# Genetic polymorphisms and association of *KIR-HLA* system of Chinese Henan Han population and an extensive *KIR* gene diversity study between populations distributed worldwide

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## ABSTRACT

**Killer-cell immunoglobulin-like receptors are expressed on the plasma membrane of natural killer cells and a minority of T cells, which can regulate the killing function of these cells by interacting with their special ligands. The major ligands for them are the human leukocyte antigen class I molecules. Combinations of human leukocyte antigen class I molecules and Killer-cell immunoglobulin-like receptor variants contribute to the intensity of acquired immune, resistance to infections, susceptibility to autoimmune disorders, complications of pregnancy, cancers and so on. In order to reveal this appropriate functional interaction of these two markers, some previous studies have revealed the co-evolution of these two markers within and across populations in disease researches. To our knowledge, the polymorphism data of two markers of Henan Han population haven't yet been available to date. In this study, we obtained their allelic frequencies of the two markers, on this basis, we obtained 26 Killer-cell immunoglobulin-like receptor genotypes, the extensive Killer-cell immunoglobulin-like receptor gene diversity between populations distributed worldwide, and the frequencies of the estimated main human leukocyte antigen haplotypes. And we also conducted the correlation analysis to investigate population-level evidence for co-evolution of the two markers based on their frequencies and the receptor-ligand pairs. This present study could provide basic and valuable polymorphism data of the two markers and their combinations for anthropological analysis and associated disease studies. In addition, it may provide some valuable clues to the co-evolution of these two complex genetic systems based on the study of the two marker pairs.**

## INTRODUCTION

Killer-cell immunoglobulin-like receptors (*KIRs*), which are encoded by one of the very complex and polymorphic gene families located on chromosome 19q13.4 are expressed on natural killer (NK) cells and a subset of T cells and can be activated or inhibited [1]. The *KIR* genes exhibiting substantial segmental or near-identical sequence copy number variations show extensive variability in terms of gene structures and gene content across haplotypes, probably because of non-allelic homologous recombination occurring between pairs of homologous *KIR* genes which generate novel expanded and contracted haplotypes, multiple genes and formation of novel fusion genes [2, 3]. Nomenclature of *KIRs* is based on the number of the extracellular immunoglobulin-like domains (2D or 3D) and the length of the cytoplasmic tail (L for long and S for short) or the pseudogene (P). To date, 15 distinct *KIR* gene loci have been identified namely *KIR2DL1, 2DL2/3, 2DL5A, 2DL5B, 3DL1/S1, 3DL2, 3DL3, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 2DL4* and two pseudogenes (*KIR2DP1* and *3DP1*) [4]. According to distinctly different gene contents, *KIR* gene combinations can be divided into two specific form haplotypes (*KIR* A and B haplotype). The *KIR* A haplotype has largely fixed gene content, with mostly genes encoding inhibitory *KIRs* and only one activating gene *KIR2DS4*; the *KIR* B haplotype has a more variable gene content [1]. Two haplotypes are always maintained in every human population, but at different frequencies in most populations distributed worldwide. Based on the above principles, we distinguished between the AA and Bx (AB or BB) genotypes of our studied population. As of March 8, 2017, 573 different *KIR* genotypes are found in 18,783 individuals from 155 populations [5].

Within distinct regions of the *KIR* cluster in classic linkage disequilibrium (LD) studies, there were two distinct regions namely centromeric region and telomeric region in the *KIR* cluster. *KIR3DL3* is located in the end of centromeric region, *KIR3DL2* is located in the end of the telomeric region, the *KIR3DP1* and *2DL4* are located in the middle of the *KIR* cluster. The *KIR2DS3*, *2DS5* and *2DL5* can be present in the centromeric and telomeric regions of the *KIR* cluster. Except for the genes described above, the centromeric region is considered to contain the *KIR2DS2, 2DL2, 2DL3, 2DP1* and *2DL1*; and the telomeric region is considered to contain *KIR3DL1, 3DS1, 2DS1* and *2DS4*. The *KIR2DL3*, *3DL1* and *2DS4* belong to A-motif genes; and the *KIR2DS2, 2DL2, 3DS1, 2DS1, 2DS3, 2DS5*, and *2DL5* belong to B-motif genes [6–8]. In our study, we also distinguished between the centromeric motif and the telomeric motif.

The *KIRs* are the critical regulators for the development, activation and tolerance of NK cells. NK cells are bone marrow-derived lymphocytes, which comprise about 10–15% of all circulating lymphocytes and are crucial components of the early innate immune response system, providing a first line of defense against transformed and virus infected cells [9]. NK cells play the function by *KIRs* binding to specific human leukocyte antigen *(HLA)* class I molecules and other unknown ligands on target cells. The major ligands of *KIRs* are the *HLA* class I (*HLA-A, -B* or *-C*) molecules which located on chromosome 6p21.31 is one of the other most polymorphic regions of the human genome [10, 11].

The *HLA-C* alleles consist of two different groups of ligands *C1* (HLA-$C^{asp80}$) and *C2* (*HLA-*$C^{lys80}$) on the basis of a dimorphism at position 80 of the α1 domain. In general, *HLA-C1* group is the ligands for *KIR2DL2/3* and *2DS2*; and *HLA-C2* group is the ligands for *KIR2DL1* and *2DS1* loci, respectively. Recently, it has been shown that *KIR2DL1* has only interacted with *HLA-C2* group [12]. *KIR3DL2* interacts with *HLA-A3* and *A11* alleles. *KIR3DL1* binds to *HLA-Bw4* allotype that contains the *Bw4* epitope, which is present on some of the *HLA-A* and *HLA-B* molecules, defined by amino acid variation at positions 77–83 [13, 14]. According to the difference of the amino acids encoded by the 80th position (Ile80 or Thr80) of the second exon of the *HLA-B* locus, *HLA-Bw4* can be divided into *Bw4-Ile80* and *Bw4-Thr80* alleles. The previous studies have shown that *Bw4-Ile80* alleles were the better ligands for *KIR3DL1* than *Bw4-Thr80* alleles [15, 16]. The ligand for *KIR3DS1* is known as *Bw4-Ile80*, which may be due to the strong similarity of the extracellular domains of *KIR3DS1* and *3DL1* [17]. A recent study indicated that full-length *KIR2DS4* binded specifically to the subsets *HLA-C1, C2*, and *A11* alleles, whereas deleted *KIR2DS4* was nonfunctional [18, 19]. But, until now, the *HLA* ligands for the other *KIRs* have not been completely identified.

Up to now, no *KIR* gene and *HLA* gene polymorphism data of the Chinese Han population from Henan province have been reported. In this study, we investigated the diversity and distributions of the 19 *KIR* genes (*KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5A, 2DL5B, 2DS1, 2DS2, 2DS3, 2DS4*FUL, 2DS4*DEL, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1, 3DP1*FUL* and *3DP1*DEL*) and five *HLA* loci (*HLA-A, -B -C, -DRB1* and *-DQB1*) of 145 individuals from Henan Han population. We also evaluated the correlation and co-evolution of *KIR-HLA* system for the first time in the population. Furthermore, *KIR* gene diversity has been studied in a large number of populations distributed worldwide.

## RESULTS

### *KIR* gene polymorphisms

In this study, 16 *KIR* genes and 3 pseudogenes (*KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5A, 2DL5B, 2DS1, 2DS2, 2DS3, 2DS4*FUL, 2DS4*DEL, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1; 2DP1, 3DP1*FUL and 3DP1*DEL*)

were tested in 145 unrelated healthy individuals of the Han population from Henan province, China. The observed carrier frequencies (OFs) and the estimated gene frequencies (GFs) for the 19 *KIR* genes were shown in Table 1. The *KIR2DL1*, the framework genes (*KIR2DL4, 3DL2* and *3DL3*) and the pseudogenes (*KIR2DP1* and *3DP1*DEL*) had OF and GF values of 1. The OF and GF values of the other functional KIR genes ranged from 0.08 (*KIR3DP1*FUL*) and 0.99 (*KIR2DL3*); from 0.04 (*KIR3DP1*FUL*) to 0.92 (*KIR2DL3*), respectively.

## *KIR* genotypes

As shown in Figure 1, twenty-six genotypes were identified in the 145 Han individuals. Among the 26 genotypes, 3 genotypes observed in 81 (55.86%) individuals belonged to AA genotype, and the other 23 genotypes observed in 64 (44.14%) individuals belonged to Bx genotype. The most common genotype consisting of nine *KIR* genes (*KIR3DL3, 2DL3, 2DP1, 2DL1, 3DP1*DEL, 2DL4, 3DL1, 2DS4*FUL* and *3DL2*) belonged to the AA genotype, which was found in 42 individuals, accounting for 28.97% of the total.

As mentioned above, the previous studies showed that there were two distinct regions namely centromeric region and telomeric region in the *KIR* cluster [6, 7]. The genotypes of the centromeric motif and telomeric motif were also analyzed in this study based on the classification standard: cA01 (*KIR3DL3*, *2DL3*, *2DP1*, *2DL1* and *3DP1*), tA01 (*KIR2DL4*, *3DL1*, *2DS4* and *3DL2*), cB01 (*KIR3DL3*, *2DS2*, *2DL2*, *2DL5*, *2DS3*, *2DS5*, *2DP1*, *2DL1* and *3DP1*), cB02 (*KIR3DL3*, *2DS2*, *2DL2* and *3DP1*), cB03 (*KIR3DL3*, *2DL3*, *2DL5*, *2DS3*, *2DS5*, *2DP1*, *2DL1* and *3DP1*), tB01 (*KIR2DL4*, *3DS1*, *2DL5*, *2DS3*, *2DS5*, *2DS*1 and *3DL2*) [6]. In the study, cB01, cB02 and cB03 were treated as cB; cA01 as cA; tA01 as tA; and tB01 as tB, resepectively. Eighty-three cA/cA (57.24%), 61 cA/cB (42.07%), one cB/cB (0.69%), 85 tA/tA (58.62%), 54 tA/tB (37.24%) and six tB/tB (4.14%) were found in the 145 Henan Han individuals.

## *KIR* gene diversities of populations distributed worldwide

In this study, we also studied the *KIR* gene diversities of populations distributed worldwide. The Heatmap analysis was drawn and presented in Figure 2 based on the 13 overlapping *KIR* gene frequencies (*KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3, 2DS1, 2DS2, 2DS3, 2DS5* and *3DS1*) among

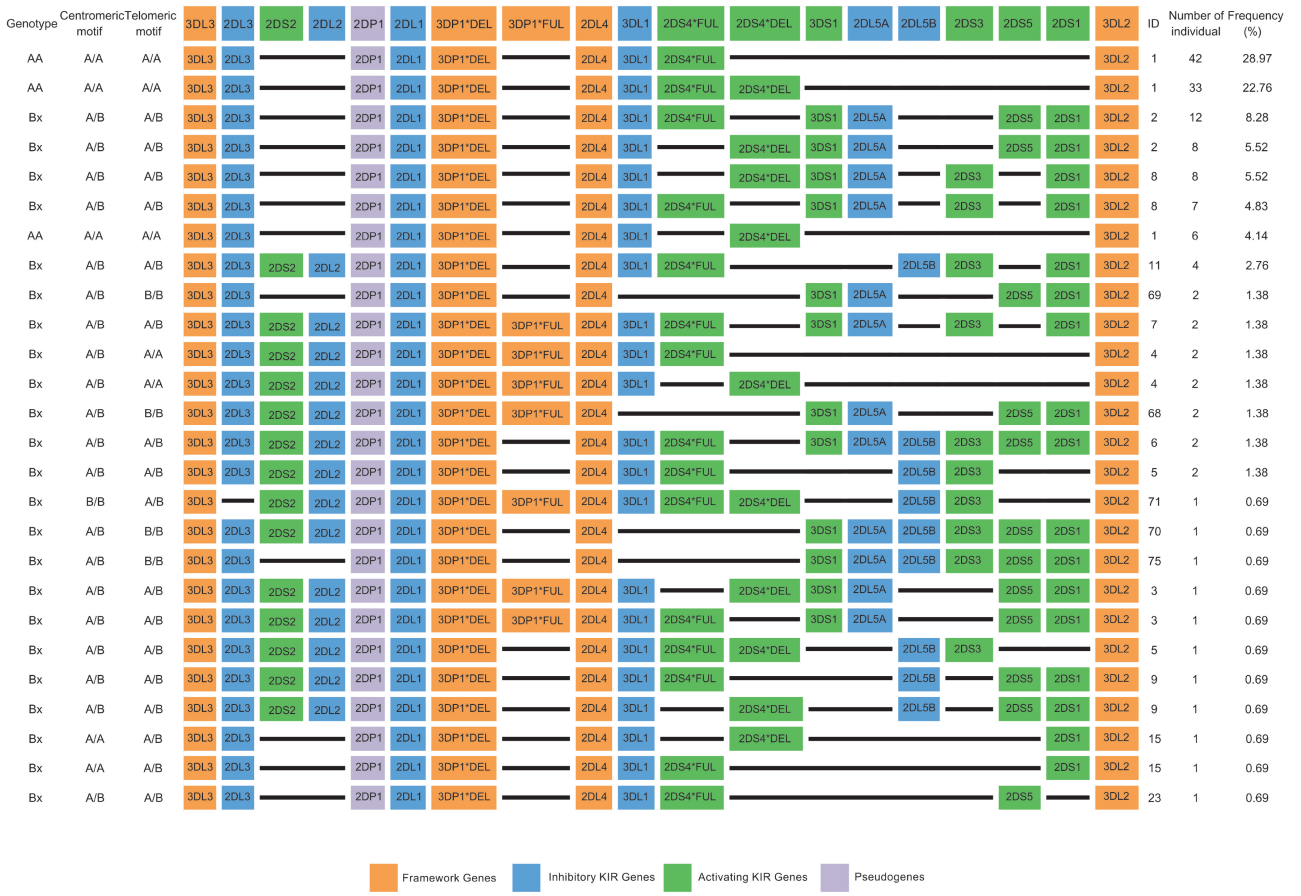


**Figure 1: Killer-cell immunoglobulin-like receptor loci profiles were observed in the Chinese Henan Han population (*n* = 145).** Genotype ID were refered to genotype classification according to www.allelefrequencies.net.

**Table 1: Observed carrier frequencies (OFs) and estimated gene frequencies (GFs) of *KIR* genes for Chinese Henan Han population ($n$ = 145)**

| Indexes | Inhibitory *KIR* Genes | | | | | | | | | Activating *KIR* Genes | | | | | | | *KIR* Pseudogenes | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *2DL1* | *2DL2* | *2DL3* | *2DL4* | *2DL5A* | *2DL5B* | *3DL1* | *3DL2* | *3DL3* | *2DS1* | *2DS2* | *2DS3* | *2DS4*$^*$*FUL* | *2DS4*$^*$*DEL* | *2DS5* | *3DS1* | *2DP1* | *3DP1*$^*$*FUL* | *3DP1*$^*$*DEL* |
| OF | 1 | 0.16 | 0.99 | 1 | 0.32 | 0.10 | 0.96 | 1 | 1 | 0.38 | 0.16 | 0.20 | 0.77 | 0.43 | 0.23 | 0.32 | 1 | 0.08 | 1 |
| GF | 1 | 0.08 | 0.92 | 1 | 0.18 | 0.05 | 0.80 | 1 | 1 | 0.21 | 0.08 | 0.11 | 0.52 | 0.24 | 0.12 | 0.18 | 1 | 0.04 | 1 |

Chinese Henan Han and 22 other populations i.e. Tujia, Shanghai Han, Shaanxi Han, Northern Han, Zhengjiang Han, Singapore Han, Yi, Chinese Han, Nu, Yunnan Han, Uygur for Urumqi, Mongolian, Yugu, Japanese, Korean, Trinidad S. Asian population, Karachi S. Asian population, Czech, Macedonian population, Pacific Islands population, Cook Islands population and Zhuang group [5, 20–36]. The languages of the 23 populations belonged to Sino-Tibetan, Altaic, Austronesian and Indo-European language families. In Figure 2, the gene frequencies of OFs from high to low were represented by the color from red to blue. All the loci could be divided into two groups roughly: the six KIR loci almost filled with red color on the left side belong to the A haplotype; the remaining seven loci almost filled with blue color on the right side belong to the B haplotype.

As shown in Figure 3, the principal component analysis (PCA) based on the OFs of the same 11 overlapping *KIRs* (*KIR3DL2* and *3DL3* were removed for the OFs in all the populations were 1.0000) was conducted for the studied Henan Han population and the above 22 other populations distributed worldwide. The distance from each locus to zero point represented the relative contribution of each *KIR* gene frequency to the variability along the first two axes (PC1 and PC2). In Figure 3, we can observe that the groups distributed around a *KIR* locus have the higher or lower OFs of this *KIR* gene than the other groups. For example, the highest value for *KIR2DL1*

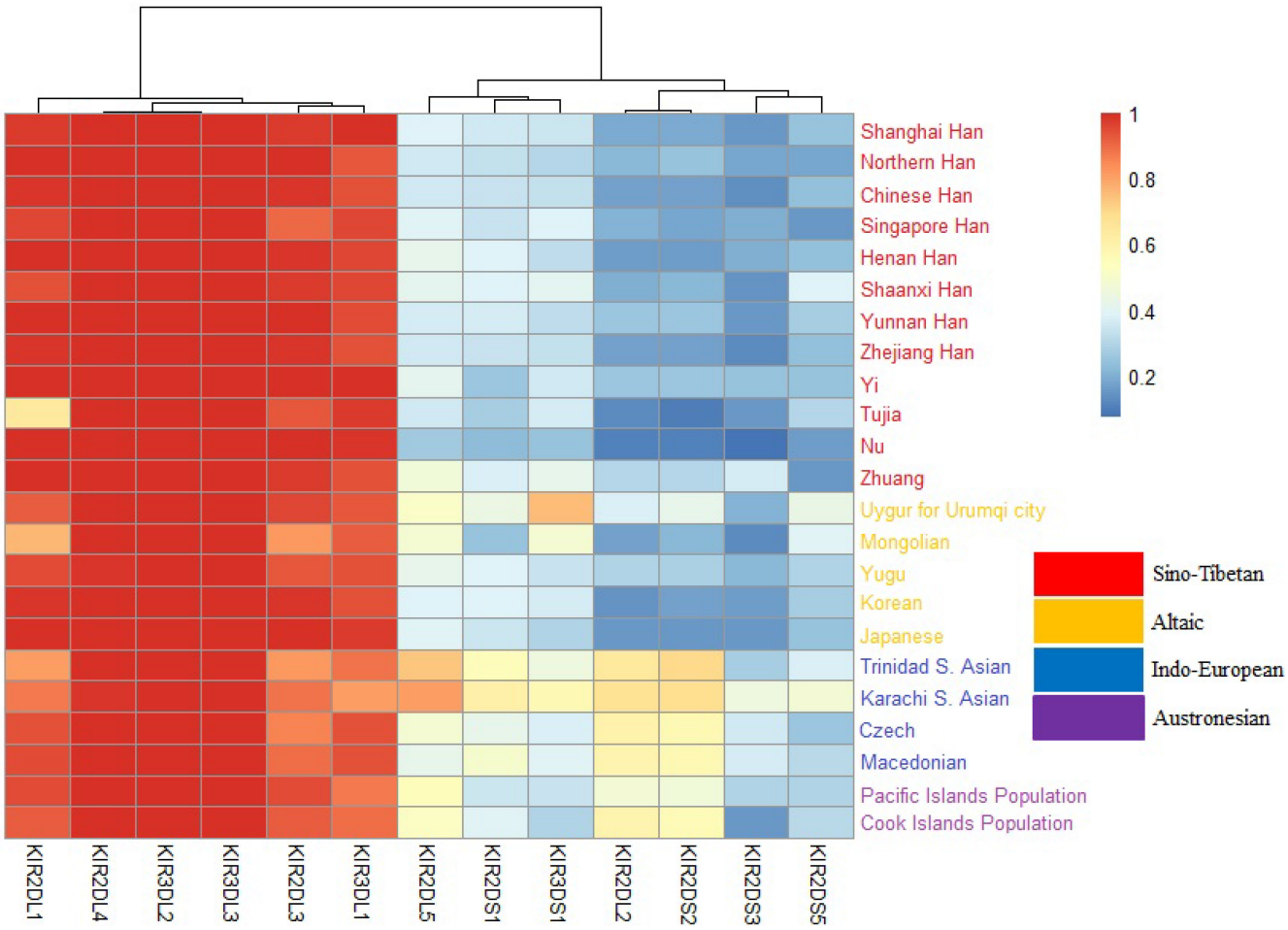


**Figure 2: A Heatmap drawn using Package 'pheatmap' containing the Chinese Henan Han and 22 other populations distributed worldwide illustrated the molecular evolutionary structure of the 13 overlapping *KIR* genes.** The deeper color indicated the higher OFs.

**Table 2: Allelic frequencies (AF) of *HLA-A, -B, -DRB1, -C* and *-DQB1* loci in the Han population from Henan province, China (*n* = 145)**

| *HLA-A* | AF | *HLA-B* | AF | *HLA-B* | AF | *HLA-DRB1* | AF | *HLA-C* | AF | *HLA-DQB1* | AF |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0101 | 4.14% | 1301 | 4.82% | 4601 | 6.55% | 1001 | 1.38% | 0102 | 13.10% | 0201 | 4.48% |
| 1101 | 16.90% | 1302 | 12.41% | 4801 | 2.07% | 0101 | 4.14% | 0103 | 0.69% | 0202 | 13.10% |
| 1102 | 0.69% | 1501 | 5.86% | 5101 | 5.17% | 1101 | 5.86% | 1202 | 3.79% | 0301 | 20.69% |
| 0201 | 18.28% | 1502 | 2.41% | 5102 | 0.34% | 1104 | 0.69% | 1203 | 1.72% | 0302 | 5.52% |
| 0203 | 1.72% | 1507 | 0.34% | 5108 | 0.34% | 1201 | 4.14% | 1402 | 3.79% | 0303 | 12.76% |
| 0205 | 0.34% | 1511 | 2.41% | 5201 | 3.10% | 1202 | 6.90% | 1403 | 1.03% | 0401 | 5.52% |
| 0206 | 2.76% | 1517 | 0.34% | 5401 | 6.55% | 1301 | 0.69% | 1502 | 1.72% | 0402 | 1.38% |
| 0207 | 5.17% | 1518 | 0.34% | 5502 | 1.03% | 1302 | 3.10% | 1505 | 1.38% | 0501 | 6.55% |
| 0211 | 0.34% | 1527 | 0.69% | 5601 | 0.34% | 1312 | 0.34% | 1602 | 0.69% | 0502 | 2.41% |
| 2301 | 0.69% | 1801 | 0.69% | 5701 | 1.38% | 1319 | 0.34% | 1701 | 0.34% | 0503 | 2.41% |
| 2402 | 16.55% | 2704 | 1.38% | 5801 | 3.79% | 1401 | 0.69% | 0202 | 1.03% | 0601 | 8.28% |
| 2420 | 0.34% | 2705 | 1.03% | 5901 | 0.34% | 1403 | 0.34% | 0302 | 3.45% | 0602 | 13.10% |
| 2601 | 3.45% | 2707 | 0.34% | 6701 | 2.76% | 1404 | 0.34% | 0303 | 6.55% | 0603 | 0.69% |
| 2901 | 1.38% | 3501 | 2.41% | 0702 | 5.17% | 1405 | 1.72% | 0304 | 9.31% | 0604 | 1.38% |
| 3001 | 10.00% | 3502 | 0.69% | 0705 | 1.38% | 1501 | 14.14% | 0401 | 9.31% | 0609 | 1.72% |
| 3004 | 0.34% | 3503 | 1.38% | 0801 | 0.69% | 1502 | 3.45% | 0501 | 0.34% | | |
| 0301 | 3.45% | 3505 | 0.34% | 8101 | 0.34% | 1506 | 0.34% | 0602 | 15.17% | | |
| 0302 | 0.34% | 3701 | 1.38% | | | 1602 | 1.38% | 0701 | 3.10% | | |
| 3101 | 2.07% | 3801 | 1.03% | | | 0301 | 4.48% | 0702 | 14.48% | | |
| 3201 | 1.03% | 3802 | 1.38% | | | 0401 | 0.69% | 0704 | 0.34% | | |
| 3303 | 8.28% | 3901 | 2.07% | | | 0403 | 0.69% | 0801 | 8.62% | | |
| 6801 | 1.03% | 3909 | 0.34% | | | 0405 | 5.17% | | | | |
| 6824 | 0.34% | 4001 | 4.83% | | | 0406 | 4.14% | | | | |
| 6901 | 0.34% | 4002 | 1.72% | | | 0407 | 1.03% | | | | |
| | | 4003 | 0.34% | | | 0410 | 0.34% | | | | |
| | | 4006 | 2.76% | | | 0701 | 14.83% | | | | |
| | | 4101 | 0.34% | | | 0802 | 1.38% | | | | |
| | | 4402 | 0.34% | | | 0803 | 5.52% | | | | |
| | | 4403 | 4.14% | | | 0901 | 11.72% | | | | |

was 1.0000 in Northern Han which was close to *KIR2DL1* in the plot. Through the principal component analysis, the variability of these 11 overlapping KIR observed carrier frequencies among the populations may be visualized.

The PCA plot was also constructed based on OF data of the 13 overlapping *KIR* genes between the Henan Han population and 22 other populations distributed worldwide (as shown in Figure 4). The first and second principle component of Figure 4 accounted for 73.97% and 11.58% of the total variance, respectively, representing the main genetic variance. In this PCA plot, the distribution of other groups was roughly consistent with their geographical distribution, and Henan Han on the right side of the figure was closed to East Asian populations.

A neighbor-joining (NJ) tree shown in Figure 5 was constructed based on OF data of the 13 overlapping

**Table 3: The frequencies of the estimated main *HLA* haplotypes (the haplotypic frequency (HF) ≥ 1.00%) in the Han population from Henan province, China**

| *A-B* | HF. | *A-DRB1* | HF. | *A-C* | HF. | *A-DQB1* | HF. | *B-DRB1* | HF. | *B-C* | HF. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3001-1302 | 9.31% | 3001-0701 | 7.90% | 3001-0602 | 9.30% | 3001-0202 | 7.91% | 1302-0701 | 10.34% | 1302-0602 | 12.41% |
| 3303-5801 | 2.97% | 2402-1501 | 5.19% | 0201-0702 | 4.16% | 0201-0301 | 6.25% | 1301-1202 | 3.45% | 5401-0102 | 6.55% |
| 0201-1301 | 2.76% | 0201-0901 | 4.31% | 2402-0304 | 3.84% | 0201-0303 | 4.62% | 4601-0901 | 3.08% | 4601-0102 | 5.52% |
| 1101-1501 | 2.72% | 0201-1202 | 3.93% | 1101-0801 | 3.63% | 2402-0602 | 4.54% | 1501-1501 | 2.91% | 0702-0702 | 5.17% |
| 2402-5401 | 2.59% | 1101-0405 | 2.40% | 2402-0102 | 3.29% | 1101-0301 | 4.02% | 0702-1501 | 2.70% | 1301-0304 | 4.48% |
| 3303-4403 | 2.41% | 1101-1501 | 2.39% | 1101-0401 | 3.20% | 2402-0301 | 3.16% | 1501-0406 | 2.07% | 1501-0401 | 4.14% |
| 0207-4601 | 2.29% | 1101-0901 | 2.32% | 3303-0302 | 3.10% | 1101-0303 | 2.94% | 4403-0701 | 2.07% | 5101-1402 | 3.79% |
| 2402-4601 | 2.15% | 0201-0701 | 2.10% | 0201-0303 | 3.04% | 1101-0601 | 2.62% | 5201-1502 | 2.07% | 5801-0302 | 3.45% |
| 0201-1511 | 2.07% | 3303-0301 | 2.07% | 0201-0304 | 3.04% | 0201-0602 | 2.43% | 5401-0405 | 2.07% | 5201-1202 | 3.10% |
| 1101-4001 | 1.99% | 3303-1302 | 2.07% | 0201-0801 | 2.74% | 2402-0501 | 2.10% | 5801-0301 | 2.07% | 4006-0801 | 2.76% |
| 0201-4006 | 1.72% | 2402-0901 | 2.06% | 1101-0102 | 2.61% | 1101-0401 | 2.07% | 4601-0803 | 1.72% | 6701-0702 | 2.76% |
| 1101-1502 | 1.72% | 1101-1101 | 1.99% | 0207-0102 | 2.60% | 2601-0301 | 2.07% | 5401-1501 | 1.53% | 1502-0801 | 2.41% |
| 1101-5101 | 1.72% | 0201-1501 | 1.93% | 2402-0401 | 2.56% | 3303-0201 | 2.07% | 4001-1201 | 1.38% | 1511-0303 | 2.41% |
| 2402-0702 | 1.72% | 1101-0406 | 1.72% | 0201-0102 | 2.47% | 2402-0302 | 2.03% | 5101-0901 | 1.38% | 4001-0304 | 2.41% |
| 2402-5101 | 1.72% | 2402-0406 | 1.72% | 1101-0702 | 2.10% | 0207-0303 | 1.87% | 5801-1302 | 1.38% | 4001-0702 | 2.41% |
| 0101-5701 | 1.38% | 0207-1101 | 1.71% | 0101-0602 | 2.03% | 1101-0302 | 1.76% | 4001-0901 | 1.22% | 4403-0701 | 2.07% |
| 0201-4601 | 1.38% | 0201-0405 | 1.59% | 0207-0702 | 1.35% | 0207-0301 | 1.75% | 1502-1501 | 1.03% | 4801-0801 | 2.07% |
| 0201-5401 | 1.38% | 1101-0701 | 1.53% | 0203-0702 | 1.33% | 0201-0401 | 1.72% | 3701-1001 | 1.03% | 0705-1505 | 1.38% |
| 0201-6701 | 1.38% | 0207-0901 | 1.49% | 2402-1402 | 1.32% | 0101-0501 | 1.69% | 3901-0803 | 1.03% | 1501-0303 | 1.38% |
| 2402-4001 | 1.34% | 1101-0803 | 1.49% | 0201-0602 | 1.29% | 3303-0602 | 1.65% | 4002-1501 | 1.03% | 3501-0303 | 1.38% |
| 0201-1302 | 1.26% | 2402-0101 | 1.41% | 1101-0602 | 1.26% | 2402-0303 | 1.47% | 4403-1302 | 1.03% | 3701-0602 | 1.38% |
| 1101-5401 | 1.20% | 2402-1502 | 1.38% | 0206-0702 | 1.20% | 0201-0202 | 1.40% | 5101-0101 | 1.03% | 3802-0702 | 1.38% |
| 2402-1501 | 1.08% | 3101-1501 | 1.38% | 2402-0702 | 1.19% | 3303-0609 | 1.38% | 1502-1202 | 1.01% | 3901-0702 | 1.38% |
| 0101-0702 | 1.03% | 2601-1201 | 1.38% | 3303-0701 | 1.16% | 0301-0601 | 1.35% | | | 5701-0602 | 1.38% |
| 0301-5201 | 1.03% | 2402-0301 | 1.03% | 2402-0303 | 1.16% | 1101-0202 | 1.30% | | | 2705-0202 | 1.03% |
| 1101-1302 | 1.03% | 3303-1501 | 1.03% | 0301-1202 | 1.03% | 3001-0602 | 1.06% | | | 3501-0401 | 1.03% |
| 1101-4801 | 1.03% | 0101-0101 | 1.00% | 1101-1502 | 1.03% | 0101-0301 | 1.03% | | | 3801-1203 | 1.03% |
| 1101-5201 | 1.03% | | | 2601-0702 | 1.03% | 0203-0301 | 1.03% | | | 4002-0304 | 1.03% |
| 2402-1301 | 1.03% | | | 2901-1505 | 1.03% | 2402-0201 | 1.03% | | | 4403-0401 | 1.03% |
| 2901-0705 | 1.03% | | | 3101-0401 | 1.03% | 2402-0502 | 1.03% | | | 4403-1403 | 1.03% |
| | | | | 3303-0102 | 1.03% | 3001-0601 | 1.03% | | | | |
| | | | | 3303-1403 | 1.03% | 3101-0602 | 1.03% | | | | |

| *B-DQB1* | HF. | *DRB1-C* | HF. | *DRB1-DQB1* | HF. | *C-DQB1* | HF. | *A-B-DRB1-C-DQB1* | HF. |
|---|---|---|---|---|---|---|---|---|---|
| 1302-0202 | 10.34% | 0701-0602 | 10.99% | 0701-0202 | 13.10% | 0602-0202 | 10.30% | 3001-1302-0701-0602-0202 | 7.93% |
| 1301-0301 | 3.31% | 1501-0702 | 3.71% | 1501-0602 | 13.10% | 0304-0301 | 4.09% | 0201-1301-1202-0304-0301 | 2.07% |
| 1501-0602 | 3.10% | 0406-0401 | 3.24% | 0901-0303 | 10.69% | 0801-0301 | 4.05% | 2402-5401-1501-0102-0602 | 1.72% |
| 4601-0301 | 2.56% | 0803-0102 | 3.04% | 1202-0301 | 6.90% | 0702-0301 | 3.46% | 0201-0702-1501-0702-0602 | 1.38% |
| 0702-0602 | 2.32% | 1202-0304 | 3.03% | 1101-0301 | 5.86% | 0702-0303 | 3.15% | 0201-1302-0701-0602-0202 | 1.38% |
| 4403-0202 | 2.07% | 0901-0801 | 2.84% | 0405-0401 | 5.17% | 0102-0301 | 2.84% | 3303-5801-0301-0302-0201 | 1.38% |
| 4601-0601 | 2.07% | 0901-0702 | 2.67% | 0803-0601 | 4.83% | 0401-0602 | 2.62% | 3303-5801-1302-0302-0609 | 1.38% |
| 5201-0601 | 2.07% | 1501-0303 | 2.52% | 0301-0201 | 4.48% | 0102-0602 | 2.54% | 0207-4601-0901-0102-0303 | 1.03% |
| 5801-0201 | 2.07% | 0901-0102 | 2.10% | 0101-0501 | 4.14% | 0401-0302 | 2.53% | 1101-1501-1501-0401-0602 | 1.03% |
| 4001-0301 | 2.07% | 1501-0401 | 2.10% | 0406-0302 | 3.79% | 0303-0602 | 2.40% | 1101-1502-1202-0801-0301 | 1.03% |
| 5401-0301 | 1.98% | 1502-1202 | 2.07% | 1201-0301 | 3.79% | 0102-0303 | 2.17% | 1101-4001-1201-0702-0301 | 1.03% |
| 1501-0302 | 1.72% | 0901-0304 | 1.91% | 1502-0601 | 2.76% | 0102-0601 | 2.14% | 2402-5201-1502-1202-0601 | 1.03% |
| 4601-0303 | 1.55% | 1501-0102 | 1.91% | 0701-0303 | 1.72% | 0702-0601 | 2.13% | 3303-4403-0701-0701-0202 | 1.03% |

**Continued**

| *B-DQB1* | HF. | *DRB1-C* | HF. | *DRB1-DQB1* | HF. | *C-DQB1* | HF. | *A-B-DRB1-C-DQB1* | HF. |
|---|---|---|---|---|---|---|---|---|---|
| 0702-0301 | 1.38% | 1101-0102 | 1.85% | 1302-0609 | 1.72% | 1202-0601 | 2.07% | | |
| 5101-0501 | 1.38% | 0301-0302 | 1.72% | 1405-0503 | 1.72% | 0303-0301 | 2.06% | | |
| 5401-0401 | 1.38% | 1201-0801 | 1.68% | 1001-0501 | 1.38% | 0702-0602 | 1.86% | | |
| 5801-0609 | 1.38% | 0803-0702 | 1.45% | 1302-0604 | 1.38% | 0304-0303 | 1.79% | | |
| 4001-0303 | 1.29% | 0701-0701 | 1.38% | 1602-0502 | 1.38% | 0302-0201 | 1.72% | | |
| 5101-0303 | 1.21% | 1302-0302 | 1.38% | 0407-0301 | 1.03% | 0602-0501 | 1.59% | | |
| 5401-0602 | 1.12% | 1501-0801 | 1.28% | 0802-0402 | 1.03% | 0302-0609 | 1.38% | | |
| 1511-0303 | 1.03% | 0405-0102 | 1.20% | | | 0701-0202 | 1.38% | | |
| 3701-0501 | 1.03% | 0405-0303 | 1.03% | | | 0602-0303 | 1.36% | | |
| 3901-0601 | 1.03% | 0802-0304 | 1.03% | | | 0801-0303 | 1.35% | | |
| 4002-0602 | 1.03% | 1001-0602 | 1.03% | | | 0401-0301 | 1.25% | | |
| 4403-0602 | 1.03% | 1101-0304 | 1.00% | | | 0303-0303 | 1.25% | | |
| 4801-0301 | 1.03% | | | | | 0702-0501 | 1.23% | | |
| 5101-0302 | 1.03% | | | | | 0102-0501 | 1.17% | | |
| 5701-0303 | 1.03% | | | | | 0102-0401 | 1.04% | | |
| 4006-0301 | 1.01% | | | | | 0702-0502 | 1.03% | | |
| | | | | | | 1402-0501 | 1.03% | | |

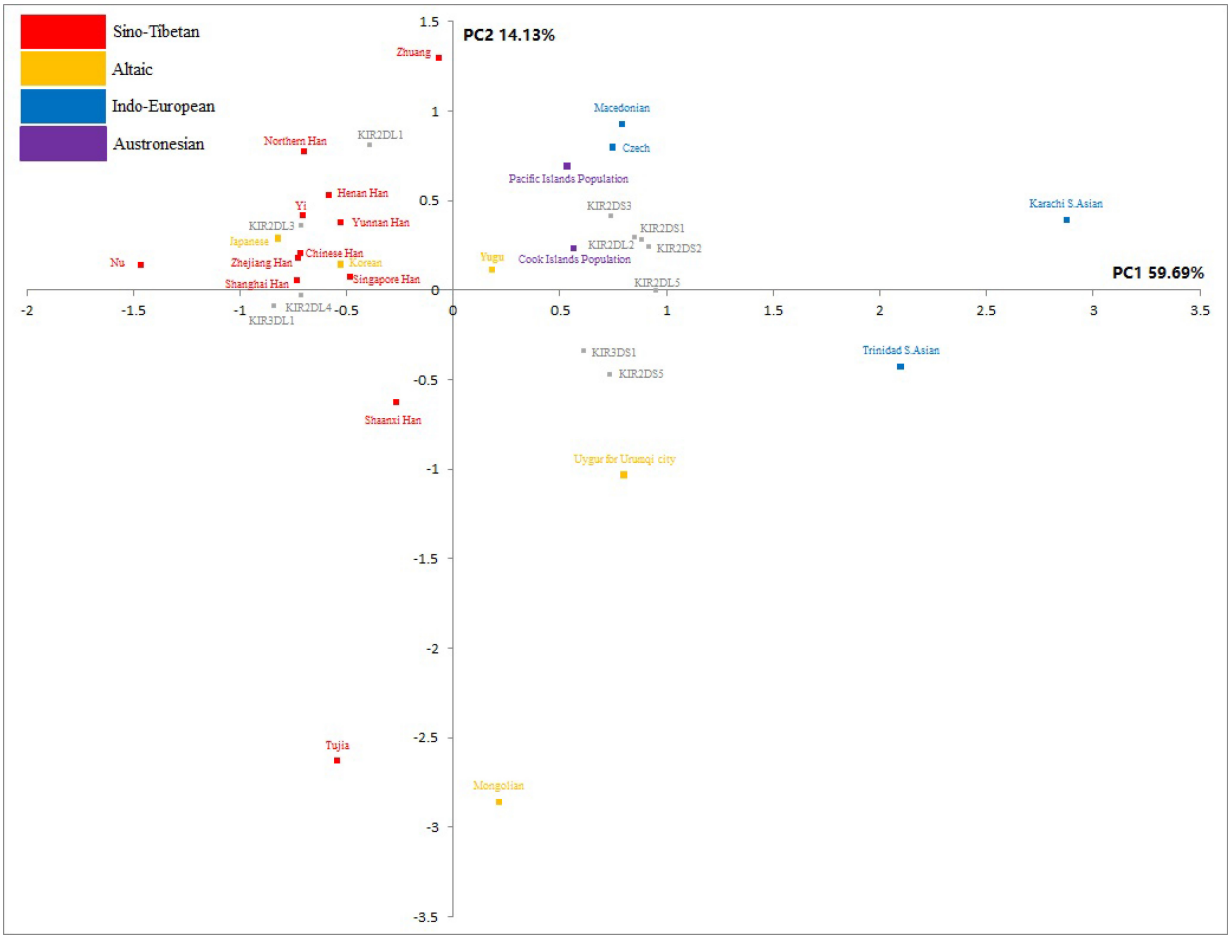


**Figure 3: The principal component analysis was constructed to study the relationships between the populations and *KIR* genes based on OFs of the 11 overlapping *KIR* genes in Chinese Henan Han and 22 other populations distributed worldwide.**

**Table 4: Distribution of the *KIR* ligands *HLA-A3, A11; HLA-Bw4, Bw4-80Ile, Bw4-80Thr; HLA-C1* and *C2* in the Henan Han population, China (*n* = 145)**

| Indexes | *HLA-A3 and/ or A11* | *HLA-Bw4*[a] | *HLA-Bw4-80Ile*[b] | *HLA-Bw4-80Thr*[c] | *HLA-Bw4-80Ile-80Thr* | *HLA-C1*[d] | *HLA-C2*[e] | *HLA-C1C1* | *HLA-C1C2* | *HLA-C2C2* |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of individuals | 55 | 119 | 74 | 68 | 23 | 92 | 67 | 25 | 30 | 8 |
| Percentage of individuals (%) | 37.93 | 82.07 | 51.03 | 46.9 | 15.86 | 63.45 | 46.21 | 17.24 | 20.69 | 5.52 |

[a]*HLA-Bw4* contained *HLA-B13, B27, B37, B38, B44, B51, B52, B57, B58, B59* and *B63*. [b,c]According to the differences of the amino acids encoded by the 80th position of the second exon of the *HLA-B* locus. [d]*HLA-C1* group contained *HLA-C1, C7 and C8*. [e]*HLA-C2* group contained *HLA-C2, C4, C5* and *C6*.

*KIR* gene between the Chinese Henan Han and 22 other populations. As shown in the figure, Henan Han was first clustered with Singapore Han and Shanghai Han, followed by other East Asian populations, and then by Pacific and Cook Islands populations, finally with the four European populations.

## *HLA* ligand polymorphisms

Five *HLA* loci were genotyped using PCR-SSO method and the allelic frequencies of *HLA-A, -B, -C, -DRB1* and *-DQB1* loci of 145 unrelated healthy Henan Han individuals were summarized in Table 2. Twenty-four alleles were detected at *HLA-A* locus in the population. The *HLA-A*02* group accounting for 28.61% of the total was found to be the most diverse allele family at *HLA-A* locus, and detected six alleles in our study: *HLA-A*0201, A*0203, A*0205, A*0206, A*0207 and A*0211*. The most common allele belonging to *HLA-A*02* group was *HLA-A*0201,* which accounted for 18.28% of the total. However, *HLA-A*0205, A*0211, A*2420, A*3004, A*0302, A*6824* and *A*6901* showed the lowest frequency of 0.34%.

*HLA-B* locus was detected with a total of 46 alleles and found to be the most diverse one of the five loci. *HLA-B*13* group accounting for 17.23% of the total was detected with the highest frequency in the locus and included two alleles: *HLA-B*1301* and *B*1302*. The most common allele belonging to *HLA-B*13* group was *HLA-B*1302*, accounted for 12.41% of the total. The *HLA-B*15* group accounting for 12.39% of the total was also found to be one of the most diverse allele family at *HLA-B* locus, and observed seven alleles: *HLA-B*1501, B*1502, B*1507, B*1511, B*1517, B*1518 and B*1527*. Nevertheless, there were 14 *HLA-B* alleles showing the lowest frequency of 0.34%.

Twenty-one alleles were detected at *HLA-C* locus in the population. *HLA-C*3* group accounting for 19.31% of the total was detected with the highest frequency and contained three alleles: *HLA-C*0302, C*0303* and *C*0304*. The most common allele belonging to *HLA-C*6* group was *HLA-C*0602* accounting for 15.17% of the total. The two other common *HLA-C* alleles with the frequencies higher than 10% were *HLA-C*0102* and *C*0702*.

At *HLA-DRB1* locus, twenty-nine alleles were detected in the population. *HLA-DRB1*15* group accounting for 17.93% of the total was detected with the highest frequency, and observed three alleles: *HLA-DRB1*1501, DRB1*1502 and DRB1*1506*. The *HLA-DRB1*4* group was found to be the most diverse allele family at *HLA-DRB1* locus and accounted for 12.06% of the total and consisted of six alleles: *HLA-DRB1*0401*, *DRB1*0403*, *DRB1*0405*, *DRB1*0406*, *DRB1*0407* and *DRB1*0410*. The three most common *HLA-DRB1* alleles with frequencies higher than 10% were *HLA-DRB1*1501, DRB1*0701* and *DRB1*0901* alleles, respectively.

*HLA-DQB1* locus was detected with a total of 15 alleles and found with the lowest polymorphism in the five *HLA* loci. *HLA-DQB1*3* group accounting for 38.97% of the total was detected with the highest frequency and contained three alleles: *HLA-DQB1*0301, DQB1*0302* and *DQB1*0303*. And the *HLA-DQB1*6* group accounted for 25.17% of the total including five alleles: *HLA-DQB1*0601, DQB1*0602, DQB1*0603, DQB1*0604* and *DQB1*0609* which was found to be the most diverse alleles at *HLA-DQB1* locus. *HLA-DQB1*0301* was found to be the most common allele, accounting for 20.69% of the total.

## Estimated *HLA* haplotype frequencies

The frequencies of the estimated main *HLA* haplotypes (the haplotypic frequency ≧ 1.00%) in the Han population from Henan province, China were shown in Table 3. A total of 129 *HLA A-B* haplotypes, 120 *A-DRB1* haplotypes, 90 *A-C* haplotypes, 92 *A-DQB1* haplotypes, 145 *B-DRB1* haplotypes, 67 *B-C* haplotypes, 127 *B-DQB1* haplotypes, 116 *DRB1-C* haplotypes, 41 *DRB1-DQB1* haplotypes, 94 *C-DQB1* haplotypes and 213 *A-B-DRB1-C-DQB1* haplotypes were estimated using the expectation maximization (EM) algorithm. *HLA A*3001-B*1302*, *A*3001-DRB1*0701*, *A*3001-C*0602*, *A*3001-DQB1*0202*, *B*1302-DRB1*0701, B*1302-C*0602*, *B*1302-DQB1*0202, DRB1*0701-C*0602, DRB1*0701-DQB1*0202*, C*0602-DQB1*0202 and A*3001-B*1302-DRB1*0701-C*0602-DQB1*0202 were the most common haplotypes in the group with the frequencies of 9.31%, 7.90%, 9.30%, 7.91%, 10.34%, 12.41%, 10.34%, 10.99%, 13.10%, 10.30% and 7.93%, respectively.

### *KIR–HLA* ligand combinations

The distribution of the *KIR* ligands including *HLA-A3, A11, Bw4, Bw4-80Ile, Bw4-80Thr, C1* and *C2* in the population was shown in Table 4. In the study, there were 119 individuals having *HLA-Bw4* epitope accounting for 82.07% of the total, which contained *HLA-B13, B27, B37, B38, B44, B51, B52, B57, B58, B59 and B63* alleles. On the basis of a dimorphism at position 80 of the α1 domain, *HLA-C* can be distinguished into two groups of ligands *C1* (HLA-$C^{asp80}$) and *C2* (HLA-$C^{lys80}$). A total of 92 individuals belonging to *HLA-C1* group accounted for 63.45% of the total which contained *HLA-C1*, *C7* and *C8*. And 67 individuals belonging to *HLA-C2* group, including *HLA-C2*, *C4*, *C5* and *C6*, accounted for 46.21% of the total.

Correlation analysis between *KIRs* and their special ligands *HLA-A3, A11, Bw4, C1* and *C2* in the population was listed in Table 5. In *KIR-C1/C2* groups, the most frequent association was *2DL3/C1*, with a frequency of 63.45%. The rarest association was *2DS1/C2C2*, with a frequency of 1.38%. In *KIR-Bw4* groups, there were 114 individuals having the association of *3DL1/Bw4* accounting for 78.62%. The association of *3DS1+/Bw4 (80Ile)+* accounted for 17.24% of the total. In the *KIR-A3/A11* groups, there were 55 individuals with the association of *3DL2+/A3* and/or *A11* accounting for 37.93% and 34 individuals with the association of *2DS4*FUL+/A11+* accounting for 23.45%.

In the study, we also conducted correlation analysis to investigate population-level evidence for co-evolution of the *KIR/HLA* loci based on the allelic frequencies of the receptor-ligand pairs including *2DL2/C1*, *2DL3/C1*, *2DS2/C1*, *2DL1/C2*, *2DS1/C2*, *3DL1/Bw4*, *3DL1/Bw4-80Ile* and *3DS1/Bw4-80Ile*. The populations included our studied Chinese Henan Han population and the other 30 geographically distinct populations distributed worldwide namely Biaka, Ethiopian, Hausa, Ibo, Mbuti and Yoruba in Africa; Adygei, CEPH_UT, Danish, European, Finns, Irish and Russian in Europe; Druze and Yemenites in Southwest Asia; Ami, Atayal, Cambodian, Hakka, Han_SF, Han_Taiwan, and Japan in East Asia; Micronesia and Nasioi in Oceania; Yakut in Northeast Asia; Maya and Pima in North America; Karitiana, Surui and Ticuna in South America [37]. Each individual was tested for the presence or absence of *HLA- C1, C2, Bw4, Bw4-80Ile*; *KIR2DL1*, *2DL2*, *2DL3*, *3DL1*, *2DS1*, *2DS2 and 3DS1*. In *KIR-C1/C2* groups, the *KIR2DS1* and *HLA-C2* ligand group showed a strong negative correlation ($r$ = -0.460; $P$ = 0.009) shown in Figure 6A and Table 6. In *KIR-Bw4* groups, *3DL1/Bw4* showed a strong positive correlation ($r$ = 0.399; $P$ = 0.026), *3DL1/Bw4 (80Ile)* also showed a strong positive correlation ($r$ = 0.447; $P$ = 0.012) and *3DS1/Bw4 (80Ile)* a strong negative correlation ($r$ = -0.656; $P$ = 0.000) which were showed in Figure 6B–6D and Table 6, respectively.

## DISCUSSION

Henan is located in the east-central part of China, along the middle and lower reaches of the Yellow River. With most parts in the history located in the south of the Yellow River, it is therefore named Henan. By the end of 2016, Henan has a resident population of 94.8 million people, ranking 3rd in China. The Han population is the main body of Henan resident population, accounting for 99.66% of the population of the whole province, while the minority population accounts for 0.34% of the province's population.

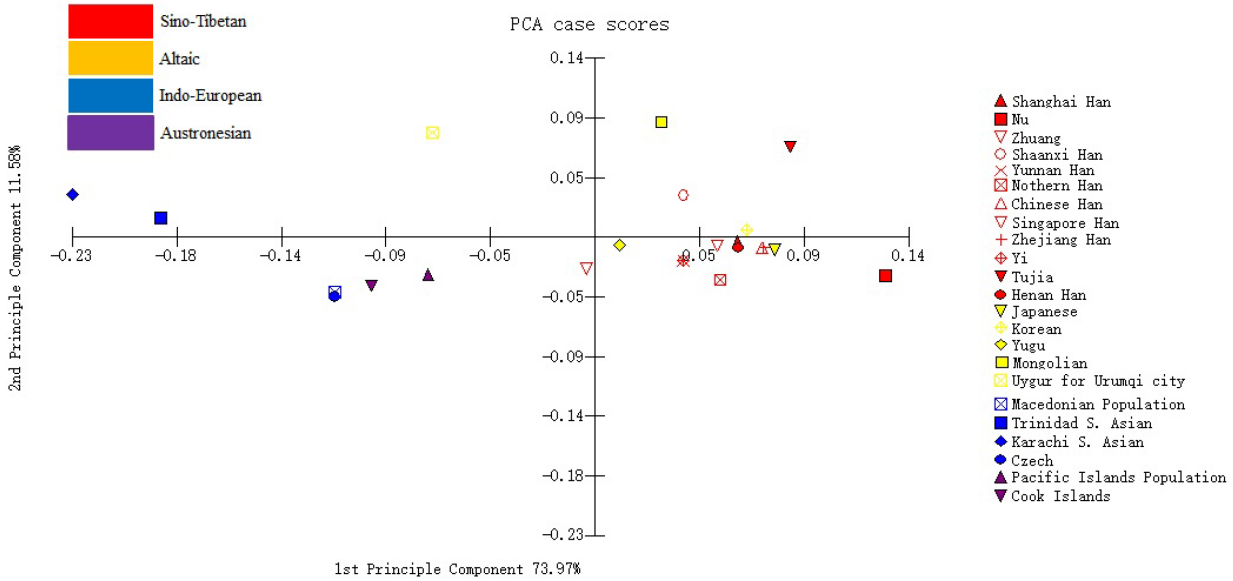


**Figure 4: The principal component analysis plot was constructed to study the genetic relationships of the Chinese Henan Han and 22 other populations distributed worldwide based on the 13 overlapping *KIR* gene frequencies.**

Henan is the birthplace of the Chinese nation and the Chinese civilization. Among the four ancient Chinese inventions, three of them, the compass, paper and gunpowder, are invented in Henan province. There are more than 20 dynasties in the history founding capital here or moving here as the capital. Henan province is the province which has the most dynasties, the longest history and the largest number of ancient capitals. There was a long time in ancient times that Henan has always been China's political, economic, cultural and transportation

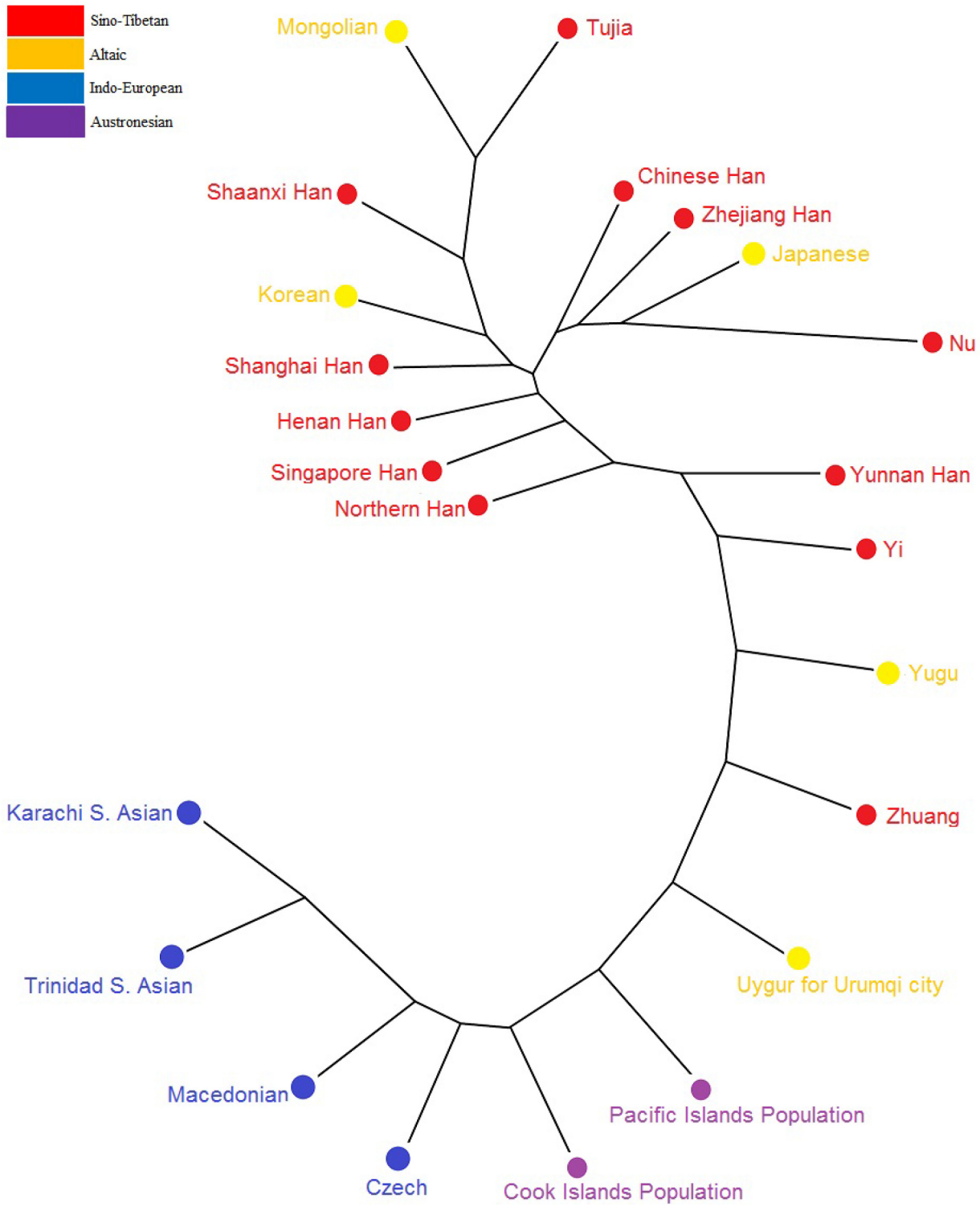


**Figure 5: A Neighbor-Joining tree was constructed between the Chinese Henan Han and 22 other populations distributed worldwide based on genotype data of the 13 overlapping *KIR* gene.**

center. So, the indigenous Han people living in this area are usually regarded as the very original Hans. That's the reason to select the Han population of Henan province, China to study. To our knowledge, to date, this was a first report about correlation analysis study of *KIR* genes and *HLA* ligands in the Henan Han population, and the present study also provided population data of 5 *HLA* loci and 19 *KIRs* for population genetics and immunogenetics.

*KIR* genes and *KIR* genotypes showed extensive genetic diversity in populations from different geographical regions and different ethnic groups [5,

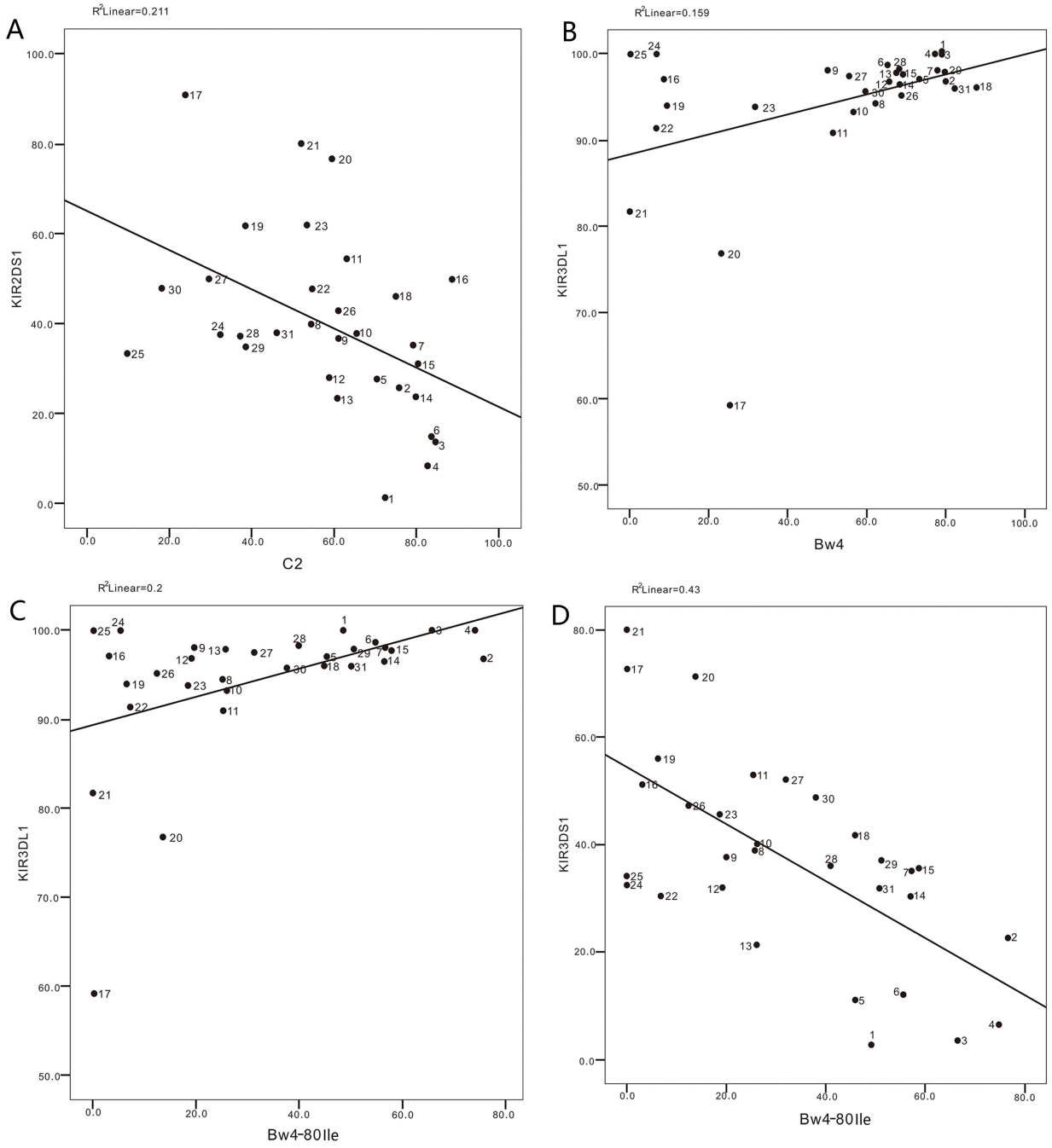


**Figure 6: Correlation analysis was constructed based on the *KIR* and *HLA* ligand frequencies.** (**A**) *2DS1/C2* showed a strong negative correlation; (**B**) *3DL1/Bw4* showed a strong positive correlation; (**C**) *3DL1/Bw4-80Ile* showed a strong positive correlation; (**D**) *3DS1/Bw4-80Ile* showed a strong negative correlation. The numbers represented the population as following: 1. Biaka, 2. Ethiopian, 3. Hausa, 4. Ibo, 5. Mbuti, 6. Yoruba, 7. Adygei, 8. CEPH_UT, 9. Danish, 10. European, 11. Finns, 12. Irish, 13. Russian, 14. Druze, 15. Yemenites, 16. Ami, 17. Atayal, 18. Cambodian, 19. Hakka, 20. Han_SF, 21. Han_Taiwan, 22. Japan, 23. Micronesia, 24. Nasioi, 25. Yakut, 26. Maya, 27. Pima, 28. Karitiana, 29. Surui, 30. Ticuna, 31. Chinese Henan Han population.

**Table 5: Distribution of the *KIRs* and their special *HLA* ligands in Henan Han population, China**

| *KIR-HLA-C1/C2* groups | NI | PI (%) | *KIR-HLA-Bw4* groups | NI | PI (%) | *KIR-HLA-A3/A11* groups | NI | PI (%) |
|---|---|---|---|---|---|---|---|---|
| *2DL2/C1* | 17 | 11.72 | *3DL1/Bw4* | 114 | 78.62 | *3DL2+/A3 and/or A11* | 55 | 37.93 |
| *2DL3/C1* | 92 | 63.45 | *3DL1/Bw4(80Ile)* | 71 | 48.97 | *3DL2+/A3+/A11-* | 11 | 7.59 |
| *2DS2/C1* | 17 | 11.72 | *3DL1/Bw4(80Thr)* | 66 | 45.52 | *3DL2+/A3-/A11+* | 44 | 30.34 |
| *2DL1/C2* | 67 | 46.21 | *3DS1+/Bw4(80Ile)+* | 25 | 17.24 | *2DS4*FUL+/A11+* | 34 | 23.45 |
| *2DS1/C2* | 25 | 17.24 | *3DS1+/Bw4(80Ile)-* | 22 | 15.17 | *2DS4*FUL +/A11-* | 78 | 53.79 |
| *2DL2/C1C1* | 4 | 2.76 | *3DS1-/Bw4(80Ile)+* | 49 | 33.79 | *2DS4*FUL -/A11+* | 10 | 6.9 |
| *2DL3/C1C1* | 26 | 17.93 | | | | | | |
| *2DS2/C1C1* | 4 | 2.76 | | | | | | |
| *2DL1/C2C2* | 8 | 5.52 | | | | | | |
| *2DS1/C2C2* | 2 | 1.38 | | | | | | |

NI: Number of individuals; PI: Percentage of individuals.

20–36]. The present study also confirmed this. In Figure 2, *KIR2DL5, 3DS1, 2DS1, 2DS5, 2DL2, 2DS2 and 2DS3* clustered together which belonged to B haplotype showed higher diversity than the other 6 *KIR* genes which clustered together belonged to the A haplotype. The results of Figure 3, 4 and 5 showed that the Henan Han was clustered with the groups from East Asian like Shanghai Han, Northern Han, Singapore Han, Shaanxi Han, etc. which were in accordance with many previous studies on different genetic markers, such as the population genetic analysis based on the 21 or 20 STRs of Chinese Henan Han, respectively [38, 39].

The sequences of human *KIR* genes in the extracellular, transmembrane and cytoplasmic domains were extremely conserved, but the *KIR* genes have evolved to be a highly polymorphic family of receptors. Genetic evidence indicated that the genes evolved through duplication and recombination, which was probably accelerated by their close proximity of head-to-tail orientation within the *KIR* cluster chromosomal locus in human genomics [40]. In addition, some alleles of individual *KIR* genes have produced through point mutations and minor sequence variations encoding one to several amino acids [41]. The extensive genetic diversity and different combinations of *KIR* genes of individuals made the diversity of the NK cell repertoire in peripheral blood, and therefore the NK cell could recognize diverse *HLA-I* allotypes and produce varying degrees of immune function.

*HLA* showed extensive genetic diversities, as did the *KIR* gene, in populations from different geographical regions and different ethnic groups [42]. The allelic frequencies of *HLA-A, -B, -C, -DRB1* and *-DQB1* loci were tested by using PCR-SSO method. A total of 135 alleles of *HLA-A, -B, -C, -DRB1* and *-DQB1* loci were detected in the study population. *HLA-B* locus was detected with a total of 46 alleles and found to be the most diverse locus in the five loci. According to the *IMGT/HLA* database (http://www.ebi.ac.uk/imgt/hla/stats.html, March 11, 2017), 3830 *HLA-A*, 4647 *HLA-B*, 3382 *HLA-C*, 2011 *HLA-DRB1* and 1054 *HLA-DQB1* alleles have been identified at *HLA* class I and class II moleculars in the world, which indicates that the *HLA* system constitutes the most complex and highly polymorphic genetic system in the human genome.

The immune function of NK cells was achieved through the signals derived from cell surface activating and inhibitory *KIR* receptors interacting with their major ligands: *HLA* class I (*HLA-A, -B and -C*) molecules. *KIR/HLA* ligand interactions were especially diverse. And a great number of previous studies have demonstrated associations between inheritance of certain combinations of *KIR* and *HLA* genes and susceptibility to many different diseases, including viral infections, autoimmune disorder, cancers, etc [43–46]. In general, *HLA-C1* was the ligand of *KIR2DL2/3* and *KIR2DS2*, and *HLA-C*2 was the ligand of *KIR2DL1* and *KIR2DS1*. The previous studies [37] have shown that *KIR3DL1* bind to *HLA-Bw4* allotype and *Bw4-Ile80* alleles were the better ligands for *KIR3DL1* than *Bw4-Thr80* alleles. And the ligand of *KIR3DS1* was known as *Bw4-Ile80*, which might be due to the strong similarity between the extracellular domains of *KIR3DS1* and *KIR3DL1*. *KIR3DL2* interacted with *HLA-A3* and *-A11* allele families. A recent study showed that *KIR2DS4* (full-length) bound specifically to the molecules *HLA-C1*, *-C2* and *-A11*, whereas *2DS4* (14bp deleted) was nonfunctional [11–18]. Several models have been proposed to explain the maintenance of this degree of diversity, including frequency dependent selection, heterozygote advantage and selection that varies in time and/or space [47, 48]. In the study, we also conducted correlation analysis to investigate population-level evidence for co-evolution of the *KIR/HLA* loci based on the frequencies of the receptor-ligand pairs including *2DL2/C1*, *2DL3/C1*, *2DS2/C1*, *2DL1/C2*, *2DS1/C2*, *3DL1/Bw4*, *3DL1/Bw4-80Ile* and *3DS1/Bw4-80Ile*. Among the 8 receptor-ligand pairs, *2DS1/C2* and *3DS1/Bw4-80Ile* showed a strong negative

**Table 6: Correlation analyses between *KIR* and *HLA* ligand based on allelic frequencies**

| *KIR-HLA-C1/C2* groups | Correlation *r* values | *P* values | *KIR-HLA-Bw4* groups | Correlation *r* values | *P* values |
|---|---|---|---|---|---|
| *2DL2/C1* | -0.274 | 0.136 | *3DL1/Bw4* | 0.399 | **0.026** |
| *2DL3/C1* | 0.184 | 0.321 | *3DL1/Bw4(80Ile)* | 0.447 | **0.012** |
| *2DS2/C1* | -0.254 | 0.168 | *3DS1/Bw4(80Ile)* | -0.656 | **0.000** |
| *2DL1/C2* | 0.019 | 0.918 | | | |
| *2DS1/C2* | -0.460 | **0.009** | | | |

Statistically significant *p* values ($p < 0.05$) are indicated in boldface type.

correlation, but *3DL1/Bw4* and 3DL1/Bw4-80Ile showed a strong positive correlation. For the *KIR2DL2* and *2DS2* loci, the correlation with *C1* group was negative (*r*=-0.274 and -0.254) but not significant. For the *KIR2DL3* locus, the correlation with C1 group was positive ($r = 0.184$) but not significant. The correlation of the *2DL1/C2* pair was also positive ($r = 0.019$) but not significant. It may show that some *KIR/HLA* pairs were possibly the dominant factor in forming the frequency distributions and the other *KIR/HLA* pairs were simply hitchhiking. Studies of the LD of the *KIR/HLA* pairs have confirmed the above phenomenon [37]. Through the studies of many groups of *KIR/HLA*, the results indicated that the *KIR* genes were evolving at a more rapid rate than the *HLA class I* ligand groups because they found that some pairs of neighboring populations shared similar *HLA* ligand group frequencies but had highly distinct phenotypic *KIR* gene frequencies. And their data provided population-level evidence for the evolution of the *KIR* gene cluster owing to selection pressure favoring frequencies of activating *KIR* that suit the specific *HLA* ligands [37]. Our data also support the above conclusions. In addition, our data will provide some immunogenetic information and supplementary data for the study of the *KIR/HLA* co-evolution. Studies performed over the last several years have revealed that the extensive genomic diversity of the *KIR/HLA* pairs and the key role of their interactions in both innate and adaptive immunity was able to explain the co-evolution of these two immunogenetics markers in order to maintain appropriate functional interaction [49, 50]. Evidence of *HLA-KIR* co-evolution within and across populations has also been suggested in disease studies [49, 50].

## MATERIALS AND METHODS

### Study population

Blood samples were obtained from 145 unrelated healthy individuals of the Han population from Henan province in central China. All the individuals provided their written informed consent for the collection of the samples and subsequent analysis. And the investigation and study were conducted in accordance with humane and ethical research principles of Henan Provincial People's Hospital and Xi'an Jiaotong University Health Science Center, China, and approved by the Ethics Committee of Henan Provincial People's Hospital and Xi'an Jiaotong University Health Science Center, China.

### Genomic DNA extraction

Whole blood samples containing ethylene diamine tetra aceticacid were utilized for DNA extraction with TIANamp Genomic DNA Kit (TIANGEN Biotech, Beijing, China) following the manufacturer's instructions. Genomic DNA samples were quantified by NanoDrop 2000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, NC, USA). The optical density values ranged from 1.6 to 1.8, evaluating the concentration and purity of the extracted genomic DNA, and the final concentration was adjusted to approximately 50 ng/μL in distilled water ($dH_2O$). All DNA samples were stored at -20°C until amplification.

### KIR genotyping

Genotypes for *KIRs* were obtained by PCR amplification with sequence specific primer methods (PCR-SSP) using the Invitrogen *KIR* Genotyping PCR-SSP Kit (Invitrogen Carlsbad, CA, USA), according to the manufacturer's instructions. The kit consists of panels of primer mixes where each primer mixture contains one or more specific primer pairs, i.e. the allele- and/or group-specific primers, as well as a control primer pair matching non-allelic sequences. And 16 *KIR* genes and 3 pseudogenes *(KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5A, 2DL5B, 2DS1, 2DS2, 2DS3, 2DS4*FUL, 2DS4*DEL, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1, 3DP1*FUL and 3DP1*DEL)* were tested in the samples. The total reaction volume was 10 μl, established on the basis of the manufacturer's instructions. All amplifications were performed in a GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) and PCR amplification parameters included a 1 min denaturing step at 95ºC, 30 cycles of 94ºC for 20s, 63ºC for 20s, 72ºC for 90s. PCR products of all samples were analyzed for *KIR* genotyping according to the manufacturer's instructions by the specific presence or absence band of each *KIR* in 2% agarose gels, which were well-mixed with ethidium

bromide. Each lane of the gel, containing a loaded PCR sample product, should be a control band, and a positive reaction band if there was presence of *KIR*, and vice versa, except for a negative control well. The false reaction, displaying no control band, was repeated.

### *HLA* genotyping

Genotypes for *HLA-A, -B -C, -DRB1* and *-DQB1* loci were obtained by PCR using sequence-specific oligonucleotide (PCR-SSO) using the LABType™ HD SSO *HLA* typing and LABType® SSO *HLA* typing Tests (One Lambda, Inc. Canoga Park, CA, USA). PCR amplifications of five *HLA* loci were in a GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA), respectively. And *HLA* genotyping was performed using LABScan™ 100 and Luminex XY platform (One Lambda, Inc. Canoga Park, CA, USA) according to the manufacturer's instructions.

### Statistical analysis

The OFs of *KIRs* in the group were determined from the number of positive typing reactions divided by the total number of individuals. GFs of *KIRs* were estimated using the formula $GF = 1-(1-OF)^{1/2}$, where OFs were the above-mentioned observed carrier frequencies of *KIRs* in studied individuals.

Based on the 13 overlapping *KIR* gene frequencies (*KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3, 2DS1, 2DS2, 2DS3, 2DS5 and 3DS1*), a heatmap containing Chinese Henan Han and 22 other populations was drawn using Package 'pheatmap' (https://cran.r-project.org/web/packages/pheatmap/index.html) based on statistical software *R* version 3.2.5 (https://www.r-project.org/). And the heatmap was constructed using Hierarchical Clustering algorithm based on Euclidean distance. *KIR2DP1, 3DP1*, *2DS4* and *ID* loci were omitted in the heatmap, because they were not previously reported in some compared populations.

The PCA in Figure 3 was conducted by the statistical software SPSS Version 13.0. based on 11 overlapping loci. The PCA plot in Figure 4 was drawn based on 13 overlapping loci by the the MVSP-A MultiVariate Statistical Package for Windows, ver. 3.1. (Kovach Computing Services, Pentraeth, Wales, U.K.).

Based on the 13 overlapping *KIR* gene genotype data mentioned above, a NJ tree (shown in Figure 5) including Central Chinese Han and 22 other populations was drawn by the Phylip 3.69 (http://evolution.gs.washington.edu/phylip.html).

Allelic frequencies of *HLA-A, -B -C, -DRB1* and *-DQB1* loci were calculated using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Haplotypic frequencies were calculated using genotype data by EM algorithm using Arlequin software package version 3.5 (Laurent Excoffier, CMPG, Zoological Institute, University of Bern, Switzerland).

The correlation analysis to investigate population-level evidence for co-evolution of the *KIR/HLA* loci between the gene frequencies of the receptor-ligand pairs including *2DL2/C1, 2DL3/C1, 2DS2/C1, 2DL1/C2, 2DS1/C2, 3DL1/Bw4, 3DL1/Bw4-80Ile* and *3DS1/Bw4-80Ile* was conducted by the statistical software SPSS Version 13.0 (SPSS Inc., Chicago, IL, USA).

## CONCLUSIONS

In summary, this study may provide basic and valuable polymorphism data of *KIR* genes, *HLA* genes and *KIR/HLA* combinations for anthropological analysis and associated disease studies. In addition, it may provide some clues of the co-evolution of these two complex genetic systems as studied the *KIR/HLA* pairs.

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## CONFLICTS OF INTEREST

None.

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