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## Human Leukocyte Antigen (HLA) Class I and II Polymorphism in Iranian Healthy Population from Yazd Province

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### ABSTRACT

The major histocompatibility complex (MHC) genes are the most polymorphic loci in the human genome and have been widely studied in various populations and ethnic groups. Investigations into the HLA genes and proteins have been useful tool for anthropological, transplantation and disease association studies.

The polymorphism of the HLA class I (A, B, C) and class II (DRB1, DQA1, DQB1) genes were investigated in 90 unrelated Iranian individuals from Yazd province located in the center of Iran using sequence-specific primers (PCR-SSP). Allele and haplotype frequencies, expected/observed heterozygosity, unbiased expected heterozygosity, number of effective alleles, deviations from Hardy-Weinberg (HW) equilibrium and genetic diversity were computed.

A total of 23, 48, 23, 24, 13 and 16 alleles for HLA-A, -B, -C, -DRB1, -DQA and -DQB loci were determined, respectively in the population study. The most frequent allele identified in this study were A\*02:01 (18.889%), HLA-B\* 51:01 (12.778%), HLA-C\* 12:03 (17.033%), HLA-DRB\* 11 (24.4%), HLA-DQA\* 05:05 (20.55%) and HLA-DQB\*03:01 (22.8%). Furthermore, the most frequent 3-locus haplotypes were DRB\*11-DQA\*05:01-DQB\*03:01 (21.1%), HLA-A\*02:01- B \*50:01- DRB\*07:01 (4.9%) and A\*26:01 –B\* 38:01 –C\*12:03(5%). The most 4-locus haplotype were A\*11:01 –B\* 52:01 –C\*12:03 –DRB\*15(2.5%) and A\*02:01 –B\* 50:01 –DRB1\*07:01 –DQB1\*02:01(4.5%). The heterozygosity of the study population was confirmed the expected value and not deviated from Hardy-Weinberg equilibrium for all loci ( $p>0.05$ ).

Our study shows a close relatedness between Yazd population and other ethnic group of Iran despite some differences, which may be due to admixture of each one of these groups with each other or foreigner subpopulations during centuries. Moreover, the results of this study suggest that the Iranian population from Yazd province is in close vicinity with the Caucasians populations and far from the Korean and Japanese populations.

**Keywords:** Human leukocyte antigen (HLA); Polymorphism; Class I and II; Yazd province

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## INTRODUCTION

The Human Leukocyte Antigen (HLA) is a highly polymorphic locus in human's genome and composed of a closely related set of genes. HLA genes have been validated as useful tools for distinguishing and/or relating populations (and individuals) and for medical purposes such as: transplantation (establishing bone marrow donor registries);<sup>1</sup> HLA associated disease (autoimmune diseases, allergies and infectious disease);<sup>2-6</sup> anthropological; forensic fields,<sup>7-10</sup> and also designing vigorous vaccines with broad coverage against pathogens.<sup>11</sup> HLA gene frequencies correlate with ethnicity groups and geographic origin of populations. Therefore, basic data on the HLA allele and haplotype frequencies among different populations are needed. Even in the beginning of our era the Silk Road connected such powerful ancient empires as Rome, Parthia, Kushan and China, Iran is also an important constituent of the Silk Road had an extremely advantageous position between Asia and Europe, between the Mediterranean, the Caspian, the Black and the Azov seas playing an important role in international trade (<http://www.advantour.com/silkroad/iran.htm>).

Phylogenetic studies of human populations revealed that Iranian are a highly mixed population of Caucasoid origin and their ancestral genetic makeup of Aryans has broadly been mixed with different populations during previous centuries.<sup>12</sup> HLA and other genetic markers showed that most of current Iranians are Aryan but during the history, they have been encountered with different foreigners e.g. Macedonians (334 to 331 BC), Arabs (7<sup>th</sup> century), Turks (10<sup>th</sup> century), and Mongols (13<sup>th</sup> to 15<sup>th</sup> centuries).<sup>13</sup>

Therefore, the population living in this country might be admixed due to encounter with other populations and immigrants from neighboring populations.<sup>14</sup>

Iranians considered as non-European Caucasian population share HLA similarities with resident populations of the eastern and southern European countries. There are reports of studies on the Iranian sub-populations studies such as: Bakhtiari, Lurs<sup>15</sup> Arabs and Jews,<sup>16</sup> Balochs, Zabolis,<sup>17,18</sup> Parsees and Zoroastrians,<sup>19</sup> Kurds, and Azeris.<sup>20</sup>

Yazd province is located in the center of Iran (Figure 1) and due to this geographical location has been far from race-mixing events in the history; therefore its population has remained relatively

unmixed. The present study was conducted to investigate the relative contribution of different alleles, haplotypes and genetic diversity in the Yazd genetic pool of HLA class I and II to provide valuable data for further comparison with other populations of Iran and the world.

## MATERIALS AND METHODS

### Study Population

The population studied included 90 unrelated healthy males and females randomly selected from, who were blood donors of the Yazd regional blood transfusion center, Yazd, Iran. All participants were more than fifth generation natives of the Yazd province. We compared our population data of Yazd with others Iranians ethnics, Mediterranean and Caucasian European, African and American African, Latin American, Siberian and Oriental populations (Table1).

Research Ethics Committee of Tehran University of Medical Sciences approved the study protocol and written informed consents were obtained from all of the participants.



**Figure 1. Geographical location of Yazd province in Iran. HLA samples were collected from Yazd regional blood transfusion center.**

## HLA Allele and Haplotype Frequencies in Yazd Province Population in Iran

### HLA Class I and II Typing

Peripheral blood samples (10 mL) were collected in EDTA treated tubes. Then, genomic DNA was extracted by proteinase-K treated peripheral blood leukocytes using a modified salting out method.<sup>21</sup>

HLA-A, -B, -C, -DRB1, -DQAA and -DQB1 genes were amplified by polymerase chain reaction (PCR) using sequence-specific primers (PCR-SSP). PCR-SSP kits were supplied by CTS (Heidelberg, Germany) for intermediate resolution typing. Taq DNA polymerase was purchased from Roche (Basel, Switzerland). Samples were amplified in thermal cyclers (Techne genius, England), after initial denaturation at 94°C for 2 minutes, followed by 10 cycles of 94°C denaturation for 10 seconds, 65°C annealing and extension for 60 seconds, and finally 20 cycles of 94°C denaturation for 10 seconds, 61°C annealing for 50 seconds and 72°C extension for 30 seconds. PCR products were electrophoresed on 2% agarose gels containing ethidium bromide, and visualized under ultraviolet light (UV) detector and the alleles at each locus were detected for each individual.

### Population Statistics Analysis and Measurement of Genetic Variability

The Arlequin v3.5 software provided by Excoffier and Slatkin, estimated allele and Haplotype frequencies.<sup>22</sup> In addition, Expected/observed heterozygosity (He/Ho) according to the Nei, 1973 method,<sup>23</sup> were estimated. Unbiased Expected Heterozygosity (uHe), Number of Effective (NE) alleles, Deviations from Hardy-Weinberg (HW) equilibrium and genetic diversity were computed by taking advantages of Arlequin v3.5 software. Phylogenetic tree was constructed using POPTREE2 software that can perform evolutionary analyses of allele frequency data (It was provided by Nao Takezaki in Japan)<sup>24</sup> based on neighbor-joining method.<sup>25</sup> Correspondence analysis, which is a complementary analysis to genetic distances and neighbor-joining trees, displays a global view of the relationships among populations according to HLA that is more informative when there is considerable genetic exchange between close geographic neighbors. Correspondence analysis in three dimensions and its bi-dimensional representation was performed according to HLA-DRB1 allele frequencies using R-3.2.3 software packages. R software was designed and implemented

by Ross Ihaka and Robert Gentleman in New Zealand.<sup>26</sup>

### RESULTS

The HLA genotypes of 90 individuals from Yazd province of Iran were investigated. The results of this study showed a total of 23, 48, 23, 14, 13 and 16 alleles for HLA-A, -B, -C, -DRB1, -DQA and -DQB in the Yazd population, respectively. Also, the number of effective alleles for each of the above mentioned locus was computed 10.814, 18.684, 10.228, 7.937, 8.549, and 7.334, respectively.

### Allele Frequencies

Table 2 shows the allele frequencies of HLA-A, -B, -C, -DRB1, -DQA, and -DQB loci in the Yazd population.

The most frequent identified HLA-A alleles in descending order, were A\*02:01 (18.889%), A\* 11:01 (12.22%), A\* 26:01 (9.44%), A\* 01:01 (8.889%) and A\*03:01 and \*24:02 (8.33%).

In HLA-B locus B\*51:01 (12.778%), \*35:01 (10.556%), \*50:01 and \*38:01 (7.22%), \*52:01 (4.4%) and \*13:01 (3.88%), in the HLA-C locus \*12:03 (17.033%), \*06:02 (15.384%), \*07:01 (9.89%), \*14:02 (9.341%) and \*18:01 (8.791%) were the most frequent alleles.

HLA-DRB1 \*11 (24.4%), \*15 (13.3%) and \*07 (13.3%), \*01:01 (8.8%) and \*03:01 (8.889%), and \*04 (7.22%) were recognized as frequent alleles at HLA-DRB1 locus.

Additionally, in case of HLA-DQA \*05:05 (20.556%), \*01:03 (13.3%) and \*02:01 (13.3%) and HLA-DQB \*03:01 (22.8%), \*02:01 (18.9%), \*05:01 (13.8%) and \*06:02 (13.3%) were frequent alleles in Yazd province population.

### HLA haplotype Frequencies in Yazd Individuals

In present study as it is shown in Table 3 the most frequent three-locus haplotypes among the 90 individuals in case of DRB-DQA-DQB were DRB\*11-DQB\*0301-DQA\*0501 with a frequency of 21.1% followed by DRB1\*07-DQB\*02:01-DQA\*02:01 (11.1%), DRB\*0101-DQB\*0501-DQA\*0101 (8%) DRB\*03:01-DQB\*02:01-DQA\*05:01 (7.9%) and DRB\*15 -DQB\*0601 -DQB\*0103 (5.5%). The frequency of the remaining haplotypes was below 4%.

In case A-B -DRB1, the most common three-locus haplotypes were A \*02- B \*50- DRB\*07 with a

frequency of 4.9% followed by A\*02- B\*51- DRB\*15 (3%) and A\*11- B\*52- DRB\*15 (2.5%). Remaining haplotypes were identified with a frequency  $\leq 2$ .

Furthermore, frequent haplotypes for HLA A-B-C were A\*02 -B\*50 -C\*06(6%), A\*26 -B\*38 -C\*12(5%), A\*11 -B\*52 -C\*12(4%) and A\*02 -B\*51 -C\*14 (2%).

The most frequent four locus haplotype HLA-A,B,C and DRB were HLA-A\*11,B\*52,C\*12,DRB\*15 (2.5%) and HLA-A\*24,B\*40,C\*12,DRB\*11 (2%) and HLA-A\*26,B\*38,C\*12,DRB\*04 (2%).

Moreover, evaluation of deviation from Hardy-Weinberg equilibrium using chi-square test showed departure of population in all loci (Table 4) in Yazd population of Iran.

### Observed and Expected Heterozygosity and Genetic Diversity

The expected heterozygosity value is computed in a population which is in Hardy-Weinberg equilibrium. The observed heterozygosity/ homozygosity value is the real rate of heterozygosity/homozygosity individuals in the examined populations. The ratio between the two values is able to show the value of difference from genetic balance of examined population.

Analysis of Nei expected heterozygosity and unbiased heterozygosity for all loci in comparison with

observed heterozygosity revealed the population was less heterozygote than it was expected in all loci (Table 5).

Genetic diversity was measured by the Shannon's information index and percentage of polymorphic loci. The calculated Shannon's index (I) ranged was from 2.292 (HLA-DRB) to 3.407(HLA-B). Among these five locus, HLA-B exhibited the greatest level of variability (Percentage of polymorphic loci (PPB)=100%, Number of effective alleles (NE) 19.684, Unbiased Expected heterozygosity (uHE)=0.949, Index (I)=3.407). By contrast, lowest genetic diversity was found in locus HLA-DRB (with PPB=100%, NE=7.937, HE=0.874, I=2.292) (Table 5).

As shown in Figure 2 the Correspondence analysis and Figure 3 Neighbor-Joining (NJ) dendrogram, relatedness was observed between Yazd province population of Iran and other Iranian populations and Chuvashia population from Russia, Greece, Macedonia, Italy and Russian populations. On the other hand it was far from the Moroccan Jews, Saudia Arabia, Korean and Japanese populations. In addition Table 6 shows the allele frequencies of Yazd population with other Iranians and populations of Macedonia, Greece and Korea. The allele frequencies indicate the similarity between all the populations except the Baluch and Korean population.

**Table 1. Worldwide populations data used in the analysis**

Population	N	Reference	Population	N	Reference
Algerians	51	(27)	Lebanese KZ	94	(28)
Ashkenazi Jews	80	(29)	Macedonians	178	(30)
Baloch	100	(31)	Mansi	68	(32)
Brazil	108	(33)	Moroccans	98	(34)
Chile	70	(35)	Moroccan Jews	113	(36)
China	121	(37)	Negidal	35	(32)
Chuvash	82	(38)	Non Ashkenazi Jews	80	(29)
Cretan	135	(8)	Palestinians	165	(39)
Evenks	35	(40)	Russians	200	(41)
Germans	111	(42)	Saudi Arabia	18	(43)
Gorgan	69	(44)	Spanish Basques	83	(45)
Greece	246	(46)	South African Blacks	200	(47)
Iranians 1 <sup>a</sup>	73	(35)	South Korea	485	(48)
Iranian Jews	101	(49)	Tofalar	43	(32)
Italians	100	(50)	Tuvinians	197	(51)
Japanese	50	(52)	Ulchi	73	(32)
Kets	22	(40)	USA African	2411	(53)
Kurds	60	(54)	Yazd	90	Present Study

<sup>a</sup>Iranians from Fars province .

## HLA Allele and Haplotype Frequencies in Yazd Province Population in Iran

**Table 2. HLA-A, -B,-C and HLA-DRB1, -DQA1 and -DQB1 Frequencies (F) in the population of Yazd province in Iran**

No.	HLA Class I						HLA Class II					
	HLA-A		HLA-B		HLA-C		HLA-DRB		HLA-DQA		HLA-DQB	
	Allele*	F(%)	Allele*	F(%)	Allele*	F(%)	Allele*	F(%)	Allele*	F(%)	Allele*	F(%)
1	01:01	<b>16 (0.08889)</b>	07:02	4 (0.022)	01:02	3(0.01648)	01:01	16(0.08889)	01:01	16(0.08889)	01:01	1(0.006)
2	02:01	<b>34 (0.18889)</b>	07:03	2 (0.01111)	01:04	4(0.02198)	03:01	16 (0.08889)	01:02	<b>20(0.11)</b>	02:01	<b>34(0.189)</b>
3	03:01	15 (0.08333)	08:01	6 (0.033)	02:01	2(0.01099)	04	13(0.07222)	01:03	<b>25(0.13856)</b>	02:03	1(0.006)
4	11:01	<b>22 (0.12222)</b>	08:04	1 (0.00556)	02:02	3(0.01648)	07	<b>24(0.133)</b>	01:04	18(0.1)	03:02	6(0.033)
5	23:01	8 (0.04444)	13:01	<b>7 (0.03889)</b>	02:03	2(0.01099)	08	7(0.03889)	<b>02:01</b>	<b>24(0.133)</b>	03:03	7(0.039)
6	24:02	15 (0.08333)	14:01	4 (0.02223)	03:02	4(0.02198)	09:01	1(0.00556)	03:02	1(0.00556)	03:04	1(0.006)
7	24:07	3 (0.01667)	14:02	1 (0.00556)	03:03	1(0.00549)	10:01	5(0.02778)	03:011	15(0.0833)	03:05	5(0.028)
8	24:08	3 (0.01667)	14:05	1 (0.00556)	03:08	4 (0.02198)	11	<b>44(0.244)</b>	03:04	2(0.011)	03:01	<b>41(0.228)</b>
9	24:19	5 (0.02778)	15:01	1 (0.00556)	04:01	2(0.01099)	13:03	1(0.00556)	04:01	5(0.02778)	03:06	1(0.006)
10	24:24	1 (0.00556)	15:24	1 (0.00556)	06:02	<b>28 (0.15384)</b>	13:01	11(0.0611)	05:011	14(0.07778)	04:01	4(0.022)
11	26:01	<b>17 (0.09444)</b>	18:01	2 (0.011)	06:04	5(0.02747)	13:02	6(0.033)	05:03	2(0.011)	05:01	<b>25(0.138)</b>
12	26:08	3 (0.01667)	18:09	4 (0.02222)	07:01	<b>18(0.0989)</b>	14:01	8(0.044)	05:05	<b>37(0.20556)</b>	05:03	8(0.044)
13	26:13	1 (0.00556)	27:01	4 (0.022)	07:08	6(0.03297)	15	<b>24(0.133)</b>	06:01	1(0.00556)	05:03	1(0.006)
14	29:01	1 (0.00556)	32:27	2 (0.011)	08:01	4(0.02198)	16	4(0.022)	-	-	05:05	3(0.017)
15	30:01	7 (0.03889)	35:01	<b>19 (0.10556)</b>	12:02	10(0.05495)	-	-	-	-	06:01	12(0.066)
16	31:01	3 (0.01667)	35:10	2 (0.011)	12:03	<b>31(0.17033)</b>	-	-	-	-	06:02	<b>24(0.133)</b>
17	31:02	1 (0.00556)	35:26	1 (0.00556)	12:04	2(0.01099)	-	-	-	-	-	-
18	32:01	7 (0.03889)	35:27	3 (0.01667)	13:01	4(0.02198)	-	-	-	-	-	-
19	32:04	2 (0.01111)	37:01	4 (0.02222)	14:02	<b>17(0.09341)</b>	-	-	-	-	-	-
20	33:01	2 (0.01111)	38:01	13 (0.07222)	16:01	1(0.00549)	-	-	-	-	-	-
21	68:01	11 (0.06111)	39:01	3 (0.01667)	16:02	8(0.04396)	-	-	-	-	-	-
22	68:02	2 (0.01111)	40:01	2 (0.011)	17:01	7(0.03846)	-	-	-	-	-	-
23	69:01	1 (0.00556)	40:02	2 (0.01111)	18:01	<b>16(0.08791)</b>	-	-	-	-	-	-
24	-	-	40:07	1 (0.00556)	-	-	-	-	-	-	-	-
25	-	-	40:08	1 (0.00556)	-	-	-	-	-	-	-	-
26	-	-	40:10	2 (0.011)	-	-	-	-	-	-	-	-
27	-	-	41:05	1 (0.00556)	-	-	-	-	-	-	-	-
28	-	-	44:02	4 (0.02222)	-	-	-	-	-	-	-	-
29	-	-	44:05	2 (0.01111)	-	-	-	-	-	-	-	-
30	-	-	44:10	5 (0.02778)	-	-	-	-	-	-	-	-
31	-	-	45:04	1 (0.00556)	-	-	-	-	-	-	-	-
32	-	-	49:02	1 (0.00556)	-	-	-	-	-	-	-	-
33	-	-	50:01	13 (0.0722)	-	-	-	-	-	-	-	-
34	-	-	50:02	1 (0.00556)	-	-	-	-	-	-	-	-

35	-	-	51:01	<b>23 (0.12778)</b>	-	-	-	-	-	-	-	-
36	-	-	51:02	3 (0.01667)	-	-	-	-	-	-	-	-
37	-	-	51:06	4 (0.02222)	-	-	-	-	-	-	-	-
38	-	-	51:08	3 (0.01667)	-	-	-	-	-	-	-	-
39	-	-	52:01	8 (0.044)	-	-	-	-	-	-	-	-
40	-	-	53:03	1 (0.00556)	-	-	-	-	-	-	-	-
41	-	-	55:01	3 (0.01667)	-	-	-	-	-	-	-	-
42	-	-	55:08	1 (0.00556)	-	-	-	-	-	-	-	-
43	-	-	57:01	6 (0.03333)	-	-	-	-	-	-	-	-
44	-	-	57:05	2 (0.01111)	-	-	-	-	-	-	-	-
45	-	-	58:01	1(0.00556)	-	-	-	-	-	-	-	-
46	-	-	78:01	1 (0.00556)	-	-	-	-	-	-	-	-
47	-	-	67:01	2 (0.011)	-	-	-	-	-	-	-	-
48	-	-	1517	1 (0.00556)	-	-	-	-	-	-	-	-

Frequent alleles in each locus are given in bold.

**Table 3. The most frequent three- and four-locus extended haplotypes in Yazd province population in Iran**

HLA-A/HLA-B/HLA-Cw haplotypes				HLA-A/HLA-B/HLA-DRB1 haplotypes				HLA-DRB1/HLA-DQB1/HLA-DQA1 haplotypes			
HLA Alleles			HF%	HLA Alleles			HF%	HLA Alleles			HF%
HLA-A	HLA-B	HLA-Cw		HLA-A	HLA-B	HLA-DRB1		HLA-DRB1	HLA-DQB1	HLA-DQA1	
20	50	60	6	02	50	07	4.9	11	0301	0501	21.1
26	38	12	5	02	51	15	3	07	0201	0201	11.1
11	52	12	4	11	52	15	2.5	01	0501	0101	8
10	35	40	3	01	44	11	2	03	0201	0501	7.9
11	38	12	2.9	01	57	07	2	15	0601	0103	5.5
01	57	06	2	03	35	01	2	1301	0602	0103	5
02	51	14	2	11	38	04	2	04	0302	0301	4
03	35	12	2	24	40	11	2	07	0303	0201	3.5
HLA-A/HLA-B/HLA-Cw/HLA-DRB1 haplotypes											
HLA Alleles			HF%								
HLA-A	HLA-B	HLA-Cw		HLA-DRB1							
11	52	12		15		2.5					
24	40	12		11		2					
26	38	12		04		2					
26	38	12		1301		2					
01	57	06		07		1.5					
03	35	04		01		1.5					
24	35	04		07		1.5					
68	18	12		11		1.5					

HF: Haplotype frequency

## HLA Allele and Haplotype Frequencies in Yazd Province Population in Iran

**Table 4. Hardy-Weinberg equilibrium in HLA classes of Yazd province population in Iran**

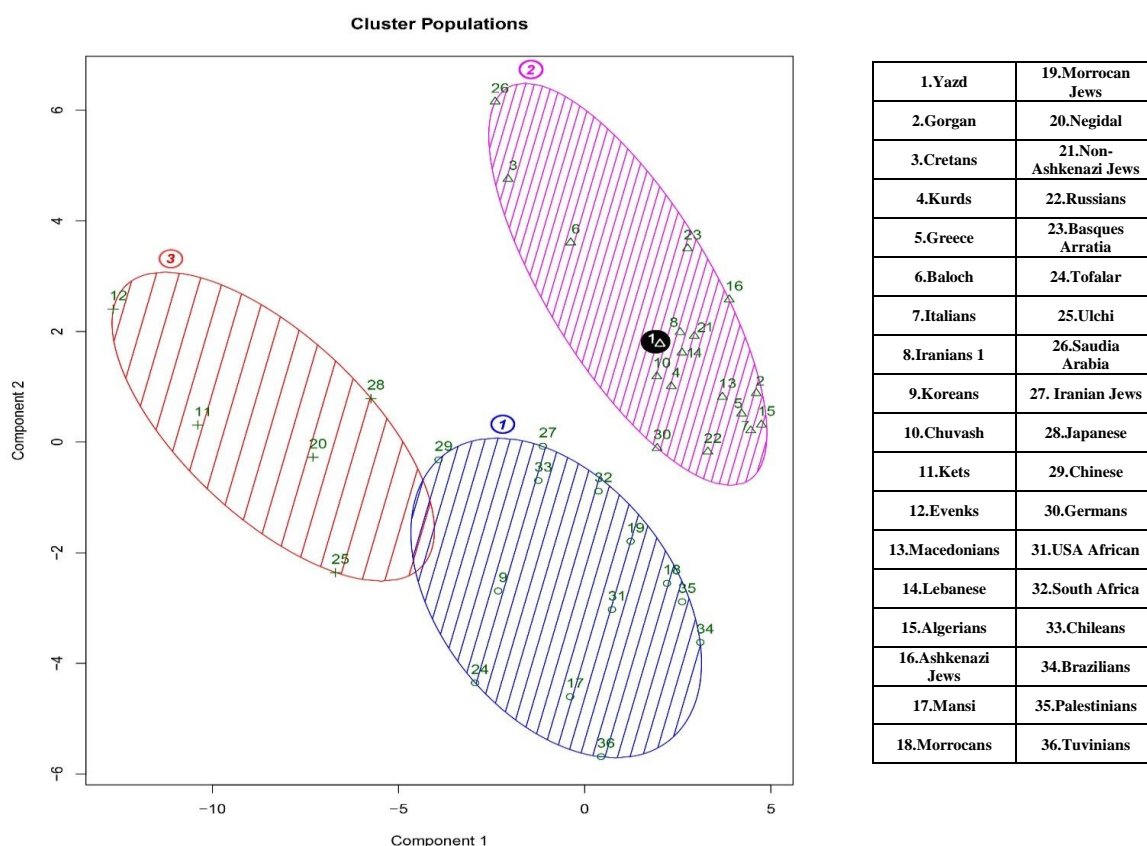
Locus	Sample Size	Obs.Het	Exp.Het	P-value	s.d.
HLA-A	90	0.92929	0.89314	0.28403	0.00029
HLA-B	90	0.91919	0.91227	0.24592	0.00021
HLA-Cw	90	0.91919	0.86925	0.51712	0.00041
HLA-DRB1	90	0.92929	0.88730	0.54298	0.00030
HLA-DQB1	90	0.82828	0.83813	0.90842	0.00020
HLA-DQA1	90	0.86869	0.82433	0.74757	0.00034

Obs.Het: Observed heterozygosity; Exp.Het: expected heterozygosity; s.d.: Standard deviation

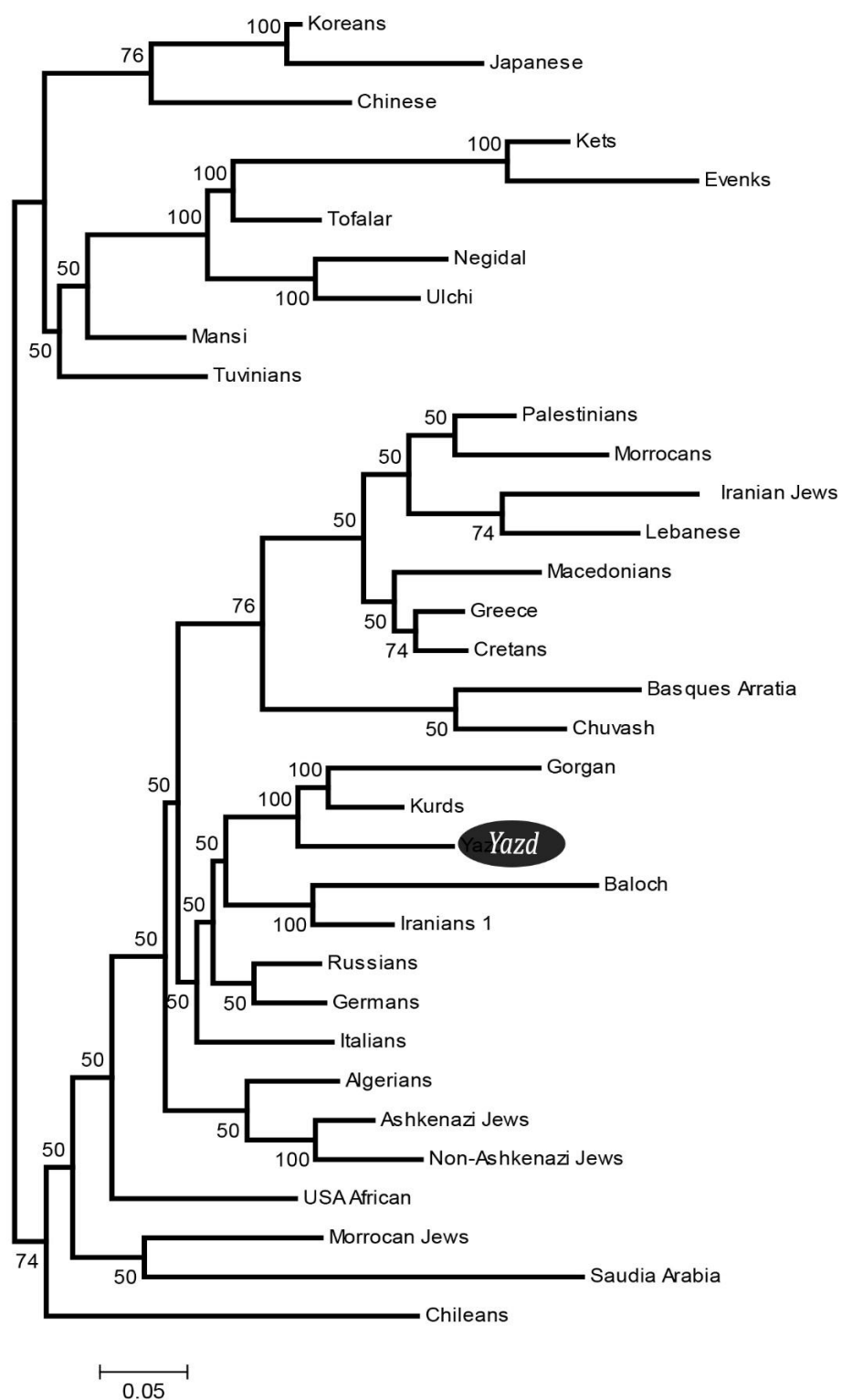
**Table 5. Observed and expected heterozygosity and genetic diversity in Yazd province population in Iran**

Locus	Sample Size	Na	Ne	I	uHe	PPB
HLA-A	90	23	10.814	2.652	0.913	100%
HLA-B	90	48	19.684	3.407	0.955	100%
HLA-C	90	23	11.228	2.720	0.9159	100%
HLA-DRB	90	14	7.937	2.292	0.879	100%
HLA-DQA	90	13	8.549	2.295	0.868	100%
HLA-DQB	90	16	7.334	2.270	0.868	100%

Na: no. of different alleles; Ne: no. of effective alleles; I: Shannon's information index; uHe: unbiased expected heterozygosity; F: fixation index; PPB: percentage of polymorphic loci



**Figure 2. Correspondence analysis showing the relationship between Iranian Yazd Province population in Iran and other World populations according to HLA-DRB1 allele frequency.**



**Figure 3. Neighbor-Joining (NJ) dendrogram showing relatedness between Yazd Province population in Iran and other World populations based on the allele frequencies of HLA-DRB1 and HLA-DQB1. Bootstrap values from 1000 replicates are shown.**



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Figure 4. Migration of Aryan during the ancient time; indo-Aryan and indo-european ([https://upload.wikimedia.org/wikipedia/commons/a/ae/Centum\\_Satem\\_map.png](https://upload.wikimedia.org/wikipedia/commons/a/ae/Centum_Satem_map.png))

Table 6. Comparison of some frequent HLA alleles between different populations

HLA	Yazd	Fars	Bakhtiyari	Baluch	Macedonia	Greece	Korean
	Present study	Ref 14	Ref 15	Ref 26	Ref 8	Ref 27	Ref 28
DRB1*0101	8.8	5.5	8	15	5.2	6.3	6.8
DRB1*0301	8.8	10	10	27	9	10.4	2.9
DRB1*04	7.2	10.5	12	10.5	7.9	7.1	21.5
DRB1*0701	13.3	6.5	12	3	6.4	6.7	7.2
DRB1*1001	2.7	4	5	0.0	2	2.5	1.6
DRB1*11	24.4	25	16	8.5	24.8	25.4	4.5
DRB1*15	13.3	12	14	12.5	11.9	8.6	10.7
DQB1*0201	18.9	19	17	32	14.5	15	9.4
DQB1*0301	22.8	31	22	10.5	25	30.4	13.5
DQB1*0302	3.3	5.5	-	1.5	6.4	5.4	10.3
DQB1*05	13.8	22	21	28	30.3	34.2	16.4
DQB1*0601	6.6	7	12	8	2.9	1.3	9.6
DQB1*0602	13.3	8.5	6	3.5	6.9	3.3	7.1
DQA1*0101	8.8	5.5	-	-	-	6.3	-
DQA1*0103	13.3	10.5	13	8	-	4.6	-
DQA1*0201	13.3	7.5	10	3.5	-	6.7	-
DQA1*05	20.5	39	32	35	-	39.6	-

## DISCUSSION

Yazd city is located in the center of Iran (Figure 1) almost within the great desert of this country, the Loot Desert. Because of the vast distances which this city has with the frontiers of the country and due to very hot climate and shortage of water in the area, the foreign invaders were not attracted to travel to reach this city and to occupy it for settling there, even for a short period. Therefore its safe location saved the city and its population during the centuries.

The selection of co-dominant DNA markers to estimate genetic polymorphism between individuals and populations is a promising approach as many polymorphic loci can be analyzing relatively easily, in a short time and at a low cost.

Our analysis of HLA class II and I (DRB, DQA, DQB and A, B, C) polymorphism in Yazd population indicates that the frequent alleles in HLA-DRB1, DQA, DQB locus do not show much difference among most Iranian ethnic groups; however, there is not enough data on HLA – A, B and C in different Iranian ethnic groups for comparison (Table 6). The frequency of these alleles in different ethnic Iranian normal populations was not statistically significant except for Balochi population. The most frequent alleles in Balochi population were DRB1 \*0101 and \*15 (15% and 12.5%), DQA\*05:05 (35%), DQB \*02:01 (32%) and A\*02 and 11 (20% and 14%), HLA-B \*51 and \*35 (5.60% and 8.10%), and HLA-C \*12:03 and \*06:02 (4.2%), which is different from the Yazd population.<sup>18,19</sup>

In current study, the most frequent three-locus haplotypes of DRB1-DQB-DQA were DRB\*11-DQB\*03:01-DQA\*05:01 (21.1% versus 19.7% in Greece and 2.56% in Japanese) followed by DRB\*07-DQB\*02:01-DQA\*02:01 (11.1% vs 4.9% in Greece) and DRB\*03:01-DQB\*02:01-DQA\*05:01 (7.9% versus 9% in Russia and 6.3 % in Greece and 0.21% in Japanese) and DRB\*13:01 –DQB\*06:02 -DQA\*01:03 (5%). In our previous study on Fars province population (14) in 2001 DRB\*11-DQB\*03:01-DQA\*05:01 with a frequency of 25.0% followed by DRB\*03:01-DQB\*02:01-DQA\*05:01 (10.0%) was reported. Also, DRB\*11-DQB\*03:01-DQA\*05:01 was the predominant haplotype in the Luristan (31.1%), Kohkiluyeh/Boyerahmad (23.1%), populations as well as Famoori-Arabs (18.5%) and Jews (29.7%) of Iran. Moreover, it was frequent haplotype in Parsees,

Kurds and Azeris populations.<sup>(19, 20)</sup>. This haplotype is the most frequent haplotype in almost all Iranian ethnic groups.

In case A-B-C, the most common three locus haplotypes were A\*02-B\*50-C\*06 (6%) followed by A\*26- B \*38- C \*12 (5% versus 1.26% in Italy) and A\*11- B \*52- C \*12 (4% versus 1.2% in Iranian Baluch).

The only report on HLA class I haplotypes of Iran (in Baluch population) shows frequent haplotypes [\*11:011-B\*40:06-C\*15:021 (5.8%); A\*02:011-B\*35:27-C\*04:011 (3.5%); and A\*33:03-B\*58:01-C\*03:02 (3.5%)], which are different from Yazd population.<sup>31</sup>

In case A-B -DRB1, the most common three locus haplotypes were A \*02- B \*50- DRB\*07 (4.9% vs. 1.7% in Iranian Kurds, 2% in Jews and 3.4 % in Saudi) followed by A \*02- B \*51- DRB \*15 (3%) and A\*11- B \*52- DRB \*15 (2.5%).

In the present study the most common haplotypes for HLA-A,B,C,DR were HLA-A\*11, B\*52, C\*12, DRB\*15 (2.5%) and A\*26, B\*38, C\*12, DRB\*04 (2% vs. 1.2% in macedonia), A\*03, B\*35, C\*4, DRB\*01 (1.5%), these haplotypes have not been reported in previous studies in Iranian population.

As expected our data in this study showed that HLA allele and haplotype differences between various Iranian ethnic groups are generally smaller than those between Iranian and other countries.

Considering the Hardy-Weinberg equilibrium analysis, our study in Yazd population, did not showed deviation from Hardy-Weinberg equilibrium in HLA class I and II loci.

Aryans are considered as common ancestors of the modern Iranian who migrated from southwestern steppes of the Russia toward south into Iran around 2000 BC.<sup>32</sup> The name of Iran meaning the land of Aryans has been used since the Sassanid's dynasty. Aryan people lived about eight thousand years ago in southern Siberia and around the Urals Lake. Due to the extreme temperature they migrated to southern pastures and resided in western and eastern parts of Iran plateau and made the Persian and median Tribe.<sup>32</sup> After the advent of Zoroastrian part of them migrated to Sand rivers and made the Indo-Iranian branch and a group of Aryan migrated to eastern and a part of southern Europe and the Balkans (Figure 4).

The geopolitical situation of Iran as an important constituent of the Silk Road had an extremely advantageous position between Asia and Europe,

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between the Mediterranean, the Caspian, the Black and the Azov seas playing an important role in international trade and causing genetic admixture with Roma and Greece as important empires in the ancient time and also the migration of Indo-European branch of Aryan to the south and east of Europe continent.

In the phylogenetic tree of different human populations, as we have stated in our previous study Iranian are considered as a highly mixed population of Caucasoid origin in whom their ancestral genetic background of Aryans has extensively been mixed with the populations from Mediterranean and due to the invasion and occupancies by Mongolian and Arabian and Turk origins during the centuries.<sup>14</sup> Comparing Iranian Yazd population with other populations and constructing the phylogenetic tree and corresponding analysis, shows that Yazd population is in close vicinity with other Iranian ethnic groups and also with Russian ,German , Italian , Macedonian and Cretan population and far from the Orientals like Korean, Japanese and Chinese populations.(Figures 2,3)

Iranian population shared some frequent alleles (HLA-A2 18.8% vs 225.6, HLA-B35 12.5% vs 14.8% and HLA-B51 16% vs 14.8%, HLA-DRB1\*11 24.4% vs 24.8% in macedonia) and haplotypes (A\*26:01 –B\*38:01 –C\*12:03(5%), A\*02 –B\*51 –C\*14 (2%)), with European Caucasians in the southern and eastern parts of Europe (Table 6).

Low sample size is one of the limitations of this study and collecting larger sample size with more precise demographic data in the future study is necessary.

In conclusion, our results show a close relatedness between Yazd population and other ethnic group of Iran, but there are also some significant differences, which may have been caused by admixture of each one of these groups with other or foreigner subpopulations during centuries. Yazd population when comparing with other world populations is in close vicinity with the Caucasian population such as Russian ,German , Italian Macedonian and Cretan populations in the south east of Europe.

Complementary information based on mitochondrial DNA (mtDNA), Y chromosome in male and other genetic markers will help to better understandings of genetics background of Yazd population.

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