) ile major doku uygunluk kompleksi (MHC) sınıf I molekülleri arasındaki etkileşimler, tümör hücrelerine karşı bağışıklık tepkilerini belirler. Önceki çalışmalar, NK hücrelerinin alloreaktif potansiyelinin hematolojik maligniteli hastalar için umut verici bir tedavi seçeneği sunduğunu göstermiştir. Bu çalışmanın amacı akut miyeloid lösemi (AML) ve kronik miyeloid lösemi (KML) hastalarında KIRgenlerini ve genotiplerini araştırmaktır.

**Gereç ve Yöntem:** Türkiye'nin güney (Akdeniz) bölgesinde AML (n=34) ve KML (n=56) hastalarında KIR sıklığı araştırıldı ve bu sonuçlar 100 sağlıklı kontrolle karşılaştırıldı. Periferik kan örneklerinden KIR genlerini tanımlamak için polimeraz zincir reaksiyonu (PCR) ve dizi spesifik oligonükleotid (SSO) yöntemi kullanıldı.

**Bulgular:** Myeloid lösemi hastalarında (%91,3) kontrollerle (%78) karşılaştırıldığında inhibitör KIR2DL3'ün artmış sıklığını gözlemledik. Ayrıca KIR2DL3 sıklığı KML hastalarında kontrol grubuna göre anlamlı derecede yüksekti (%93,5'e karşı %78). Özellikle KML hastalarında kontrollerle karşılaştırıldığında inhibitör KIR2DL2 ve onun aktive edici karşılığı olan KIR2DS2'nin insidansında azalma da mevcuttu (sırasıyla %34,8'e karşı %52, her ikisi de). Ek olarak, myeloid lösemi hastalarında inhibitör KIR genlerinin baskın olma eğilimi mevcuttu.

**Sonuç:** Bulgularımız miyeloid lösemilerin patogenezinde KIR genlerinin potansiyel bir rolüolduğunu göstermektedir. Lösemilerde KIR gen polimorfizmleri ile insan lökosit antijeni (HLA) ligandları arasındaki ilişkileri ve NK hücre yanıtlarının klinik sonuçlar üzerindeki etkisini araştıran daha fazla çalışma yapılması gerekmektedir.

**Anahtar kelimeler**: Lösemi, KIR, miyeloid, NK hücresi, genotip

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## INTRODUCTION

Natural killer (NK) cells play a role in tumor immunology by targeting neoplastic cells that lack major histocompatibility complex-1 (MHC-1) expression. The function of these cells, which are involved in the innate immune response, is to act on tumor cells and virus-infected cells through the interaction of activating and inhibitory signals. The cell surface receptors that provide the signals are known as killer cell immunoglobulin-like receptors (KIRs) and play an important role in regulating immune responses by interacting with specific human leukocyte antigen (HLA) class I molecules. The KIR genetic locus encodes two major haplotypes, haplotypes A and B, and has 16 highly polymorphic genes[1,2]. Variations in KIR genotypes were found to be associated with cancer treatment outcomes[3,4]. Clinical data demonstrating the activity of NK cells against hematological malignancies has also increased interest in the role of NK cells in the pathogenesis of leukemia. Therefore, the role of KIR genes in different leukemia subtypes is being investigated[2]. Preclinical studies have shown that NK cells may have the capacity to inhibit the growth of clonogenic leukemic cells in acute myeloid leukemia (AML), chronic myeloid leukemia (CML), and preleukemic patients[5]. Furthermore, NK-cell therapy is effective in patients with high-risk hematological malignancies[6]. It is reported in the literature that NK cell activity determined by KIR/HLA class I ligand polymorphisms affects susceptibility to myeloid leukemia but not lymphoblastic leukemia[8]. It is thought that NK cells are inhibited in leukemias due to many inhibitory KIRs[2]. In contrast to the study showing that KIR genes are associated with AML[11], a study on AML patients and healthy controls reported no difference between the presence or absence of individual KIR genes[12]. KIR genes that protect against CML are being identified[7], and it is stated that certain KIR genotypes predict cytogenetic response and survival[10]. Although it includes results from different populations, the data in the literature on KIR genes in myeloid leukemias are still inconclusive and contain contradictory results. This clearly shows that more studies are needed to clarify the exact role of KIR genes in myeloid leukemia, which is one of the leukemia subtypes. The frequencies and genotype distributions of polymorphic KIR genes may differ in myeloid malignancies (AML and CML) from healthy individuals, and these genes may have a determining role in the pathogenesis of the disease. Therefore, we aimed to present new and original data on this subject by determining the frequencies of polymorphic KIR genes and the distribution of KIR genotypes in myeloid malignancies, including AML and CML, in a Turkish patient cohort.

## MATERIALS AND METHODS

### Sample

In this cross-sectional study, KIR gene distribution was investigated in patients with myeloid malignancy who were followed up at the Department of Hematology, Çukurova University Faculty of Medicine between 2016 and 2017. The institution is specialized in its field and all data is stored using the institution's secure electronic archive system and access permissions are restricted. Eighty unrelated Turkish individuals diagnosed with myeloid leukemia were included in the study. Of these, 34 had AML (18 males, 16 females) and 46 had CML (31 males, 15 females). The control group consisted of 100 healthy, unrelated Turkish volunteers (61 males, 39 females) matched to the patient group regarding age and sex. Patients diagnosed with myeloid leukemia (AML or CML) and who gave written informed consent were included in the study. Patients with another hematological disease, malignancy, and autoimmune disease were excluded from the study.

### Procedure

The study was approved by the Ethics Committee of Çukurova University Faculty of Medicine (Decision Date: 10.06.2016, Decision No: 26). All procedures were carried out in accordance with the ethical standards of the Declaration of Helsinki.

Written informed consent was obtained from all participants before the study. Data of the participants included in the study were obtained from medical record archives. Participants were first grouped as myeloid leukemia and healthy controls. Then, the patient group was grouped as AML and CML. The flow chart of the study is given in Figure-1.

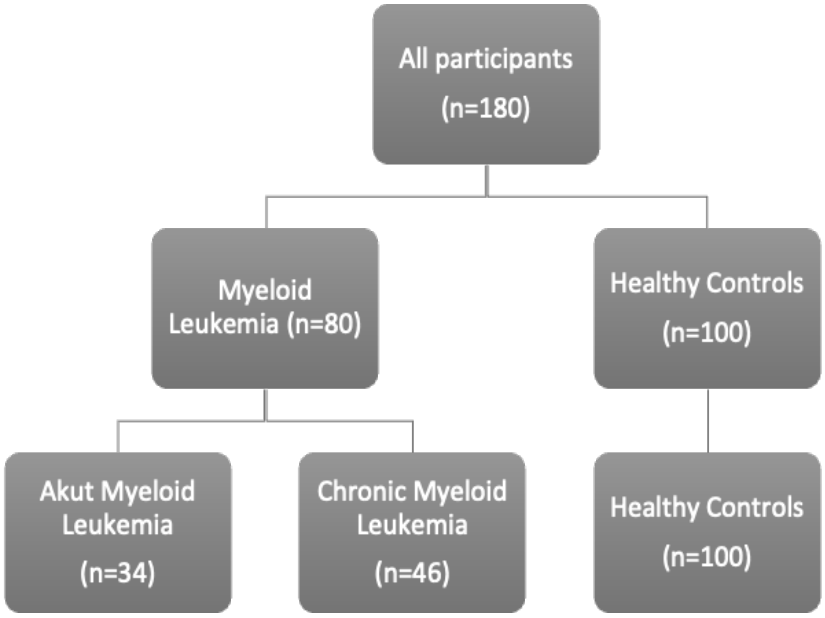


**Figure 1. Flow chart of the study**

### Sample collection and DNA isolation

Peripheral blood was collected from all patients and the control group into ethylenediaminetetraacetic acid (EDTA)-containing tubes for KIR genotyping. The samples were stored at 2-8°C until the day of study. Genomic deoxyribonucleic acid (DNA) was extracted from whole blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN) according to the manufacturer's instructions. Purified DNA samples were stored at -20°C until further analysis. Targeted DNA regions were amplified using two polymerase chain reaction (PCR). Sequence-specific oligonucleotides (SSOs) were used to detect KIR alleles in PCR-amplified DNA samples. The multiplex KIR PCR-SSO genotyping kit (Tepnel Lifecodes, Immucor, Inc., CT, USA) was used for this step. PCR products hybridized with the SSOs were analyzed on the Luminex platform to determine specific KIR gene profiles. Locus-specific oligonucleotide probes conjugated to color-coded microspheres (Luminex Corp., USA) were used for KIR genotyping. DNA isolation, PCR and genotyping procedures were performed by an experienced biologist at the Çukurova University Internal Medicine Rheumatology/Immunology Department Tissue Typing Laboratory.

### Killer cell immunoglobulin-like receptor genotyping

Allele frequency data for Group A and Group B haplotypes KIR were obtained from the Allele Frequency Net Database (*http://www.allelefrequencies.net*)[13]. While those with the characteristic gene content of Group A haplotypes KIR2DL1, 2DL3, 2DL4, 2DP1, 3DP1, 3DL1, 3DL2, 3DL3 and 2DS4 were evaluated as AA genotype, if any of the genes KIR2DL2, 2DL5, 2DS1, 2DS2, 2DS3, 2DS5 and 3DS1 were present, they were evaluated as haplotype B (genotype Bx)[13]. The genotype Bx is divided into AB and BB genotypes, with the presence of KIR2DS4, KIR3DL1, KIR2DL1, and KIR2DL3 all distinguishing the AB genotype from the BB genotype[7,14].

### Statistical analysis

Killer-cell immunoglobulin-like receptor genes were directly recorded for each individual. Statistical analysis was performed using IBM SPSS Statistics (version 21, Statistical Package for Social Sciences). Descriptive statistics for age are presented as median and range, as the variable did not show a normal distribution. The percentages of each KIR gene in the patient and control groups were determined by direct

counting (individuals positive for the gene per population/tested individuals × 100). The frequencies of each KIR genotypes were evaluated separately for all groups and given as n (%). The Pearson Chi-Square test was used to compare the frequency of KIR genes between the patient groups (AML/CML) and the control group. Fisher's exact test was applied to 2x2 contingency tables where the expected KIR gene cell frequencies were less than 5. In multiple comparisons of all KIR genes between all three groups, Bonferroni correction was applied to reduce the false positive (Type I error) rate in multiple comparisons. $P < \alpha'$ was considered significant; $\alpha' = 0.0167$ (0.05/3, where 3 was the number of multiple comparisons). $P < 0.05$ was considered statistically significant.

## RESULTS

The median age of the AML and CML cohort was 52 years (range, 21–77 years) and 56.5 years (range, 20–86 years), respectively. The median age of the control group of 100 unrelated healthy Turkish volunteers was 53 years (range 32-86 years) and 61% were male. The distribution of KIR gene frequencies in all groups is presented in Table 1. Four framework loci were positive in all patients, namelyKIR3DL3, KIR3DP1, KIR2DL4, and KIR3DL2. The frequencies of these genes in the control group were 99% for KIR3DL3, 100% for KIR3DP1, 98% for KIR2DL4, and 99% for KIR3DL2 (Table 1).

**Table 1. Comparison of KIR genes, and KIR genotypes in AML, CML patients and controls**

| KIR Genes | CML (n=46) (%) | AML (n=34) (%) | Myeloid Leukemia (n=80) (%) | Controls (n=100) (%) |
|---|---|---|---|---|
| Inhibitory | | | | |
| 2DL1 | 97.8 | 94.1 | 96.3 | 96 |
| 2DL2 | 34.8* | 50 | 41.3 | 52 |
| 2DL3 | 93.5** | 88.2 | 91.3 | 78 |
| 2DL4 | 100 | 100 | 100 | 98 |
| 2DL5 | 54.3 | 50 | 52.5 | 53 |
| 3DL1 | 91.3 | 91.2 | 91.3 | 94 |
| 3DL2 | 100 | 100 | 100 | 99 |
| 3DL3 | 100 | 100 | 100 | 99 |
| Activating | | | | |
| 2DS1 | 43.5 | 47.1 | 45 | 35 |
| 2DS2 | 34.8* | 52.9 | 42.5 | 52 |
| 2DS3 | 30.4 | 17.6 | 25 | 32 |
| 2DS4 | 91.3 | 91.2 | 91.3 | 95 |
| 2DS5 | 34.8 | 47.1 | 40 | 33 |
| 3DS1 | 45.7 | 41.2 | 43.8 | 39 |
| Pseudogene | | | | |
| 2DP1 | 97.8 | 94.1 | 96.3 | 96 |
| 3DP1 | 100 | 100 | 100 | 100 |
| KIR genotypes | | | | |
| AA | 34.8 | 38.2 | 36.3 | 30 |
| Bx | 65.2 | 61.8 | 63.8 | 70 |

KIR: Killer-cell immunoglobulin-like receptor, AML: Acute myeloid leukemia, KML: Chronic myeloid leukemia

* $P<0.05$ but are taken as non-significant after correction (Difference compared to controls).

** $P<\alpha'$ significant between the patient group and control after Bonferroni correction. $\alpha' = 0.017$

**Table 2. KIR genotype profiles in AML, CML patients and controls**

| Genotype ID | 3DL1 | 2DL1 | 2DL3 | 2DS4 | 2DL2 | 2DL5 | 3DS1 | 2DS1 | 2DS2 | 2DS3 | 2DS5 | 2DL4 | 3DL2 | 3DL3 | 2DP1 | 3DP1 | Control (n=100) n (%) | AML (n=34) n (%) | CML (n=46) n (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AA1 | ■ | ■ | ■ | ■ | | | | | | | | ■ | ■ | ■ | ■ | ■ | 28 (28) | 13 (38.2) | 16 (34.8) |
| AA180 | ■ | ■ | | ■ | | | | | | | | ■ | ■ | ■ | ■ | ■ | 1 (1) | 0 (0) | 0 (0) |
| AA407 | ■ | ■ | ■ | ■ | | | | | | | | ■ | ■ | | ■ | ■ | 1 (1) | 0 (0) | 0 (0) |
| Bx2 | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | | | ■ | ■ | ■ | ■ | ■ | ■ | 8 (8) | 1 (2.9) | 8 (17.4) |
| Bx3 | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | 2 (2) | 6 (17.7) | 3 (6.5) |
| Bx4 | ■ | ■ | ■ | ■ | ■ | | | | ■ | | | ■ | ■ | ■ | ■ | ■ | 11 (11) | 2 (5.9) | 2 (4.3) |
| Bx5 | ■ | ■ | ■ | ■ | ■ | ■ | | | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | 4 (4) | 1 (2.9) | 4 (8.7) |
| Bx6 | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 4 (4) | 2 (5.9) | 1 (2.2) |
| Bx7 | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | 5 (5) | 0 (0) | 2 (4.3) |
| Bx8 | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | | ■ | | ■ | ■ | ■ | ■ | ■ | 1 (1) | 0 (0) | 1 (2.2) |
| Bx9 | ■ | ■ | ■ | ■ | ■ | ■ | | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | 1 (1) | 1 (2.9) | 0 (0) |
| Bx10 | ■ | ■ | ■ | ■ | | | | | ■ | | | ■ | ■ | ■ | ■ | ■ | 0 (0) | 1 (2.9) | 0 (0) |
| Bx13 | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | 1 (1) | 0 (0) | 0 (0) |
| Bx14 | ■ | ■ | ■ | ■ | | | ■ | | | | | ■ | ■ | ■ | ■ | ■ | 0 (0) | 0 (0) | 1 (2.2) |
| Bx15 | ■ | ■ | ■ | ■ | | | | ■ | | | | ■ | ■ | ■ | ■ | ■ | 0 (0) | 0 (0) | 1 (2.2) |
| Bx17 | ■ | ■ | ■ | ■ | | ■ | ■ | | | | ■ | ■ | ■ | ■ | ■ | ■ | 2 (2) | 0 (0) | 0 (0) |
| Bx27 | ■ | ■ | ■ | ■ | | ■ | ■ | | | ■ | | ■ | ■ | ■ | ■ | ■ | 2 (2) | 0 (0) | 1 (2.2) |
| Bx68 | | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | 1 (1) | 0 (0) | 0 (0) |
| Bx69 | | ■ | ■ | | | ■ | ■ | ■ | | | ■ | ■ | ■ | ■ | ■ | ■ | 2 (2) | 2 (5.9) | 0 (0) |
| Bx70 | | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 1 (1) | 1 (2.9) | 1 (2.2) |
| Bx71 | ■ | ■ | | ■ | ■ | ■ | | | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | 7 (7) | 0 (0) | 1 (2.2) |
| Bx72 | ■ | | | ■ | ■ | | | | ■ | | | ■ | ■ | ■ | | ■ | 1 (1) | 1 (2.9) | 1 (2.2) |
| Bx73 | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 3 (3) | 2 (5.9) | 0 (0) |
| Bx75 | | ■ | ■ | | | ■ | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 0 (0) | 0 (0) | 2 (4.3) |
| Bx81 | | ■ | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 0 (0) | 0 (0) | 1 (2.2) |
| Bx89 | ■ | ■ | | ■ | ■ | | | | ■ | | | ■ | ■ | ■ | ■ | ■ | 1 (1) | 0 (0) | 0 (0) |
| Bx90 | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | 1 (1) | 0 (0) | 0 (0) |
| Bx91 | ■ | ■ | | ■ | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 1 (1) | 0 (0) | 0 (0) |
| Bx106 | ■ | | | ■ | ■ | ■ | | ■ | ■ | ■ | | ■ | ■ | ■ | | ■ | 1 (1) | 1 (2.9) | 0 (0) |
| Bx118 | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | 1 (1) | 0 (0) | 0 (0) |
| Bx200 | ■ | ■ | ■ | ■ | | ■ | | | | | | ■ | ■ | ■ | ■ | ■ | 1 (1) | 0 (0) | 0 (0) |
| Bx400 | ■ | ■ | ■ | ■ | ■ | | ■ | | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | 2 (2) | 0 (0) | 0 (0) |
| Bx401 | ■ | ■ | | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 1 (1) | 0 (0) | 0 (0) |
| Bx403 | ■ | ■ | | ■ | | | | ■ | | | | ■ | ■ | ■ | ■ | ■ | 1 (1) | 0 (0) | 0 (0) |
| Bx404 | | ■ | | | ■ | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 1 (1) | 0 (0) | 0 (0) |
| Bx405 | | ■ | ■ | | | ■ | ■ | ■ | | | ■ | ■ | | ■ | ■ | ■ | 1 (1) | 0 (0) | 0 (0) |
| Bx406 | ■ | | | ■ | ■ | | | | ■ | | | | ■ | ■ | | ■ | 1 (1) | 0 (0) | 0 (0) |
| Bx408 | ■ | | | ■ | ■ | ■ | | ■ | ■ | | ■ | | ■ | ■ | | ■ | 1 (1) | 0 (0) | 0 (0) |

KIR: Killer-cell immunoglobulin-like receptors, AML: Acute myeloid leukemia, CML: Chronic myeloid leukemia

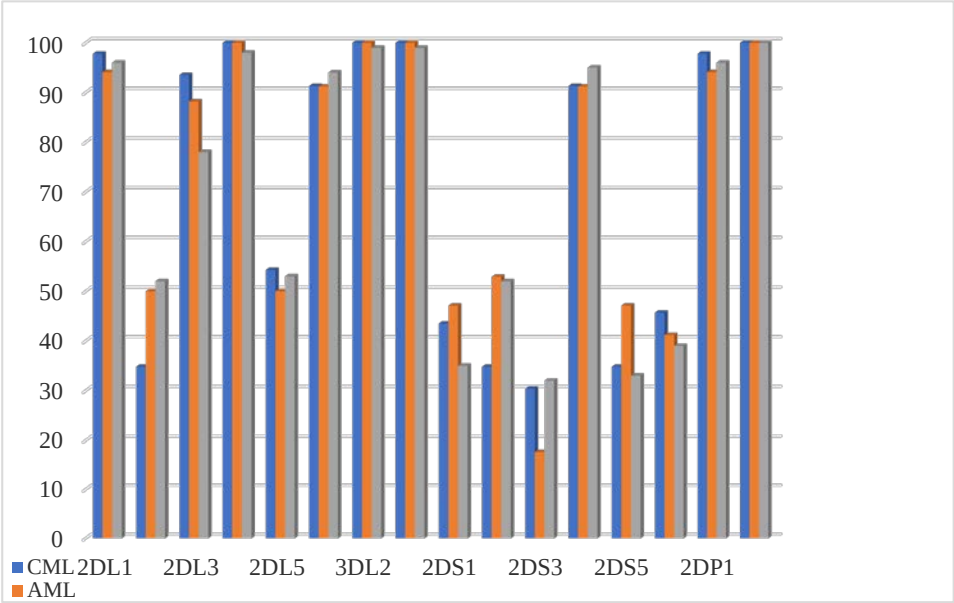


**Figure 2. KIR genotype profiles in AML, CML patients and controls**

KIR3DP1 was present in all the patients and controls. The frequency of inhibitor KIR2DL3 was significantly higher in all of the leukemia patients compared to controls (*P*=0.016), but these data were not statistically different in the AML group compared with the healthy control group, probably due to the small sample size. The frequency of KIR2DL3 was significantly higher in CML patients compared with controls (93.5% vs 78%, *P*=0.021), which was still significant according to the correctedBonferroni *P* value. In comparison, the frequencies of KIR2DL2 (34.8% vs 52%, *P*=0.053) and KIR2DS2 (34.8% vs 52%, *p*=0.053) were lower in these patients than in controls, but the statistical difference disappeared after *P* correction (*P* >α'). Although KIR2DS3 was more common in control group than in AML patients (32% vs. 17.6%), this difference was insignificant. The graphical representation of KIR gene frequencies in study groups is shown in Figure 2.

We identified a total of 21 different KIR genotypes in leukemia patients (16 and 13 KIR genotypes in CML and AML patients, respectively) and 33 genotypes in controls. No statistically significant difference was found in the frequency of AA and Bx genotypes in the AML, CML, and control groups (P>0.05). Haplotype Bx was the most common genotype with rates of 61.8%, 65.2%, and 70% in AML, CML patients, and controls, respectively. Additionally, 38.2% of AML patients, 34.8% of CML patients and 30% of the control group had the AA genotype (AA1), with the only activating gene being KIR2DS4. Three leukemia patients (2 AML, 1 CML) had the Bx6 genotypecontaining all 16 KIR genes. The frequencies of Bx genotypes were highly variable in patients and healthy controls ranging from 1% to 17.7%. The frequencies of AA, AB, and BB genotype were 36.3%, 47.5%, and 16.3%, respectively. Seven and 9 genotypes were observed only once in AML and CML patients, respectively (Table 2).

## DISCUSSION

In this study, we shared the KIR gene repertoire and genotypes in myeloid leukemias and also compared them with the healthy group. When leukemia patients were compared with healthy controls, the inhibitory KIR2DL3 allele was observed to be significantly more frequent in the leukemia group, and this difference was found to be more pronounced in CML patients. KIRs expressed in NK cells have an essential role in the function of NK cells [15]. These receptors are genetically highly polymorphic, as are their equally polymorphic ligands, termed MHC class

1 molecules. The possible role of NK cell dysfunction as an underlying mechanism in the development of hematological malignancies has gained particular interest following the emergence of data demonstratingthe alloreactivity of NK cells via KIRs against AML in the setting of allogeneic hematopoietic transplantation[16-18]. NK receptor mismatch in the graft-versus-host orientation was found to be associated with lower leukemia relapse rate and graft-versus-host disease[19]. A previous in vitro study has also demonstrated that cord blood-derived KIR+NK cells effectively inhibited acute lymphoblastic leukemia (ALL) and AML cell lines by inducing the expansion and differentiation of KIR+NK cells using cytokines, thereby restoring NK cell function[15]. KIR2DL3 is among the major NK cell inhibitory receptors in the KIR family. Among *human leukocyte antigen* (HLA) ligands, HLA-C1 serves as an epitope for KIR2DL2/DS2 and KIR2DL3/DS3[20] KIR2DL2 and KIR2DL3 bind to HLA-C1 ligands to deliver inhibitory signals[21]. There is a weak interaction between C1 and KIR2DL2 or KIR2DL3. The net effect of KIR2DL3 on NK function is inhibitory, which is suggested to be protective[6]. A recent study investigating arsenic-induced carcinogenesis demonstrated suppression of interleukin (IL)-2-activated cytotoxic activity of NK cells by aresenic, which impairs recognition and killing of target tumor cells through multiple pathways, including increased expression of the inhibitory receptors KIR2DL2 and KIR2DL3[22]. Accordingly, high KIR2DL3 gene frequencies in our patients may have impaired the elimination of leukemic cells by inhibiting NK activity. An Italian study, found that increased frequency of the activating receptor KIR2DS1 and a decreased frequency of the KIR-ligand combination KIR2DS2/2DL2 were significant in patients with CML[8]. On the other hand, the absence of the inhibitory KIR2DL2 and its activating counterpart KIR2DS2 have been reported as risk factors for acute leukemias in several studies[20,23,24]. A recent study including an in-depth KIR genotype analysis revealed that in AML patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT), protection against relapse was dependent on donor having the inhibitor KIR2DL2 and/or activating KIR2DS2, and was enhanced by the donor lacking inhibitors KIR2DL1, KIR2DL3 and KIR3DL1[24].

There was a trend towards lower allelic frequencies of KIR2DS2 and KIR2DL2 in our CML cohort. These data, consistent with previous leukemia studies, may indicate increased susceptibility to myeloid leukemias in the absence of KIR2DL2/DS2. Expression of activating KIRs, particularly KIR2DS2, has been associated with reduced relapses after transplantation in haematologic malignancies and has been suggested to be protective, particularly in childhood ALL[25]. However, another study of Belgian Caucasians with leukemia reported an increased frequency of KIR2DL2 compared to healthy individuals[26]. The heterogenous findings from these preliminary studies clearly indicate the need for further studies involving the regulation of NK cell function in leukemias. A case-control study in Turkish leukemia patients indicatedthat KIR3DL2(+)/HLA-A3/11(-) behaved as a myeloid leukemia-associated genotype, most likely reflectedby the lack of NK cell activity mediated by this inhibitory receptor[7]. The frequency of KIR3DL2 was similar in this cohort to our leukemia patients and controls. In line with these observations, our results suggest that cumulative effects of KIR signaling may modulate the function of NK cells in myeloid leukemias. The rate of activating KIR2DS4 was found to be much higher in CML patients than in healthy controls, suggesting that it may serve as a CML susceptibility gene that triggers the development of leukemia[27], but the opposite was observed in our study, although not statistically significant. Activating KIR2DS3 has been shown to be protective in patients with ALL, AML and CML[23]. The decreased frequency of KIR2DS3 in myeloid leukemias may indirectly support its protective effect. In our study, there was a trend for lower prevalance for KIR2DS3 in AML patients than in healthy controls (17.6% vs. 32%), but the difference was not statistically significant. An association between KIR3DS1 and reduced risk of AML has been shown[28], but there are also studies, including our study, that show no significant association between KIR3DS1 and AML[23].Contrary to the view that KIR genes are associated with other hematological diseases including AML[11,26-28], there is one study that suggests that individual KIR genotype does not constitute a risk factor for the development of AML. This has been attributed to small sample size, heterogeneous patient groups, and/or lack of *P*-value correction[12]. We also observed differences in KIR genes in AML patients compared to controls, although insignificant. We attribute this result to our small sample size. However, despite the small number of patients, only the increase in KIR2DL3 freguency in CML patients was still significant after Bonferroni *P* value correction. It is well known that

genotype-phenotype correlations in KIR genes also differ at the population level, and this is a confounding factor when comparing KIR frequencies across ethnic groups[29]. The frequencies of KIR genes and phenotypes observed in our control population were not significantly different from the frequencies previously reported in other studies of individuals with similar genetic backgrounds. In a population genetic study of 200 healthy individuals from southern Türkiye, the frequencies of A and Bx haplotypes were reported as 50.5% and 49.5%, respectively[30]. In our study, a lower prevalence of group A haplotypes (AA) was observed among the reported KIR haplotypes, the frequency of group A was quite close to our in CML (34.8%), AML (38.2%), and our healthy controls (30%). In general, activating KIR genes are encoded more in group B haplotypes than in group A haplotypes. In a study conducted in Turkish AML (n=54) and CML (n=52) patients and examining KIR gene frequencies, three genotypes were identified as AA, BB, and AB[7]. For comparison, the AB and BB genotypes, referred to as the KIR genotype Bx used in our study, were reported as 70.37% and 61.54% in AML and CML patients, respectively. This supports the dominance of Bx haplotype in a similar cohort with the same genetic background[7]. The distribution of the main genotypes AA and Bx was comparable to our results. In-depth analysis of the KIR gene content in our patients revealed the dominance of the AB haplotype in the Bx repertoire. The prevalance of the AB haplotype was similar for healthy controls in both studies[7]. Considering the allele frequencies within each haplotype, these results suggest the dominance of the inhibitory genotype in our patients. Similarly, a study in the literature reported an increased risk of leukemia with KIR genotypes associated with inhibitory KIR[26].Another interesting study showed a association between KIR genotypes and treatment outcomes in phase-chronic (CP)-CML patients[31]. In this study, an association between the presence of KIR2DL5B and low moleculer reponse, as well as loe transformation-free survival and event-free survival, was reported in CML patients[31]. In a report evaluating treatment response in CML patients with KIR gene profiling, it was stated that the presence of KIR2DS2/KIRSDL2 was associated with lower molecular response in 12 months of tyrosine kinase inhibitor therapy[32]. However, data from other groups showed different results in CML patients treated with imatinib or dasatinib[9,10,31]. In our study, we cannot providedetailed results demonstrating the relationship between treatment response and KIR genes due to the small number of patients for both CML and AML and the existenceof different immunophenotypes, especially in AML.The first and most important limitation of our study is that, since it is a single-center study, leukemias with different clinical and biological features were included and the number of patients in each group was limited. Therefore, comparison of variable parameters such as KIR genotypes between leukemia subgroups and healthy controls could not be made. Our second limitation is that we cannot provide detailed information on treatment responses with KIR genes**.** In conclusion, this study suggests that a functional NK cell repertoire shaped by the diversity and distribution of KIR genes may influence the pathogenesis of myeloid leukemias such as AML and CML. A more comprehensive understanding of KIR-HLA interactions and phenotypic characterization of NK cell receptors may help elucidate immune mechanisms in leukemia. Future studies should focus on larger, ethnically diverse cohorts to confirm the observed associations and clarify population-specific KIR profiles. These studies may ultimately guide the development of personalized, NK cell-based therapies and improve clinical outcomes in myeloid leukemia patients.

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## REFERENCES

1. Kulkarni S, Martin MP, Carrington M. The yin and yang of HLA and KIR in human disease. Semin Immunol. 2008;20:343-52.
2. Agnello L, Masucci A, Tamburello M, Facciotti F, Santoni A, Moretta L et al. The role of killer Ig-like receptors in diseases from A to Z. Int J Mol Sci. 2025;26:3242.
3. Purdy AK, Campbell KS. Natural killer cells and cancer: regulation by the killer cell Ig-like receptors (KIR). Cancer Biol Ther. 2009;8:2211-20.

4. Zitvogel L, Apetoh L, Ghiringhelli F, Smyth MJ, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol. 2008;8:59-73.
5. Lotzova E, Savary CA, Herberman RB. Inhibition of clonogenic growth of fresh leukemia cells by unstimulated and IL-2 stimulated NK cells of normal donors. Leuk Res. 1987;11:1059-66.
6. Ruggeri L, Capanni M, Casucci M, Aversa F, Moretta L, Moretta A et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood. 1999;94:333-9.
7. Middleton D, Diler AS, Meenagh A, Farrell L, Curran MD, Barnardo MC et al. Killer immunoglobulin-like receptors (KIR2DL2 and/or KIR2DS2) in presence of their ligand (HLA-C1 group) protect against chronic myeloid leukaemia. Tissue Antigens. 2009;73:553-60.
8. Varbanova VP, Mihailova S, Naumova E, Pavlova E, Ivanova M, Kostova E et al. Certain killer immunoglobulin-like receptor (KIR)/KIR HLA class I ligand genotypes influence natural killer antitumor activity in myelogenous leukemia but not in acute lymphoblastic leukemia: a case control leukemia association study. Turk J Haematol. 2019;36:238-46.
9. Ali S, Sergeant R, O'Brien SG, Goldman JM, Melo JV, Apperley JF et al. Dasatinib may overcome the negative prognostic impact of KIR2DS1 in newly diagnosed patients with chronic myeloid leukemia. Blood. 2012;120:697-8.
10. Marin D, Gabriel IH, Ahmad S, Mahmud N, Agrawal V, Rea D et al. KIR2DS1 genotype predicts for complete cytogenetic response and survival in newly diagnosed chronic myeloid leukemia patients treated with imatinib. Leukemia. 2012;26:296-302.
11. Alavianmehr A, Mansouri M, Ramzi M, Ghavami S, Shirazi F, Farsinejad A et al. Association of killer-cell immunoglobulin-like receptor genes with acute myelogenous leukaemia. Int J Immunogenet. 2020;47:512-21.
12. Heidenreich F, Falk B, Baldauf H, von Bonin M, Witzens-Harig M, Heuser M et al. Large case-control study indicates no association of KIR genotype and risk of developing acute myeloid leukemia. Blood Adv. 2023;7:2994-3004.
13. Middleton D, Menchaca L, Rood H, Komerofsky R, Mao M, Vierra-Green C et al. New allele frequency database: http://www.allelefrequencies.net. Tissue Antigens. 2003;61:403-7.
14. Jiang M, Guo X, Yuan L, Xu Y, Han M, Zhang Z et al. Killer cell immunoglobulin-like receptor gene cluster predisposes to susceptibility to B-cell acute lymphoblastic leukemia in Chinese children. Int J Clin Exp Pathol. 2020;13:536-42.
15. Dizaji Asl K, Rafat A, Mazloumi Z, Ghaderi A, Soleimani M, Dalirfardouei R et al. Cord blood stem cell-generated KIR(+)NK cells effectively target leukemia cell lines. Hum Immunol. 2023;84:98-105.
16. Debska-Zielkowska J, Moszkowska G, Zielinski M, Baranska M, Bednarz-Knoll N, Tafil-Klawe M et al. KIR receptors as key regulators of NK cells activity in health and disease. Cells. 2021;10:1777.
17. Makanga DR, Jullien M, David G, Damoiseaux J, Six A, Schleinitz N et al. Low number of KIR ligands in lymphoma patients favors a good rituximab-dependent NK cell response. Oncoimmunology. 2021;10:1936392..
18. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;295:2097-100.
19. Schellekens J, Gagne K, Marsh SG. Natural killer cells and killer-cell immunoglobulin-like receptor polymorphisms: their role in hematopoietic stem cell transplantation. Methods Mol Biol. 2014;1109:139-58.
20. Misra MK, Prakash S, Moulik NR, Kumar S, Singh M, Agarwal S et al. Genetic associations of killer immunoglobulin like receptors and class I human leukocyte antigens on childhood acute lymphoblastic leukemia among north Indians. Hum Immunol. 2016;77:41-6.
21. Parham P. MHC class I molecules and KIRs in human history, health and survival. Nat Rev Immunol. 2005;5:201-14.
22. La Nasa G, Caocci G, Littera R, Campus F, Usai P, Carcassi C et al. Homozygosity for killer immunoglobin-like receptor haplotype A predicts complete molecular response to treatment with tyrosine kinase inhibitors in chronic myeloid leukemia patients. Exp Hematol. 2013;41:424-31.
23. Varbanova V, Naumova E, Mihaylova A. Killer-cell immunoglobulin-like receptor genes and ligands and their role in hematologic malignancies. Cancer Immunol Immunother. 2016;65:427-40.
24. Guethlein LA, Beyzaie N, Nemat-Gorgani N, Horowitz A, Trachtenberg EA, Parham P et al. Following transplantation for acute myelogenous leukemia, donor KIR Cen B02 better protects against relapse than KIR Cen B01. J Immunol. 2021;206:3064-72.
25. Blunt MD, Vallejo Pulido A, Fisher JG, Peppa D, Frazier S, Costantini S et al. KIR2DS2 expression identifies NK cells with enhanced anticancer activity. J Immunol. 2022;209:379-90.
26. Verheyden S, Bernier M, Demanet C. Identification of natural killer cell receptor phenotypes associated with leukemia. Leukemia. 2004;18:2002-7.
27. Zhang Y, Wang B, Ye S, Gao X, Tang K, Wang J et al. Killer cell immunoglobulin-like receptor gene polymorphisms in patients with leukemia: possible association with susceptibility to the disease. Leuk Res. 2010;34:55-8.
28. Shahsavar F, Tajik N, Entezami KZ, Rezaei N, Khosravi A, Shokri F et al. KIR2DS3 is associated

with protection against acute myeloid leukemia. Iran J Immunol. 2010;7:8-17.
29. Middleton D, Gonzelez F. The extensive polymorphism of KIR genes. Immunology. 2010;129:8-19.
30. Ozturk OG, Polat G, Atik U. Diversity of killer cell immunoglobulin-like receptor genes in Southern Turkey. Mol Biol Rep. 2012;39:1989-95.
31. Yeung DT, Tang C, Vidovic L, Ku E, Hiyama K, Kroger N et al. KIR2DL5B genotype predicts outcomes in CML patients treated with response-directed sequential imatinib/nilotinib strategy. Blood. 2015;126:2720-3.
32. Kreutzman A, Jaatinen T, Greco D, Syrjälä K, Rajala H, Partanen J et al. Killer-cell immunoglobulin-like receptor gene profile predicts good molecular response to dasatinib therapy in chronic myeloid leukemia. Exp Hematol. 2012;40:906-13.e1.