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 studied 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/H11002/3DL1/H11001/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.

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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
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 positions 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 67°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 2DL3 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 (GFs) were calculated using the formula $GF = \frac{1}{2 \ OF}$, 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 $\chi^2$ 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 contain 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
receptor (KIR2DS4) [24]; and (4) the B haplotype includes various combinations of stimulatory receptors [25].

Linkage disequilibrium (LD) between pairs of gene loci was estimated by the classical LD coefficient (D), another conventional measure of linkage disequilibrium ($r^2$), and statistical significance ($p$) using the Arlequin software 3.0.

3. Results

3.1. Observed KIR carrier frequencies

Table 1 lists the distributions of the OF and the GF of the 15 KIR genes and 2 pseudogenes in the study population. Seventeen KIR genes were observed in 104 unrelated healthy individuals from the Chinese Han population living in Shaanxi Province. All of the individuals were determined to have five ubiquitous KIR genes, including the three framework genes KIR2DL4, 3DL2, and 3DL3 and the 2 pseudogenes KIR2DP1 and 3DP1. Some variations occurred in the frequency distributions of the other KIR genes. Among the nonubiquitous KIR genes, KIR2DL3 showed the highest observed gene frequency (98%). The other most frequent nonubiquitous KIR genes were KIR3DL1 (96%) and KIR2DL1 (94%). KIR2DS3 showed the lowest observed carrier frequency (14%).

The observed carrier frequencies in this study in comparison with those reported in literature [27–44] are presented in Table 2. The $p$ values were calculated by $\chi^2$ analysis for the significance evaluation of the observed carrier frequencies, with $p$ values of less than 0.05 considered statistically significant. Statistical analysis shows that KIR 2DL1, 2DL2, 2DL3, 2DL5, 3DL1, 2DS1, 2DS2, 2DS3, 2DS5, and 3DS1 had significant observed carrier frequency distributions among different populations, whereas KIR2DL4, 3DL2, and 3DL3 did not.

The framework gene KIR3DL2 was found to have a frequency of 100% in all of the populations listed in Table 2. Interestingly, however, the framework genes KIR2DL4 and 3DL3 did not have a frequency of 100% in all the populations. The KIR2DL4 gene had a frequency of 99% in the South Asian population, and the KIR3DL3 gene had a frequency of 99% in the Pacific Island population.

A neighbor-joining phylogenetic tree (Fig. 1) was constructed based on the observed carrier frequency data of 13 KIR loci (KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3, 2DS1, 2DS2, 2DS3, 2DS5, and 3DS1) in different populations, for comparison of the genetic distances. The neighbor-joining phylogenetic tree clearly shows the relationships between the Shaanxi Han population and other populations, including the Hong Kong Han, the Iranian, Tarahumara, Purepecha, Huichol, Mestize, African, South Asian, Macedonian, Chinese Han (as described by Jiang K et al.), Réunion, Brazilian, Oman Basin, SE French, Finnish, Indian, Malaysian, Yunnan Han group in southwest China, Han population from Singapore, Lebanese, Pacific Island population, Northern Ireland population, Czech, Caucasian, Comorian, French, Senegalese, Japanese, Korean, Shanghai Han, Zhejiang Han, and Chinese Yi ethnic minority group [27–44]. Four main clusters were found in this neighbor-joining phylogenetic tree. The Hong Kong Han, the Chinese Han, the Yunnan Han, the Singapore Han, the Japanese, the Korean, the Shanghai Han, the Zhejiang Han, the Yi ethnic group in China and the Shaanxi Han were in the same cluster on the top of the tree. The populations in this cluster were all from Asia. In this Asian cluster, the Shaanxi Han population was the nearest to the Yunnan Han group in southwest China. The European and African population belonged to the second and third clusters, respectively. The populations from Tarahumara and Purepecha in the fourth cluster were on the bottom of the phylogenetic tree and had the farthest genetic distances from the study population.

3.2. KIR genotypes

The KIR gene profiles, the KIR genotypes, the genotype ID number, the number of the individuals studied, and the percentage distribution of the individuals, which were obtained on the basis of the Allele Frequencies KIR database (http://www.allelefrequencies.net/), are shown in Fig. 2. In this KIR database, genotypes are defined according to the presence or absence of the 16 KIR genes (KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1, and 3DP1), and 369 different KIR genotypes have been found in 11,267 individuals from 102 populations. The KIR database has not attempted to distinguish between AB and BB genotypes. Therefore, we distinguished the AB and BB genotypes according to the following principle: If KIR3DL1, KIR2DL1, KIR2DL3, and KIR2DS4 are all present in the KIR gene profiles, the genotype is considered as AB [24,25]; the rest of the genotypes are taken as BB. In our present study, a total of 51...
different KIR gene profiles were observed in the tested population samples.

Three individuals were found to possess 15 of the tested KIR genes; however, they showed different KIR gene profiles. The rest of the individuals had 5–14 KIR genes. The most common genotype profile observed included nine KIR genes (KIR2DL1, 2DL3, 3DL1, 2DS4, 2DP1, and the four framework KIR genes). This gene profile was observed in 20 individuals, which accounted for 19% of the total study population. Thirty-five individuals had an AA genotype (33%), 19 individuals had a BB genotype (18%) and 50 individuals

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**Fig. 2.** Killer cell immunoglobulin-like receptor (KIR) locus profiles observed in Chinese Shaanxi Han population (n = 104), the number and percent of individuals displaying each profile. *Shaded parts in the table stand for the genes present in tested individuals. *Genotype ID refers to genotype classification according to the Allele Frequency Net Database (www.allelefrequencies.net).
had an AB genotype (48%). Thirty-one individuals (30%) had the same genotype ID 1, which belongs to the most common genotype AA. No prominent gene profile of AB and BB were observed in the study population. Most importantly, five new gene profiles of AB and six new genotype profiles of BB were observed. In addition, the A haplotype was predominantly observed, and accounted for 58% of the total haplotypes.

### 3.3. Interactions of KIRs and their ligands

KIRs and their HLA ligands are highly polymorphic and inherited independently, which resulted in difficulty in finding a common KIR and HLA ligand combination in any two individuals. The HLA-A3, -A11, and -Bw4 were typed. Correlation between KIRs and their special ligands HLA-A3, -A11, -Bw4 in our study population is listed in Table 3. The most frequent association in the study population was 3DS1/3DL1/Bw4, with a frequency of 62.7%; and the rarest association was 3DS1/3DL1/Bw4, with a frequency of 3.4%.

#### 3.4. Linkage disequilibriums

The LD parameters for KIR gene pairs are shown in Table 4. Because KIR2DL4, 3DL2 and 3DL3 were present in all samples, they were excluded from the analysis. Values of \( p < 0.05 \) were considered statistically significant and are indicated in boldface type in Table 4. Significant positive associations were found in 21 pairs of KIR genes. KIR2DS1, 3DS1, and 2DS5 were found to be in positive LD with one another. KIR2DS3 were also found to be in positive LD with KIR2DS1 and 3DS1. In addition, positive LD between KIR2DS2 and 2DL2 was also observed. The descriptions above were consistent with associations between the constituents of B haplotypes.

Significant negative LD was observed in KIR2DL2/2DL3 and KIR3DL1/3DS1. These two pairs of KIR genes were not found together on one haplotype as previously described [35]. A haplotype genes KIR2DL1 and 2DL3 were also found in significant negative LD with all B haplotype genes.

These observations of positive and negative LD were consistent with the descriptions of A and B haplotypes.

### 4. Discussion

KIR genes show considerable genetic diversity, and the presence or absence of different KIR genes leads to a variety of haplotypes. Because of this diversity, populations from different geographic regions and different ethnic origins possibly show different features of the KIR genotype [27–44]. The present study of the Shaanxi Han population is expected to provide novel information

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### Table 1

<table>
<thead>
<tr>
<th>Frequencies</th>
<th>Inhibitory KIR</th>
<th>Activating KIR</th>
<th>Pseudogenes</th>
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<td>KIR2DL1</td>
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### Table 2

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<th>KIR2DS4</th>
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<tr>
<td>Shaanxi Han</td>
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<td>1</td>
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</table>

\( p \) Values were calculated from \( R \times C \) contingency of each locus, corrected for the 13 KIR genes investigated and shown with the highest and lowest frequencies (%); compared; only those \( p \) values that are statistically significant are shown in the table.
for the genetic characteristics of different populations. KIR genotypes of originally related populations are known to demonstrate some common genetic features [38]. This study is also beneficial to the recognition of the common features of different individuals of the same or originally related populations. A large number of studies have shown that KIRs and KIR genes play an important role in some physiologic and pathologic processes. KIRs are involved in susceptibility to diseases, such as celiac disease [6], rheumatoid arthritis [7], systemic lupus erythematosus [8], and infectious diseases [9]. KIRs also play a role in the process of pregnancy [10], the potentially beneficial graft versus leukemia responses [11], and the HLA-matched hematopoietic cell transplantation [12]. Recent studies have also revealed that KIR polymorphism in patients with leukemia is correlated with susceptibility to this disease [46] and that the NK cell can be used for leukemia immunotherapy [47]. This study may provide a better understanding of some specific biologic functions and regulatory mechanisms of NK and T cells at the molecular level.

All 17 KIR genes were detected in the Shaanxi Han population. Besides the four framework genes (KIR2DL4, 3DL2, 3DL3, and 3DP1), KIR2DP1 was also found in every individual. The four framework genes also existed in every individual of the other populations listed in Table 2, except for the South Asian and Pacific Island populations. KIR2DL4 locus had an observed carrier frequency of 99% in the South Asian population and KIR3DL3 locus also had an observed carrier frequency of 99% in the population from Pacific Island. The two nonubiquitous KIR genes KIR2DL2 and KIR2DS3 (19% and 14%, respectively) in the study population had observed carrier frequencies of less than 20%. The other populations with observed carrier frequency lower than 20% at KIR2DL2 locus were Chinese Han (described by Jiang Ke et al.; 17%), Japanese (15%), Korean (14%), Shanghai Han (18%), and Zhejiang Han (17%). However, South Asian and Indian populations had observed carrier frequencies of up to 64% and 69% at KIR2DL2 locus, respectively. Except for the southeastern France and Indian populations, which had observed carrier frequencies higher than 40% at the KIR2DS3 locus, all other populations had observed carrier frequencies lower than 40% at this locus. The other KIR genes were highly polymorphic, with their observed carrier frequencies ranging from 21% to 96%. The observed carrier frequencies of the activating receptors excluding KIR2DL2 were lower than those of the inhibitory receptors. This polymorphic feature also present in other populations listed in Table 2.

Table 3
Correlation between KIRs and their special ligands HLA-A3, -A11, -Bw4 in Chinese Shaanxi Han population (n = 104)

<table>
<thead>
<tr>
<th>Locus pair</th>
<th>Number of individuals (n)</th>
<th>Percentage of individuals (%)</th>
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<tr>
<td>KIR/Bw4 group (n = 59)</td>
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</tr>
<tr>
<td>3DS1/3DL1/Bw4*</td>
<td>20</td>
<td>33.9</td>
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<tr>
<td>3DS1/3DL1/Bw4*</td>
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<td>6.7</td>
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<td>3DL2/A3/A11*</td>
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<td>KIR2DS4/HLA-A group (n = 87)</td>
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<td>2DS4/A11+</td>
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<td>17.2</td>
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* HLA-Bw4 contained HLA-B13, B27, B37, B38, B44, B51, B52, B57, and HLA-B58.

Table 4
Linkage disequilibrium parameters for pairs of KIR genes in Shaanxi Han population

| Loci | Parameter | 2DL1 | 2DL3 | 2DS4 | 2DL5 | 2DS1 | 3DS1 | 2DS5 | ID | 2DS2 | 2DL2 | 2DS3 |
|------|-----------|------|------|------|------|------|------|------|----|------|------|------|------|
| 2DL1 | D | 0.00022 | 0.00007 | 0.0263 | 0.00226 | 0.00237 | 0.01317 | 0.0237 | 0.0126 | 0.0015 | 0.0022 | 0.0137 |
| p | 0.0024 | 0.00088 | 0.0081 | 0.00216 | 0.00015 | 0.00035 | 0.00006 | 0.00001 | 0.00001 | 0.00001 | 0.00015 | 0.00021 |
| r2 | 0.0133 | 0.0072 | 0.0351 | 0.0151 | 0.0099 | 0.1376 | 0.0099 | 0.1552 | 0.8477 | 0.7653 | 0.0389 |
| 2DL3 | D | 0.0068 | 0.00202 | 0.0046 | 0.0030 | 0.0027 | 0.0010 | 0.00001 | 0.00001 | 0.00001 | 0.00001 | 0.00001 |
| p | 0.0067 | 0.0582 | 0.6814 | 0.7903 | 0.7532 | 0.7903 | 0.3877 | 0.9192 | 0.2182 | 0.3002 |
| r2 | 0.0076 | 0.0345 | 0.0106 | 0.0007 | 0.0010 | 0.0071 | 0.0164 | 0.0146 | 0.0103 | 0.0103 |
| 2DS4 | D | 0.0035 | 0.0080 | 0.0074 | 0.0076 | 0.0063 | 0.0063 | 0.0063 | 0.0051 | 0.0004 | 0.0004 | 0.0004 |
| p | 0.0047 | 0.0030 | 0.0138 | 0.0123 | 0.0123 | 0.0123 | 0.0123 | 0.0123 | 0.0123 | 0.0123 | 0.0123 |
| r2 | 0.0571 | 0.2305 | 0.2589 | 0.2493 | 0.2589 | 0.3196 | 0.3131 | 0.4859 | 0.5577 | 0.5577 |

Statistically significant p values (p < 0.05) are indicated in boldface type.
D = classical linkage disequilibrium coefficient; r2 = another conventional measure of linkage disequilibrium.
By χ² analysis, we demonstrated that the observed carrier frequencies at 10 loci (KIR2DL1, 2DL2, 2DL3, 2DL5, 3DL1, 2DS1, 2DS2, 2DS3, 2DS5, and 3DS1) in Shaanxi Han population were significantly different from those in the other populations (p < 0.05). The results indicate that the same KIR gene showed substantial diversity among different populations. Therefore, the differences of the observed carrier frequencies of the KIR gene among populations will help in interpreting national and racial traceability, as previously reported by several research groups [34,48,49].

A total of 51 different KIR gene profiles were detected in the 104 Han individuals in Shaanxi, China (Fig. 2). Three individuals possessed 15 of the tested KIR genes but different KIR gene profiles. The other individuals had 5–14 KIR genes. The most common gene profile consisted of nine KIR genes (KIR2DL1, 2DL3, 3DL1, 2DS4, 2DP1, and the four framework KIR genes), and 20 individuals had this profile, accounting for 19% of the total population. All of the gene profiles were divided into three main categories (AA, AB, and BB) based on the Allele Frequencies KIR database. A haplotype was predominantly observed that accounted for 58% of the total haplotypes. The study of the KIR gene profiles showed a diverse distribution of KIR genes.

The neighbor-joining phylogenetic tree shows that the Shaanxi Han population belonged to the Asian cluster. Hong Kong Han, Chinese Han, Yunnan Han, Singapore Han, Japanese, Korean, Shanghai Han, Zhejiang Han, and Yi ethnic group in China were also in this cluster. The result shows that Han populations in different regions may possibly have a common ancestor and have a lower genetic distance with Japanese, Korean, and Yi ethnic group, which agrees with the findings of the previous studies on short tandem repeat (STR) and HLA loci[50–53].

In conclusion, the characteristics of KIR gene frequency in the Shaanxi Han population are similar to those in other Han populations previously reported. The new gene profiles observed can provide information on novel racial/ethnic characteristics of the Han population. These gene profiles can be used to characterize KIR haplotypes for detailed studies of the allelic polymorphism of KIR loci in individuals, families, and races/ethnicities. Our study also shows that PCR-SSP remains a useful method for KIR gene typing. Moreover, neighbor-joining phylogenetic tree analysis indicates that population genetic analysis of KIR genes can be of help, from an anthropological perspective, in understanding genetic as well as the geographic history of world populations. However, KIR genes may have some other features, so further studies should be done in more populations.

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