The association of KIR locus with breast cancer risk in Kermanshahi women population

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Abstract

Killer cell immunoglobulin-like receptors (KIRs) regulate the antitumor effect of Natural killer cells. This study aims to compare the frequency of KIR genes distribution in women with breast cancer and the control group in Kermanshah province. This study was performed on 53 women with BC and 37 healthy women. The KIR gene content was determined by polymerase chain reaction with sequence-specific primers (PCR-SSP). The frequency of the KIR-2DL5B gene was significantly different between the two groups (P: 0.037), and this locus increases the risk of disease (OR: 2.491). The following results were associated with breast cancer risk: the cBx-tAtA distribution (OR: 5.122), the B content score 1 (OR: 5.122), tA01|tA01 (OR: 5.122) and inversely the following results were associated with protection: the cBx-tBx distribution (OR: 0.176), the B content score 2 (OR: 0.176), cA01|cB0X (OR: 0.287) and tA01|tB0X (OR: 0.301). These results suggested that KIR-2DL5B, the cBx-tAtA, the B content score 1 and tA01|tA01 were associated with increased susceptibility while the cBx-tBx, the B content score 2, cA01|cB0X, and tA01|tB0X were associated with protection for BC in kermanshahi women population.

1. Introduction

Despite the varying incidence of breast cancer in different regions of the world, breast cancer is the most common type of cancer among all women worldwide. According to the International agency for cancer research (IARC), breast cancer is responsible for 25% of all cases of cancer diagnosed in women worldwide. The incidence rate of breast cancer is increasing with the highest in the United States. According to statistics provided by the Iranian Cancer Institute, breast cancer is the most common type of cancer in the Iranian women population, with 25% of all cancer cases in this population. In Iran, the incidence rate of breast cancer varies across geographic regions, and central provinces of Iran, such as Tehran, and Isfahan have the highest incidence rate. Generally, breast cancer risk factors are divided into two categories: genetic and non-genetic factors. The non-genetic risk factors include age, lifestyle, early menarche, late menopause, family history, weight, smoking, diet, socioeconomic condition, air pollution, and delayed pregnancy, decreased period of breastfeeding, smoking before the menopause and alcohol consumption. Among genetic risk factors, germ-line mutations in high-penetrance breast cancer susceptibility genes such as Breast Cancer gene1 (BRCA), BRCA2, p53 and phosphatase and tensin homolog (PTEN) accounts for 5–10% of all breast cancers, mutation in low-penetrance genes involved in DNA repair and cell cycle Checkpoints such as ataxia telangiectasia mutated (ATM), BRCA1 interacting protein (BRIP1), checkpoint kinase 2 (CHEK2), Nibrin (NBN) (previously known as NBS1), partner and localizer of BRCA2 (PALB2), and RAD50 which they increase the risk of breast cancer by 2 to 4 times, and also single nucleotide polymorphisms (SNPs) in genes: trinucleotide-repeat-containing 9 (TNRC9), fibroblast growth factor receptor2 (FGFR2), mitogen-activated protein kinase kinase kinase1 (MAP3K1), H19 and lymphocyte-specific protein 1 (LSP1) which are associated with increased susceptibility to breast cancer. The identification of genetic risk factors for breast cancer is an ongoing endeavour so that through Genome-wide association studies (GWAS), more than 170 genomic loci harboring common variants associated with breast cancer risk have been identified. Information on cancer-susceptibility
genes may help in improving the prevention, early detection, and treatment of some cancers,\textsuperscript{11} including breast cancer.\textsuperscript{12}

Of the genes that association between their polymorphisms with susceptibility to breast cancer has been investigated in a limited number of studies are KIR genes.\textsuperscript{9,13,14} These genes are located on 19q13.4 chromosome,\textsuperscript{15} and so far, 17 genes and pseudogenes have been identified.\textsuperscript{16} The KIR3DS1, KIR2DS1–5 genes, encode activatory receptors, KIR3DL1–3, KIR2DL1–3, KIR2DL5A-2DL5B codes for inhibitory receptors, KIR2DL4 code for a receptor with both activatory and inhibitory function, and KIR2DP1 and KIR3DP1 are pseudogenes, which do not encode cell-surface receptors.\textsuperscript{17} The KIRs are receptors that expressed on Natural killer (NK) cells, and they are also present on subsets of T cells (as co-receptors).\textsuperscript{18} NK cells, as 10–15\% of circulating lymphocytes,\textsuperscript{14} form the primary line of defense against malignant cells.\textsuperscript{19} The killing activity of NK cells is mediated by a series of transmembrane receptors from several different families.\textsuperscript{14} Among the NK cells receptors, KIRs are the critical regulators of their activities.\textsuperscript{18} The nomenclature of KIRs is based on the number of their extracellular Ig-like domains (2D or 3D) and by the length of their cytoplasmic tail (long (L), short (S), or pseudogene (P)).\textsuperscript{16} So far ligands for most KIRs have been recognized to a certain extent.\textsuperscript{20} Specific patterns of the HLA class I molecules are ligands for most KIRs,\textsuperscript{16} and HLA-C and HLA-B are well-recognized ligands for inhibitory KIR receptors.\textsuperscript{20} Recognition of HLA class I molecules on target cells by inhibitory KIRs on NK cells regulates NK cell functions and kept NK cell tolerant and unresponsiveness to healthy tissues. On the contrary, the target cells which downregulate HLA-I molecules at their surface, like most tumorous cells, are susceptible to attack by NK cells.\textsuperscript{21–25} Concerning the gene content, two haplotypes (A and B) and genotypes (AA and Bx, where x can be A or B) have been identified for KIR.\textsuperscript{26} Framework genes (KIR2DL4, KIR3DL2, KIR3DL3, and KIR3DP1) are present in both A and B haplotypes. Gene content of A haplotype consists of eight genes, including KIR2DL1, KIR2DL3, KIR2DS4 and KIR3DL1 in addition to framework genes and gene content of B haplotype consist of activating KIR genes, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, and KIR3DS1, as well as the inhibitory KIR genes, KIR2DL5A/B and KIR2DL2 in addition to framework genes.\textsuperscript{27} One of the characteristics of the family of KIR receptors, especially the inhibitory KIRs, is the presence of allelic polymorphisms (high numbers of variants) and haplotypes’ variation (different numbers of gene loci for inhibitory and activating receptors on individual chromosomes).\textsuperscript{28} Great heterogeneity in the number and type of KIR genes is observed within human populations,\textsuperscript{29} and the distribution of KIR haplotypes varies among different ethnic groups.\textsuperscript{30–33} KIR haplotypes comprise centromeric and telomeric regions, the centromeric region from 3DL3 to 3DP1, and the telomeric region from 2DL4 to 3DL2; and depending on the haplotype both regions can be cenA or cenB, and telA or telB. The 2DL5, 2DS3, and 2DS5 genes have been identified in centromeric and/or telomeric regions. According to gene content 9 centromeric regions (cA01, cA02, cA03, cB01, cB02, cB03, cB04, cB05, and cB06) and 8 telomeric regions (tA01, tB01, tB02, tB03, tB04, tB05, tB06, and tB07) have been described.\textsuperscript{34–37} KIR B haplotype can also be classified according to B content genes, and the B content score is calculated by adding the number of cenB and/or telB motifs in each genotype.\textsuperscript{38} Association between KIR genes polymorphisms with susceptibility to
breast cancer has been investigated in a limited number of studies.\textsuperscript{9,13,14} Regarding the importance of KIR receptors in the anticancer function of NK cells, this study aims to compare the frequency of KIR genes distribution, KIR B score, and the centromeric and telomeric distribution of KIR in women with breast cancer.

2. Materials And Methods

2.1. Study population

We analyzed 53 Kermanshahi women with breast cancer from the oncology unit of Imam Reza Hospital and 37 healthy female individuals who randomly selected from the Kermanshah population without any family history of hereditary and autoimmune diseases or malignancies. The mean age of patients and controls was 48.98 $\pm$ 11.15 (mean $\pm$ SD) years and 46.21 $\pm$ 14.25 years, respectively. The groups were matched for age and geography. Informed consent was obtained orally from individuals and, our survey was conducted under the approval of the Ethics Committee of Kermanshah University of Medical Sciences.

2.2. DNA extraction

5 cc of venous blood was collected from all subjects in CBC tubes containing ethylene diamine tetraacetic acid (EDTA) anticoagulants. Using the standard salting-out method, genomic DNA was extracted from blood samples. The quality and purity of DNA samples were evaluated by NanoDrop (Thermo Scientific/2000C). DNA samples were stored at -20$^\circ$C for KIR genotyping.

2.3. KIR genotyping

KIR genotyping was performed with PCR-SSP commercial kit (Olerup SSP AB, KIR Genotyping Kit, Sweden, CareDx Company) according to the manufacturer's instructions. This kit enables the detection of 2DL1, 2DL2, 2DL3, 2DL4, 2DL5A, 2DL5B, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1 and 3DP1 genes. Test interpretation was performed according to the worksheet provided in the kit.

2.4. Statistical analysis

We used IBM™ SPSS software version 16 for statistical analysis. For each individual, the percentage of each KIR gene was determined by direct counting (individuals positive for the gene/individuals tested per population $\times 100$). A chi-square test was used to compare the frequency of KIR genes and genotypes between the two groups. The relationship between KIR genes and genotypes with susceptibility to breast cancer and calculation of Odds ratios (ORs) with a 95\% Confidence Interval (CIs) has been studied through logistic regression analysis. In all analysis P value < 0.05 were considered as significant.

3. Results

3.1. Distribution of KIR gene loci in patients with breast cancer and control group
The frequency of KIR genes and pseudo-genes were calculated for all individuals and compared between patient and control group (the results are presented in Table 1). The framework genes, 2DL4 (centromeric framework gene) and 3DP1 (telomeric framework gene), and also the 2DS4 gene (activating gene) and 2DP1 (pseudogene) were present in 100% of the two groups studied. The 2DL1-L3, 3DL2, and 3DL3 genes were present in 100% of the control group individuals and, therefore, for these genes, odds ratio and confidence interval were not calculated. The frequency of the KIR-2DL5B gene was significantly higher in the patient's group (P-value: 0.037), and this gene was associated with risk for breast cancer (OR: 2.491) and was statistically significant (P-value: 0.039). There was no significant difference between the frequencies of other KIR gene loci in the two studied groups.

3.2 KIR haplotypes

Based on gene content, the genotypes were grouped as A or B. In this study we were examined the association between genotypes with breast cancer. No significant association was found between genotypes and breast cancer (Table 2). Due to the different gene content of the centromere and telomeric regions, we evaluated genotypes AA and Bx based on their distribution. These evaluations showed that: the cBx-tAtA distribution was significantly associated with risk (p-value: 0.026, OR: 5.122, CI: 1.073-24.458) and cBx-tBx with protection (p-value: 0.017, OR: 0.176, CI: 0.037-0.834) for breast cancer. In the cAcA-tAtA distribution, there was no significant difference between the two groups (Table 2).

3.3 KIR B score

In the analyses of the B content score, score 1 was associated with risk (p-value: 0.026, OR: 5.122, CI: 1.073-24.458), whereas score 2 was associated with protection for breast cancer (p-value: 0.017, OR: 0.176, CI: 0.037-0.834). Definition of B score: the number of cB and/or tB motifs in each genotype. No significant association was found between other scores and breast cancer (Table 3).

3.4 The centromeric and telomeric distribution of KIR

For further analyses for the association between gene content of centromere and telomere region with breast cancer, the different combinations that have been reported were examined. The numbers were according to gene content. These examinations were indicative of the association between tA01|tA01 with risk for breast cancer (p-value: 0.026, OR: 5.122, CI: 1.073-24.458) and association between cA01|cB0X and tA01|tB0X with protection for breast cancer (p-value, OR and CI respectively for each: 0.005, 0.287, 0.119-0.693 and 0.007, 0.301, 0.124-0.727). No significant association was found between other centromeric and telomeric distribution and breast cancer (Table 4).

4. Discussion

In this study, we found the association between KIR gene family and breast cancer susceptibility in various aspects, including the presence of gene loci, centromere-telomeric regions, and B score. The KIR-2DL5B gene was significantly higher in the patient's group (P-value: 0.037), and this gene had an
association with risk for breast cancer (OR: 2.491). KIR2DL5B encodes an inhibitory receptor that its ligands have not yet been identified. The previous study showed that the NK-mediated killing of leukemia has an inverse association with KIR2DL5B presence.\textsuperscript{11} The higher frequency of the inhibitory allele in breast cancer patients might comprise cell activation and the eradication of transformed cells.\textsuperscript{9} Regarding the extraordinary diversity of the KIR system, its contribution to cancer development in the different populations remains to be firmly established. In the Turkish population, a positive association between the KIR2DS1 gene with breast cancer and a protective effect associated with KIR2DL1, and the allelic variant of KIR2DS4 was reported.\textsuperscript{13} In the other study, which was conducted in the Brazilian population, the frequency of inhibitory KIR2DL2 receptors was significantly higher in breast cancer patients also, KIR2DL2 concomitant with the HLA-C heterozygote ligand (C1/C2) was associated with increased breast cancer susceptibility.\textsuperscript{9} The results of another study in Saudi Arabia showed that KIR2DS2, 2DS3, and 2DL5A genes had a protective effect against breast cancer, for these genes synergic action was observed when occurred together, and protective effects of KIR2DL2 and 2DL3 in the absence of their HLA-C1 ligand were observed in this study.\textsuperscript{14}

Similar to our finding, The association between inhibitory KIR genes with various solid tumor, has been reported in previous studies, these include, a positive association between KIR3DL1 and risk for getting basal cell carcinoma,\textsuperscript{39} the lower frequency of KIR-2DL1,\textsuperscript{13} and higher frequency of KIR-2DL2 gene in breast cancer,\textsuperscript{9} the increased frequency of 3DL1 and its ligand HLA-Bw4 in the development of kidney cancer as well as the higher frequency of KIR-2DL1 and its ligand HLA- C2, in non–small-cell lung cancer patients.\textsuperscript{40}

The association of 2DL5 locus with some cancers has been identified in a number of studies, including protective effect of KIR-2DL5A along with 2DS2 and 2DS3 against breast cancer,\textsuperscript{14} susceptive effect of KIR-2DL5 along with 2DS1, 2DS3, 2DS5, 3DS1 for gastric cancer,\textsuperscript{15} the lower frequencies of KIR-2DL5A and 3DS1 genes along with the higher frequency of KIR2DS4*001 in myelogenic leukemia patients,\textsuperscript{16} susceptive effect of KIR-2DL5 gene along with 2DS1, 2DS5, 3DS1, 2DS4fl genes in colorectal adenocarcinoma,\textsuperscript{41} the lower frequencies of KIR-2DL5, 2DL2, 2DS1, 2SD2 and 2DS3 genes in patients with hematopoietic disorders\textsuperscript{42} and inverse association between KIR2DL5B*002 gene and cervical intraepithelial neoplasia.\textsuperscript{43}

In our study AA and Bx genotypes, no significant difference between two groups and Bx genotype in our study population were a combination of A and B haplotypes without B homozygotes haplotypes which are partly consistent with the reported results on the frequency of KIR haplotypes in Iranians, and this reports indicate that in Iranians the frequency of A haplotype was greater than B haplotype (50.9% A haplotype vs. 49.1% B haplotype in healthy control group) and also the AB genotype had higher frequency than BB genotype.\textsuperscript{44} Inconsistent with our results in the study, which were conducted in Saudi Arabia in patients with breast cancer, the frequency of AA and Bx genotypes showed no significant difference between the two groups.\textsuperscript{14}
About the distribution of KIR A and B haplotypes, we came to the following conclusions: in the patients, the A in telomere and Bx in centromere had a higher frequency, but in the control group Bx in centromere and telomere had a higher frequency. Further analysis led us to the following results: the association between tA01|tA01 with risk for breast cancer and the association between cA01|cB0X and tA01|tB0X with protection against breast cancer. Our results indicate that homozygote form of the telomeric part of the KIR A genotype may have a role in the development of breast cancer, and heterozygote forms of centromeric parts of KIR A genotype/ KIR Bx genotype and telomeric parts of KIR A genotype/ KIR Bx genotype may have roles in preventing breast cancer. However, there was no significant difference between the two groups for any of the genes found in the telomeric and centromeric regions of A haplotype. In this study, Only the frequency of the KIR-2DL5B gene, which located in the centromeric region of haplotype B,\(^{38}\) showed a significant difference between the two groups. There is no report about the analysis of gene content in centromere and telomere regions of KIR locus in breast cancer, but in gastric cancer Bx genotype, Bx-Bx centromere-telomere, cA01|cB03, tB01|tB01 gene content were found as risk factors.\(^{15}\)

We evaluated the B score in centromere and telomere of KIR locus for determining the role of B motifs in breast cancer. The score\(^{1}\) was associated with risk, whereas score 2 was associated with protection against breast cancer. In two study B score were determined, In line with our study, in patients with acute myeloid leukemia who received transplant from donors with a B-score of 2 or greater, the patients showed a better protection from relapses, and an increased disease-free survival,\(^{38}\) in gastric cancer B-score of zero was strongly protective, a score of 2 was associated with non-atrophic gastritis, and a score of 3 increased the risk for gastric cancer. In this study, it was concluded that the B haplotype contributes to the damage of the gastric mucosa toward the development of gastric cancer.\(^{15}\) Despite the limited sample size, which we encountered in this study, however, we were able to find a significant contribution of the KIR loci and its centromere-telomeric regions with breast cancer. Further studies, focusing more on analyzing the expression levels of these molecules on the surface of killer cells, may help us to understand their possible roles in the fight against cancer cells.\(^{14}\) Because inhibitory KIRs can regulate the activation of NK cells, therapeutic strategies that inhibit KIRs can improve anti-cancer immunity. Blocking KIR may be a suitable therapeutic modality to boost the NK cell-mediated cellular cytotoxicity response in breast cancer.\(^{11,45}\)

**Declarations**

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**Declaration of conflicting interests**
The authors declare that there is no conflict of interest. The authors alone are responsible for the content and writing of the paper.

**Availability of data and materials**

Data will be available on request

**Ethics approval and consent to participate**

The Ethics committee of Kermanshah University of Medical Sciences approved the experimental protocol (Permission Code No: IR.KUMS.REC.1397.158). All patients signed written informed consent to participate in the study.

**Author contributions**

Seyedeh Zahra Shahrokhvand, Zahra Samimi and Mehrdad Payandeh worked on Concepts, Design and Definition of intellectual content. Parisa Feizollahi, Farbod Ghobadinezhad and Mahdi Taghadosi wrote the main manuscript text. Payam Nikjo prepared figures. All authors reviewed the manuscript.

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### Tables

Tables 1 to 4 are available in the Supplementary Files section

### Supplementary Files

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