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Diversity distributions of killer cell immunoglobulin-like receptor genes and their ligands in the Chinese Shaanxi Han population

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ABSTRACT

In the present study, 17 killer cell immunoglobulin-like receptors (KIR) genes and KIR ligands (human leukocyte antigen [HLA] –A and -B) were detected by using a polymerase chain reaction–sequence-specific primer (PCR-SSP) method in 104 unrelated healthy Han individuals living in Shaanxi province, China. The observed carrier frequencies of the 12 KIR genes ranged from 0.14 to 0.96. KIR2DL4, 3DL2, 3DL3, 2DP1 and 3DP1 were found to be present in every individual. A total of 51 different KIR gene profiles were identified, in which 11 gene profiles exclusively belonged to the study population. Neighbor-joining phylogenetic tree between the studing population and its neighboring ethnic groups was constructed using the observed carrier frequencies of 13 KIR loci. The phylogenetic tree shows that the Shaanxi Han population, Han populations in different regions, Yi, Japanese, and Koreans were in the same cluster. KIR/HLA relationships show that KIR3DS1$^-$/3DL1$^+$/Bw4$^+$ was the most common association in the population. In conclusion, the present study findings reveal the high polymorphism of KIRs in the Shaanxi Han population, demonstrate the KIR/HLA association in the study population, and enrich the KIR and HLA gene resources. The obtained KIR data will further the understanding of genetic relationships among populations in different geographic areas, and assist in answering questions regarding KIR/HLA relationships.

1. Introduction

Human killer cell immunoglobulin-like receptors (KIRs) are members of the immunoglobulin superfamily expressed on the surface of natural killer (NK) cells and cytotoxic T cells. Each KIR molecule consists of two or three extracellular immunoglobulin domains called 2D or 3D molecules, a transmembrane part, and a short (S) or long (L) intracellular tail. The KIR molecule with a long cytoplasmic tail has an inhibitory function by virtue of the immune receptor tyrosine-based inhibitory motifs, whereas the short-tailed KIR molecule has a potential activating function by interaction with the DAP-12 adaptor molecule [1,2].

KIRs can ligate with specific human leukocyte antigen (HLA) class I molecules and other unknown ligands on target cells with the help of conserved lectin-like receptors CD94-NKG2A and CD94-NKG2C and lead to either inhibition or activation of cytotoxic cells [3,4]. The large number of interactions between KIRs and their ligands will affect the activity of NK cells. Therefore, KIRs may play a significant role in the control of immune responses [5]. KIRs have been found to be implicated in susceptibility to diseases, including celiac disease [6], rheumatoid arthritis [7], systemic lupus erythematosus [8], and infectious diseases [9], in the process of pregnancy [10], in the potentially beneficial graft versus leukemia responses after allogeneic transplantation [11], and in the HLA-matched hematopoietic cell transplantation [12].

Previous studies have revealed the specific HLA ligands of some KIRs. It is known that some KIRs can recognize HLA-C alleles, which are classified into HLA-C1 and -C2 according to the amino acid

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dimorphism in position 80 (the epitope for KIR binding). KIR2DL2, 2DL3, 2DS2, and 2DS3 bind to HLA-C1 ligands carrying asparagines at position 80, whereas KIR2DL1 and 2DS1 bind to HLA-C2 ligands carrying lysine at the same position [13–16]. The KIR3DL1 (and possibly KIR3DS1) has HLA-B as its ligand, but binds only to HLA-B alleles bearing the HLA-Bw4 epitope. HLA-B is divided into HLA-Bw4 and HLA-Bw6 according to the amino acid polymorphism at posotions 77, 80, and 81–83 [17,18]. The inhibitory KIR3DL2 interacts with HLA-A3 and -A11 [19,20]. A recent study shows that full-length KIR2DS4 binds specifically to the subsets HLA-$C1^+$, -$C2^+$, and HLA-A11, whereas deleted 2DS4 is nonfunctional [21,22]. At present, the HLA ligands for other KIRs have not been identified.

KIR genes are located at chromosome 19q13.4. Within a range of ~160 kb, KIR genes cluster together with a genetic distance of less than 3 kb [23]. The diversity of KIR gene content and expression results in the high genetic polymorphism of KIR genes. KIR genes are expressed in a variegated, overlapping pattern, such that several inhibitory and stimulatory receptors are possibly expressed on each NK cell. In addition, the number of KIR genes in different NK clone cells and individuals also show high diversity.

KIR gene haplotype structures are classified into A haplotypes and B haplotypes, which are distinguishable by the order of KIR genes and gene content of 15 loci. The A haplotype contains at least six encoding inhibitory receptors (KIR3DL3, 2DL3, 2DL1, 2DL4, 3DL1, 3DL2, 3DP1) and only one activating receptor (KIR2DS4) [24]. In contrast, the B haplotype has been described as having a great variety of subtypes that differ from each other mostly because of the various combinations of stimulatory receptors [25]. KIR allelic and genotypic variability leads to the KIR haplotypic diversity. Because of the KIR haplotypic diversity, two unrelated individuals seldom have identical HLA and KIR genotypes.

Previous studies [26–44] have identified new alleles, found new haplotypes, verified the KIR diversity in different populations, furthered the understanding of genetic relationships among populations in different geographic areas, and answered the questions regarding ethnic origins. However, no KIR gene polymorphism data of the Chinese Han population from Shaanxi province, China, have been reported. The aim of this study was to analyze the distributions of 17 KIR genes and pseudogenes in the Chinese Shaanxi Han population by polymerase chain reaction–sequence-specific primer (PCR-SSP) method, and identify haplotypic and genotypic structures of this population. We also investigated HLA-A and -B ligands of KIR genes in the study population by PCR-SSP method and evaluated the correlation between 4 KIR genes (KIR3DS1, 3DL1, 3DL2 and 2DS4) and their ligands (HLA-A3, -A11 and -Bw4).

2. Subjects and methods

2.1. Study samples

A total of 104 unrelated healthy Han individuals were randomly chosen from Shaanxi province, China. All participants were interviewed to ensure that no individuals have common ancestry going back at least three generations. This study was approved by the Ethics Committee of Xi'an Jiaotong University, China. All the participants provided their written informed consent with the assistance of medical staff, and completed a questionnaire concerning their health conditions for sample selection. The investigation was conducted in accordance with humane and ethical research principles of Xi'an Jiaotong University, China.

2.2. DNA isolation

Whole blood samples were collected from the participants and stored at −20°C until DNA extraction. Genomic DNA was extracted from 300 μl of whole blood containing ethylenediaminetetraacetic acid (EDTA) using a DNA isolation kit (Promega Biotech, Co, Ltd, Madison, WI) according to the manufacturer's instructions, and was quantified by ultraviolet spectrophotometry. The optical density values used to evaluate the concentration and purity of the extracted DNA ranged from 1.6 to 1.9.

2.3. PCR-SSP amplification

KIR genes were typed for the presence or absence of the 15 KIR genes, including. KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4 (in the full-length form), ID (in the deleted form), 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, and the two pseudogenes, including 3DP1 (putative protein product) and 2DP1 (no protein expression), using PCR-SSP reactions. Primer designs and PCR cycling conditions for all the KIR genes were adopted from Uhrberg et al. [23] and Hsu et al. [26]. An internal control primer was included in each PCR typing reaction. All PCR reactions were amplified with the Bio-Rad PCR system (Bio-Rad, Hercules, CA) in a 12-μl volume reaction mixture containing 1 μl of 10 × PCR Buffer, 0.2 mmol/l of deoxynucleoside triphosphate, 10 pmol/l of forward and reverse KIR-specific primers each, 1 pmol/l of forward and reverse internal control primers each, 50 to 100 ng of template DNA, and 2.5 U of Taq polymerase. Temperature cycling conditions for PCR reactions were as follows: denaturation for 3 minutes at 95°C, followed by 30 cycles for 30 seconds at 95°C, 30 seconds at 63°C, 1 minute at 72°C, and a final elongation step for 10 minutes at 72°C for. Annealing temperatures were modified for primers amplifying KIR2DS1 at 61°C, KIR2DL2 at 60°C, KIR2DS4 at 59°C, KIR2DS3 at 56°C, and KIR2DP1 at 55°C. Extension time was modified for long-range amplification of KIR2DS1, 2DS2, 2DS4, ID, 2DL1, 2DL2, and 3DL2 for 2 minutes. PCR products were visualized under ultraviolet light after electrophoresis in 1.5% agarose gel well mixed with ethidium bromide. Each lane of the gel should show a control band. False reactions that yielded no control bands were repeated.

The typing of HLA-A locus and HLA-B locus was performed using the PCR-SSP method. The HLA typing reagent was purchased from PEL-FREEZ (Dynal Biotech, Milwaukee, WI).

2.4. Statistical analysis

The observed carrier frequencies (OF) of the KIR genes were determined as the number of positive typing reactions divided by the total number of individuals typed. Based on the assumption of Hardy–Weinberg equilibrium, the estimated gene frequencies (GF) were calculated using the formula $GF = 1-(1-OF)^{1/2}$, where OF is the observed carrier frequency of a KIR gene in individuals. Differences in the observed carrier frequency between the observed population and other populations previously published were assessed by the standard χ^2 test using statistical software SPSS Version 13.0. A p value of less than 0.05 was considered statistically significant.

A hierarchical clustering was conducted based on the observed carrier frequencies of the KIR genes detected in the study population and in other previously published populations [27–44]. A neighbor-joining tree was constructed and is shown in Fig. 1. The KIR gene profiles of the 104 individuals established on the basis of the Allele Frequency KIR database (http://www.allelefrequencies.net/) [28] are shown in Fig. 2. Included in Fig. 2 are the genotypes of the study population, the KIR genotype ID number, the number of the individuals, and the percentage distribution. The KIR genotypes were classified into three groups (AA, AB, and BB) according to the following rules: (1) the four framework genes KIR2DL4, 3DL2, 3DL3, and 3DP1 are present in all haplotypes; (2) alleles of the same locus do not appear together on one haplotype (haplotypes contained either KIR2DL2 or 2DL3, but not both; haplotypes contain either KIR3DL1 or 3DS1, but not both) [35]; (3) the A haplotype contains at least six loci encoding inhibitory receptors (KIR3DL3, 2DL3, 2DL1, 3DP1, 2DL4, 3DL1, and 3DL2) and only one activating