Original Article

Killer cell immunoglobulin-like receptor gene cluster predisposes to susceptibility to B-cell acute lymphoblastic leukemia in Chinese children

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Abstract: B-cell acute lymphoblastic leukemia (B-ALL) is the most common malignancy in children. Killer cell immunoglobulin-like receptors (KIRs) are mainly expressed on natural killer (NK) cells and regulate killing of cancer cells. To investigate the possible association of KIR genes with B-ALL in Chinese children, we used polymerase chain reaction with sequence-specific primers (PCR-SSP) to determine the KIR genotypes of 137 B-ALL patients and 288 healthy children of Chinese Han origin. Herein we report no significant difference in the carrying frequency of individual KIR genes and haplotypes between patients and controls; however, individuals carrying C4Tx genotypes were more frequent in the B-ALL group compared with healthy controls (11.7% vs. 5.9%, P=0.038). In addition, the centromeric KIR gene cluster, KIR2DS2-2DL2-2DS3-2DL5, was significantly increased in the B-ALL group compared with healthy controls (13.9% vs. 7.3%, P=0.030). These data suggest that the C4Tx genotype and centromeric KIR gene cluster (KIR2DS2-2DL2-2DS3-2DL5) might predispose to susceptibility to B-ALL in Chinese children.

Keywords: Killer cell immunoglobulin-like receptor, gene cluster, B-cell acute lymphoblastic leukemia, genotype

Introduction

B-cell acute lymphoblastic leukemia (B-ALL) is the most common childhood malignancy and accounts for approximately 80% of all pediatric ALL cases. The causes of B-ALL have not been established. With the development of molecular biology, genetic and immune factors are known to be important in the pathogenesis of ALL [1]. NK cells, which are essential components of the innate immune system, have become the focus of research [2].

NK cells play an important role in defending against tumor cells [2]. The development and function of NK cells is governed by a dynamic balance between inhibiting and activating cell surface receptors, including killer cell immunoglobulin-like receptors (KIRs) [2, 3]. KIR genes are located on chromosome 19q13.4 and form part of the leukocyte receptor complex (LRC). To date, the family of KIR genes compromise 16 loci, including 2 pseudogenes (KIR2DP1 and KIR3DP1) and 4 framework KIR genes (KIR2DL4, KIR3DL2, KIR3DL3, and KIR3DP1). Fourteen functional and highly homologous KIR genes encode the key receptors that trigger activation (2DS1-5 and 3DS1), inhibition (2DL1-3, 2DL5, and 3DL1-3), or activation and inhibition (2DL4) of NK cells [4]. Furthermore, based on the presence of one or more activating KIR genes and linkage disequilibrium between KIR loci, KIR haplotypes can be classified into two basic groups (A and B) [5]. Briefly, in addition to the framework KIR genes, group A haplotypes have a relatively fixed content, including KIR2DL1, KIR2DL3, KIR2DS4, and KIR3DL1. In contrast, group B haplotypes have a variable gene content, comprising at least one of the activating KIR genes (except KIR2DS4) and inhibitory genes (KIR2DL5 and KIR2DL2). According to genetic studies [5], distinct populations have a different distribution of KIR haplotypes. For example, group A haplotypes are overrepresented in northeast Asians, while the A and B haplotypes are equally distrib-
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uted in Caucasians. Given the distinct combination of maternal and paternal KIR haplotypes and the multiallelic diversity of KIR genes, it is likely that individuals have quite different KIR genotypes. Consequently, the inherited diversity of KIR genes within and across populations may influence immunity and susceptibility to disease.

Several association studies have suggested a possible association between KIR genes and the development of certain forms of leukemia [6-10]. Recently, several studies have analyzed the association between KIRs genes/genotypes and childhood B-ALL in Western countries [11, 12]; however, the studies had conflicting results. In an effort to address genetic factors that might contribute to the susceptibility to childhood B-ALL, we performed a case-control study and evaluated the KIR genes/genotypes with the aim of investigating the association between KIR genes/genotypes and B-ALL in Chinese children.

Materials and methods

Patients and controls

We performed a case-control study at the West China Second University Hospital. One hundred thirty-seven unrelated children diagnosed with B-ALL (89 boys and 48 girls; mean ± S.D.: 6.15 y ± 4.11 y) were enrolled from the Pediatric Hematology/Oncology Department between September 2011 and July 2013. There were 288 gender- and age-matched unrelated controls (164 boys and 124 girls; mean ± S.D.: 6.27 y ± 3.81 y) from the same hospital who underwent health examinations during the same time period. B-ALL patients were diagnosed according to established criteria that included morphology, immunophenotyping, cytogenetics, and molecular biology (MICM), and treated based on therapeutic guidelines (Chinese Children Leukemia Guideline [CCLG-ALL 2008]). Control subjects and their relatives had no history of malignant diseases or autoimmune disorders. The study was approved by the Institutional Ethics Committee of West China Second University Hospital and informed consents were obtained from parents or guardians.

KIR genotyping

Genomic DNA was extracted from peripheral blood samples with a DNA extractor kit (RelaxGene Blood DNA System; Tiangen Biotech Co., LTD., Beijing, China) according to the manufacturer’s instructions. The quality and quantity of the extracted DNA samples were determined by UV spectrophotometry. DNA samples in sterile water were stored at -20°C for further studies.

Genotyping of the KIR was performed to detect the presence or absence of 16 KIR loci (KIR2DL1-5, 2DS1-5, 3DL1-3, 3DS1, 2DP1, and 3DP1) using PCR amplification with sequence-specific primers (PCR-SSP), as described previously [13] with minor modifications [14]. We amplified 100-200 ng of DNA in a 12.5-µl PCR mixture containing 6.25 µl of 2×PCR buffer (2×Taq PCR MasterMix, KT201; Tiangen Biotech Co., LTD.) between 4 pm and 10 pm for each specific primer. All amplifications were performed in a programmable PCR thermal cycler (Bio-Rad C1000 Touch Thermal Cycler; Richmond, CA, USA). The reaction products were run on 2.5%-3% agarose gels for electrophoresis to determine if the genes were present. The gel was visualized using a UV light source and the gel was photographed for a permanent record. The appearance of the amplicon band of the expected molecular weight was considered to indicate the presence of the gene in the genomic DNA sample. Each PCR experiment included one negative and one positive control.

Prediction of haplotypes from genotypes

The KIR gene content was used to determine KIR genotypes and haplotypes, as previously detailed [15]. Haplotypes 1 and 2 were referred to as haplotype A; all of the other haplotypes were designated as haplotype B. We assigned each child to one of three genotypes (AA, BB, and AB). The AB and BB genotypes were previously referred to as KIR genotype Bx [16].

Classification of genotypes on the basis of centromeric and telomeric gene clusters

Based on linkage disequilibrium, the KIR cluster is subdivided into two regions [17]. One cluster comprises the KIR2DS2-2DL2-2DS3-2DL5 genes and is located at the centromeric half of the KIR gene complex, whereas another cluster comprises the KIR3DS1-2DL5-2DS1-2DS5 genes and is located at the telomeric half of the complex. For simplicity, Ashouri et al. [18] refer to these clusters as C4 and T4 (C represents centromeric, T represents telomeric, and
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Table 1. Comparison of carrying KIR genes, genotypes, and gene clusters in B-ALL and controls

<table>
<thead>
<tr>
<th>Types</th>
<th>B-ALL (n=137)</th>
<th>Control subjects (n=288)</th>
<th>OR (95% CI)</th>
<th>P</th>
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<tr>
<td></td>
<td>%F</td>
<td>(N)</td>
<td>%F</td>
<td>(N)</td>
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<td>(201)</td>
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<td>(28)</td>
<td>19.1</td>
<td>(55)</td>
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</table>

Carrying frequency (%F) of each gene is defined as the number of individuals carrying the gene (N) divided by the number of individuals studied (n) in the given group. The p values are shown in bold, indicating significant (<0.05) differences. *KIR2DS4 was considered absent when the PCR amplified only the 22-bp deleted amplicon.

4 indicates the number of genes). On the basis of the presence and absence of C4 and T4 clusters, the Bx genotypes were further divided into the following four subsets: C4Tx (presence of C4 and absence of T4); CxT4 (absence of C4 and presence of T4); C4T4 (presence of both C4 and T4); and CxTx (absence of both C4 and T4).

Statistical analysis

The observed carrying frequency of each KIR gene was calculated as the percentage of positive numbers among all specimens. The frequencies of genotypes and gene clusters were calculated as the percentage of the numbers of AA, AB, and BB genotypes and gene clusters among all specimens. Pearson's Chi-squared test or Fisher's exact test were used to analyze the differences between B-ALL patients and controls in the frequencies of carrying KIR genes, genotypes, and gene clusters. We also assessed the combined odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for each variable. All data analyses were performed with SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA). A P-value <0.05 was considered significant.

Results

No difference between B-ALL patients and healthy controls in carrying frequencies of individual KIR genes

To better understand the impact of the underlying biology, 16 KIR genes were assessed in Chinese children with B-ALL (n=137) and healthy controls (n=288). A univariate comparison of KIR carrying frequencies between the B-ALL patients and healthy controls was performed (Table 1). As expected, KIR3DL2, 3DL3,
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3DP1, 2DP1, and 2DL4 were present in almost every sample (data not shown). In addition, the frequency of KIR2DL1, KIR2DL3, and KIR3DL1 genes within the A haplotype were >90% in both groups. Conversely, marked variation was apparent in the genes associated with the B haplotype. Compared with healthy controls, the B-ALL group exhibited an increased presence of B haplotype centromeric genes (KIR2DL2, KIR2DS2, and KIR2DL5) as well as KIR2DS3 (Table 1). The carrying frequencies for all of the KIR genes were not statistically different between children with B-ALL and the healthy controls (Table 1). Conversely, there was no significant difference in the frequency of the telomeric KIR gene cluster (KIR3DS1-2DL5-2DS1-2DS5) between the 2 groups (20.4% vs. 19.1%, P>0.05; Table 1).

Discussion

We report herein the first case-control association study to show an impact of KIR gene clusters on childhood B-ALL. Indeed, this is one of the largest studies focusing on the association between KIR-gene clusters and childhood B-ALL. Within the study panel of 137 B-ALL Chinese children and 288 healthy controls, we identified 41 distinct KIR gene content profiles (genotypes) carrying a different number and combination of 16 KIR genes (Figure 1) that comprised 27 genotypes in the B-ALL group and 34 genotypes in the healthy controls. Fifteen genotypes designated as NNx were not reported by Hsu et al. [15]. The dominant genotypes (AF, AG, and AJ) comprised the homozygous combination of A haplotypes. The combined frequencies in the 2 groups (54.7% and 56.6%, respectively) were comparable to those reported in the Chinese population [19]. Conversely, the BB genotypes were more frequently represented in B-ALL patients than controls but did not reach statistical significance (13.9% vs. 8.3%, P=0.077). The C4Tx genotype was significantly increased in the B-ALL patients compared with the healthy controls (11.7% vs. 5.9%, P=0.038). Consequently, the frequency of the C4 gene cluster was significantly increased in B-ALL patients compared with the healthy controls (13.9% vs. 7.3%, P=0.030). Conversely, there was no significant difference in the frequency of the telomeric KIR gene cluster (KIR3DS1-2DL5-2DS1-2DS5) between the 2 groups (20.4% vs. 19.1%, P>0.05; Table 1).
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B-ALL. Our analysis provides the first evidence of an association between the C4Tx genotype and the centromeric KIR gene cluster (KIR2DS2-2DL2-2DS3-2DL5) and susceptibility of Chinese children to B-ALL.

In previous case-control studies involving KIRs, small sample sizes, age-of-onset effects, different types of leukemia, and heterogeneity of the populations made it impossible for researchers to draw definitive conclusions [12]. Several previous studies focused on adult leukemia patients [6-8, 20]. Specifically, one group [20] has shown the involvement of an inhibitory KIR-HLA gene in susceptibility to chronic lymphoid (n=31) and myeloid leukemia (n=48) in a Belgian population, but did not demonstrate an association between KIR-HLA ligand interactions and acute lymphoid leukemia. In addition, Verheyden et al. [6] reported that KIR2DS2, a gene characteristic of the C4 KIR gene cluster, had a significantly higher frequency among patients with various types of leukemia (ALL n=8). The data support the possibility that KIR2DS2 increases the risk of leukemia [6]. In another study, Giebel et al. [7] demonstrated a protective role for KIR2DS4 in the regulation of NK cell-mediated immunosurveillance against chronic myeloid leukemia (n=31) in patients of Polish and German origin, but no associations with AML (n=38) and ALL (n=21) were evident. Yet another study [8] showed that the frequency of KIR2DS4 in Chinese patients with chronic myeloid leukemia (n=135) was higher than in healthy controls, but a significant protective association existed between KIR2DS3 and ALL (n=61).

B-ALL is the most frequent form of leukemia affecting children. Almalte et al. [11] performed a case-control study in Canadian children of French origin by studying the frequency of all 6 activating KIR genes (n=100 [B-ALL] and n=245 [controls]). Almalte et al. [11] found that harboring activating KIR genes is associated with a reduced risk for developing B-ALL in these children, and the higher number of activating KIR genes the lower the risk for developing B-ALL. Conversely, Babor et al. [12] reported no association between individual KIR genes and childhood B-ALL with relatively larger samples of B-ALL patients of European origin (n=185). Another study [21] found that the KIR A/A genotype frequency was significantly increased in children with ALL, which was in agreement with the Almalte et al. study [11]. One study [22] showed that KIR A homozygosity offered protection from adult leukemia in Chinese southern Han. NK cells from KIR A homozygous individuals were strongly cytotoxic to leukemic cells and the incidence of activating KIRs increased in childhood ALL cases compared to controls in northern Indians [23]. In the present study, 16 KIR genes, including activating and inhibiting KIR genes and KIR pseudogenes, were typed by PCR-SSP in 137 B-ALL Chinese Han children and compared with 288 healthy controls. The analysis enabled the identification of extended KIR genotypes. We observed that none of the individual KIR genes, whether activating or inhibiting KIR genes, tended to increase or reduce the risk for developing this type of leukemia (P>0.05). Our results were quite different from the report by Almalte et al. [11], but consistent with the results of Babor and colleagues [12], suggesting no association between individual KIR genes and childhood B-ALL. Due to the strong similarity and linkage disequilibrium between KIR genes it may be necessary to assess extended KIR genotypes to provide an important plausibility control [12]. Moreover, given the historic patient sample collections in the Almalte et al. study [11], high-quality sample collections are quite demanding for increasing amplification efficiency.

It is noteworthy that we are the first group to study the impact of KIR gene clusters on the susceptibility to childhood B-ALL in Chinese children. The B-ALL group had an increased frequency of the C4Tx genotype compared to healthy controls (Table 1). The KIR centromeric genecluster [KIR2DS2-2DL2-2DS3-2DL5(C4)] was significantly increased in the B-ALL group. Whatever the mechanism, the increased C4 gene cluster frequency in the B-ALL group suggested that the prevalence of B centromeric genes is a risk factor. As hypothesized, specific KIR genes or combinations of activating and inhibiting KIR genes may participate in the pathogenesis of leukemia, potentially explaining the remarkably different susceptibility to B-ALL in Chinese children.

The ligands and functions of activating KIRs are not documented as well as inhibiting KIRs. Thus, the mechanism involved in the regulation
of NK cell functions by activating KIRs is unclear. In addition, distinct expression characteristics of KIR, down-regulation of natural cytotoxicity receptors, and the lack of expression of ligands for activation receptors may contribute to disease progression [2, 24]. In fact, the cumulative evidence suggests that the interaction of KIR receptors and HLA-I ligands plays an important role in triggering an immune response against leukemia [25]. The leukemic cells can down-regulate the expression of HLA class I molecules, and in some situations, show a complete loss of a HLA class I allele [26]. Not all inhibitory signals mediated by the various KIRs are of equal weight [27]. Our findings shed light on the biologic implications of KIR gene associations in terms of the potential balance between inhibitory and activating effects. Perhaps all of the above factors would lead to one of only two outcomes (activation or inhibition of effector cells), a characteristic that is entirely predictable of a multigenic, functionally closely related, highly polymorphic family of genes.

In conclusion, we report here the first case-control association study to show the impact of KIR gene clusters on childhood B-ALL. Our results provide the first evidence for an association between childhood B-ALL and certain KIR genotypes and gene clusters. The C4Tx genotype, which encoding the C4 KIR gene cluster (KIR2DS2-2DL2-2DS3-2DL5), might predispose to susceptibility to B-ALL in Chinese Han children. In addition, we recommend further investigation of the expression and function of KIR genes, HLA ligands, and the expression of HLA class I molecules on leukemic cells to better understand the underlying mechanism(s) of possible NK cell dysfunction in B-ALL.

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Disclosure of conflict of interest

None.

References


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