Genetic Polymorphisms at *HLA-A, -B*, and *-DRB1* Loci in Han Population of Xi'an City in China

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Aim To determine genetic polymorphisms at human leukocyte antigen (*HLA*)*-A, -B*, and *-DRB1* loci in Han population of Xi'an city in China.

Methods Polymerase chain reaction-based reverse line-strip sequence specific oligonucleotide hybridization was used to determine the alleles of *HLA-A, -B,* and *-DRB1* in 516 unrelated, healthy individuals of Han population in Xi'an. Allele frequencies at *HLA-A, -B*, and *-DRB1* loci were estimated by direct counting method. Haplotype frequencies were calculated from genotype data by expectation maximization.

Results A total of 14 alleles of *HLA-A*, 33 alleles of *HLA-B*, and 13 alleles of *HLA-DRB1* were found. The most common alleles were *HLA-A**02 (28.39%), *A**11 (19.19%), and *A**24 (16.28%); *HLA-B**13 (11.05%), *B**15 (B62: 9.30%), and *B**51 (8.53%); and *HLA-DRB1**15 (17.15%), *DRB1**09 (13.18%), and *DRB1**04 (10.85%). The most common haplotypes of *HLA-A-B-DRB1* haplotype were *HLA-A*30-B*13-DRB1*07* (3.93%), *HLA-A*02-B*46-DRB1*09* (3.20%), and *HLA-A*33-B*58-DRB1*17* (1.63%).

Conclusion The finding that the *HLA* loci are highly polymorphic in Han population of Xi'an City may be useful for population genetics, *HLA*-related studies, human identification, and paternity tests in forensic sciences.

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Human leukocyte antigen (*HLA*) is a large, closely linked cluster of genes located on chromosome 6p21.3 (1). The *HLA* system is one of the most polymorphic immunological genetic systems in human genome and usually used in the anthropological analysis, disease linkage analysis, population genetics, forensic sciences, and organ transplantation, especially bone marrow transplantation (2,3). Previous studies have shown that allele and haplotype distribution in the *HLA* system differ from one ethnic group to another or between the members of the same ethnic group living in different geographic areas. Also, certain alleles are exclusively found in some ethnic groups (4). The comparisons between different populations using genetic distances calculated from *HLA* allele or haplotype frequencies have been used to determine the genetic relationship between different ethnic groups, so the *HLA* genetic markers are valuable tools for tracing ancient human migrations and determining the origins of different ethnic groups (5-8). In the present study, we determined the genetic polymorphisms at *HLA-A, -B*, and *-DRB1* loci in a Chinese Han population in Xi'an city and compared them with other neighboring populations.

Materials and methods

Population samples

After obtaining informed consent, whole blood samples were taken from 516 unrelated healthy individuals, aged 16 to 42 years, from the Chinese Han population in Xi'an city. Study participants were randomly chosen among individuals from Chinese national marrow donor program whose ancestors had been living in the region for at least 3 generations. Two milliliters of whole blood sample were obtained by venipuncture and collected into EDTA tubes.

Genomic DNA extraction

High-concentration-salt precipitation methods (9) were used to extract genomic DNA from 200 μL whole blood sample. The purity of the extracted DNA ranged from 1.6 to 1.9 optical density value.

Polymerase chain reaction sequence specific oligonucleotide amplification and typing

Polymerase chain reaction sequence specific oligonucleotide (PCR-SSO) amplification for *HLA-A, -B,* and *-DRB1* loci was performed using INNO-LiPA *HLA-A* Update, INNO-LiPA *HLA-B* Update, and INNO-LiPA *HLA-DRB1* kits, respectively (Innogenetics, Ghent, Belgium). According to the protocols of the kit, all the reactions were in 10 μL total volume. Thermal cycling of amplification reaction in *HLA-A* and *-B* loci was conducted under the following conditions: at 96°C for 5 minutes; 5 cycles at 96°C for 30 seconds, 64°C for 50 seconds, 72°C for 50 seconds; 5 cycles at 96°C for 30 seconds, 62°C for 50 seconds, 72°C for 50 seconds; 10 cycles at 96°C for 30 seconds, 60°C for 50 seconds, 72°C for 50 seconds; 15 cycles at 96°C for 30 seconds, 55°C for 50 seconds, 72°C for 50 seconds, and a final extension at 72°C for 10 minutes. Thermal cycling of *HLA-DRB1* locus was conducted as follows: 95°C for 5 minutes; 35 cycles at 95°C for 20 seconds, 58°C for 20 seconds, 72°C for 20 seconds, and a final extension at 72°C for 10 minutes. Thermal cycling was conducted using GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA). Detection and genotyping of all PCR products were performed by the reverse dot blot hybridization assay (Innogenetics).

Statistical analysis

Allele frequencies of *HLA-A, -B*, and *-DRB1* loci were estimated by direct counting method. Haplotype frequencies were calculated from genotype data by expectation maximization method using Arlequin software package, version 3.01 (Laurent Excoffier, CMPG, Zoological Institute, University of Bern, Swit-

zerland). Distributional differences in allele frequency in different populations were compared by χ^2 test using the Statistical Package for the Social Sciences, version 11.0 (SPSS Inc., Chicago, IL, USA). Genetic distance among different populations was calculated according to Nei (10), and a phylogenetic tree based on the neighbor-joining method (neighbor-joining tree construction from allele frequency of *HLA-DRB1* loci) was constructed using the Mega3.1 software package (Center for Evolutionary Functional Genomics, The Biodesign Institute Tempe, AZ, USA). Tests for Hardy-Weinberg equilibrium were performed using Arlequin software.

Results

The genotypic frequency distribution of *HLA-A, -B*, and *-DRB1* loci was in Hardy-Weinberg equilibrium (χ^2 test, *P* values of *HLA-A, -B,* and *-DRB1* loci were 0.8285, 0.3111, and 0.5594, respectively).

Genetic polymorphisms of HLA-A,-B, and -DRB1 loci

The allele frequency distributions of *HLA-A, -B*, and *-DRB1* loci of Chinese Han population in Xi'an city are shown in Table 1. We found 14 alleles at *HLA-A* locus, 33 alleles at *HLA-B* locus, and 13 alleles at *HLA-DRB1* locus. In the population, *HLA-A**02 was the most common *HLA-A* allele (28.39%), followed by *A**11 (19.19%), and *A**24 (16.28%); on the *HLA-B* locus, the most common allele was *HLA-B**13 (11.05%), followed by *B**15 (antigen specificity 62) (9.3%), and *B**51 (8.53%); and on the *HLA-DRB1* locus, the most common alleles were *HLA-DRB1**15 (17.15%), *DRB1**09 (13.18%), and *DRB*1*04 (10.85%).

HLA haplotype frequency

We observed 1418 different *HLA* haplotypes, which is 23% of the expected theoretical haplotype number. The frequencies of 400 haplotypes in the population were more than 10^{-5}. The significant haplotype frequency of *HLA-A-B-DRB1* (haplotype frequency ≥2/3N; N = sample size) (11) in the population is shown in Table 2. The most common haplotypes of *HLA-A-B-DRB1* haplotype were *HLA- A*30-B*13-DRB1*07* (3.93%), *HLA-A*02-B*46-DRB1*09* (3.20%), and *HLA-A*33-B*58-DRB1*17* (1.63%).

Construction of phylogenetic tree

Neighbor-joining dendrogram of DRB1 loci showed the relationship between Han population in Xi'an and other neighboring ethnic groups or areas (7,12-22). The phylogenetic tree (Figure 1) showed that the Xi'an Han population had the smallest genetic distance to the Shanxi Han (0.001), followed by

Table 1. Allele frequency distribution of *HLA-A, -B,* and *-DRB1* loci of Chinese Han population in Xi'an city (n = 516)

HLA-A	Allele frequency (%)	*HLA-B*	Allele frequency (%)	*HLA-B*	Allele frequency (%)	*HLA-DRB1*	Allele frequency (%)
1	5.33	7	4.36	54	3.10	1	4.17
2	28.39	8	1.16	55	2.13	3(17)	3.29
3	4.65	13	11.05	56	0.10	4	10.85
11	19.19	18	0.68	57	1.84	7	9.98
23	0.19	27	2.62	58	4.17	8	6.30
24	16.28	35	5.33	40(60)	7.75	9	13.18
26	3.49	37	1.65	40(61)	6.69	10	1.84
29	1.16	38	3.00	15(62)	9.30	11	7.56
30	6.40	39	1.16	15(63)	0.29	12	10.56
31	4.94	44	3.78	64	0.19	13	5.43
32	1.94	45	0.10	65	0.87	14	7.56
33	6.88	46	7.56	67	0.78	15	17.15
68	1.07	48	2.23	15(71)	1.16	16	2.13
69	0.10	49	0.19	15(72)	0.10		
		50	0.39	15(75)	4.26		
		51	8.53	81	0.19		
		52	3.29				

the Lanzhou Han (0.009), while the greatest genetic distance was found to be with the Luoba Tibet (0.136) population. Location of the Chinese Han population in Xi'an City is shown in Figure 2.

Discussion

In the present study, genetic polymorphisms at *HLA-A, -B,* and *-DRB1* loci in Chinese Han population of Xi'an city were analyzed using PCR-SSO. The Han population in Xi'an and the Han population in the southern and northern China showed no obvious difference at *HLA-A* locus, and the 3 predominant alleles (*HLA-A* *02, *A**11, *A**24) in the Han population of Xi'an city were also common in the Han populations of the southern and northern China (16,23-26). At *HLA-B* locus, however, there were obvious difference between Han population in Xi'an and Han population

Table 2. Haplotype frequencies of *HLA-A-B-DRB1* in Chinese Han population in Xi'an city (haplotype frequency ≥0.129%)

HLA-A-B-DRB1	Haplotype frequency (%)	*HLA-A-B-DRB1*	Haplotype frequency (%)	*HLA-A-B-DRB1*	Haplotype frequency (%)	*HLA-A-B-DRB1*	Haplotype frequency (%)
1 8 13	0.19	2 62 11	0.58	11 60 9	0.49	24 62 12	0.73
1 13 7	0.14	2 62 15	0.70	11 60 11	0.48	24 62 14	0.60
1 37 10	0.87	2 62 4	0.46	11 60 14	0.32	24 62 15	0.42
1 44 7	0.19	2 62 8	0.17	11 60 15	0.88	24 71 16	0.17
1 52 15	0.39	2 62 9	0.53	11 61 9	0.33	24 75 12	0.72
1 57 7	0.97	2 67 12	0.19	11 61 11	0.26	26 35 1	0.19
1 58 13	0.19	2 67 16	0.19	11 62 4	0.85	26 38 1	0.19
1 60 15	0.29	2 71 15	0.14	11 62 12	0.48	26 38 13	0.19
1 60 7	0.17	2 71 9	0.29	11 62 14	0.33	26 38 16	0.19
1 63 13	0.19	2 75 9	0.69	11 62 15	1.11	26 51 9	0.19
2 8 17	0.19	2 75 14	0.29	11 67 8	0.19	26 62 8	0.19
2 13 15	0.66	2 75 15	0.80	11 75 12	1.05	26 62 14	0.26
2 13 4	0.33	3 7 14	0.19	11 75 15	0.22	29 7 8	0.39
2 18 15	0.19	3 7 15	1.07	24 7 7	0.19	30 13 1	0.20
2 27 15	0.19	3 27 15	0.19	24 8 17	0.19	30 13 7	3.93
2 35 14	0.39	3 35 7	0.19	24 13 12	0.38	30 13 11	0.60
2 35 15	0.40	3 44 1	0.19	24 13 15	0.29	30 35 15	0.19
2 35 9	0.39	3 44 13	0.29	24 18 11	0.29	30 60 14	0.18
2 37 10	0.19	3 51 1	0.39	24 27 1	0.19	30 62 15	0.14
2 38 15	0.66	3 51 16	0.19	24 27 4	0.19	31 7 1	0.19
2 38 8	0.26	3 54 9	0.19	24 35 4	0.29	31 7 15	0.19
2 44 1	0.19	11 7 1	0.48	24 35 12	0.14	31 13 7	0.19
2 46 11	0.37	11 7 15	0.19	24 35 15	0.62	31 13 15	0.29
2 46 14	0.32	11 13 4	0.23	24 38 15	0.21	31 35 4	0.29
2 46 15	0.36	11 13 7	0.20	24 46 14	0.14	31 35 11	0.19
2 46 4	0.71	11 13 8	0.20	24 46 15	0.25	31 48 14	0.19
2 46 8	0.75	11 13 12	0.50	24 48 11	0.27	31 51 4	0.12
2 46 9	3.20	11 13 15	0.47	24 48 12	0.31	31 51 7	0.38
2 48 9	0.32	11 27 8	0.19	24 48 14	0.16	31 51 11	0.19
2 50 7	0.29	11 27 12	0.39	24 48 15	0.43	31 51 14	0.26
2 51 11	0.69	11 35 1	0.24	24 51 4	0.53	31 51 16	0.17
2 51 12	0.20	11 35 12	0.25	24 51 9	0.36	31 60 11	0.17
2 51 14	0.35	11 37 10	0.29	24 51 16	0.22	31 61 12	0.19
2 51 15	0.25	11 38 4	0.19	24 52 15	0.28	31 62 4	0.19
2 51 8	0.15	11 38 8	0.23	24 54 4	0.92	31 62 12	0.39
2 51 9	1.34	11 38 14	0.19	24 54 7	0.19	31 62 14	0.21
2 54 11	0.42	11 39 8	0.19	24 54 14	0.35	32 27 7	0.19
2 54 4	0.44	11 39 11	0.29	24 55 8	0.19	32 35 13	0.19
2 55 12	0.27	11 46 8	0.44	24 55 9	0.14	32 44 7	0.29
2 55 4	0.44	11 46 9	0.19	24 58 13	0.20	32 52 15	0.97
2 55 9	0.14	11 44 13	0.19	24 60 9	0.33	33 7 15	0.29
2 60 11	0.25	11 52 4	0.64	24 60 11	0.22	33 8 4	0.19
2 60 12	0.34	11 51 9	0.47	24 60 12	0.80	33 44 7	0.78
2 60 14	0.30	11 51 11	0.19	24 60 14	0.22	33 44 13	0.48
2 60 8	0.46	11 51 14	0.32	24 60 15	0.17	33 35 1	0.15
2 61 1	0.19	11 51 15	0.38	24 61 4	0.28	33 35 4	0.19
2 61 12	0.57	11 52 15	0.27	24 61 9	0.86	33 52 15	0.15
2 61 15	0.43	11 54 14	0.20	24 61 11	0.19	33 58 17	1.63
2 61 4	0.82	11 55 9	0.25	24 61 12	0.51	33 65 1	0.48
2 61 8	0.53	11 60 4	0.75	24 61 16	0.19		
2 61 9	0.63	11 60 8	0.23	24 62 11	0.20		

in the southern China. The most common alleles in the Han population of Xi'an city were *HLA-B**13 (11.05%), B*15(62) (9.30%), and B*51 (8.53%). These were also common in the Han populations of Shanxi (16) and Shandong Province (23) in China, but were much less observed in the southern China (24-26).

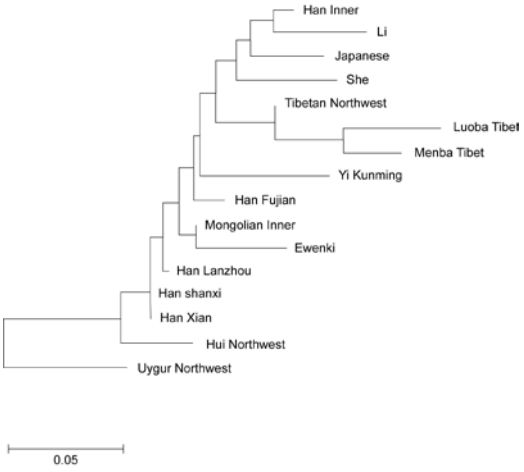

Figure 1. Dendrogram constructed by the neighbor-joining method showing the relationship between Han population with other 15 populations based on the allele frequencies of *HLA-DRB1* locus.

The most common alleles in the southern China were *HLA-B**46, *B**40(60), and *B**58. This shows that the Han populations of different Chinese geographic areas differ at *HLA-B* locus. The predominant allele *HLA-DRB1**12 in the Han population of Xi'an was less common in the Han populations of the southern and northern China.

The common frequent three-locus haplotype in Xi'an Han population was *HLA-A*30-B*13-DRB1*07* (3.93%), followed by the *HLA-A*02-B*46-DRB1*09* (3.20%) and *HLA-A*33-B*58-DRB1*17* (1.63%). Comparing the *HLA-A-B-DRB1* haplotype in Xi'an Han population with that of Chinese Han population in other areas (23-27), we found an increasing trend in the frequencies of *HLA-A*30-B*13-DRB1*07* haplotype from south to north. Frequencies of *HLA-A*02-B*46-DRB1*09* haplotype were higher in the southern than in the northern China. The haplotype frequency distributions of *HLA* loci of Han population in different regions were different.

Figure 2. Location of Chinese Han population in Xi'an City and other Chinese populations.

Neighbor-joining dendrogram showed the relationship among 15 populations based on the allele frequencies at *HLA-DRB1* locus. The cluster analysis of 15 populations showed significant differences between different regions and nationalities, with the differences between different ethnic groups having been more significant than those between different regions. Han population in Xi'an formed the strongest cluster with Shanxi Han population, followed by Lanzhou Han population, and northwestern Han population. Xi'an Han population was close to the Shanxi, Lanzhou, and northwestern Han population, but far from the Fujian and Inner Han population. Results are in accordance with geographic and historical features of Han population (28).

Results of the present study indicate that alleles and haplotypes at *HLA* loci in Xi'an Han population were highly polymorphic. Genetic characteristics of *HLA* loci in Xi'an Han population are similar to northern Han, but have some specificities in genetic polymorphisms.

In organ transplantation, compatibility between donor and recipient *HLA* haplotypes is more important than compatibility between the phenotypes. *HLA* haplotypes, therefore, could be used in search for the suitable donor in bone marrow bank. At present, there is a lack of *HLA* haplotype data, which warrants further studies on genetic polymorphisms of *HLA*.

Beside their usefulness in organ transplantation, the results of this study could enrich Chinese ethnic gene information resources and provide valuable basic population data for human population genetics, disease linkage analysis, forensic identity, and paternity testing.

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