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Distribution of *HLA-DRB1* and *HLA-DQB1* alleles in Lak population of Iran

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ABSTRACT

Human leukocyte antigen (*HLA*) genes are the most polymorphic loci in the human genome and encode the highly polymorphic molecules critically involved in immune responses. Anthropological studies based on highly polymorphic *HLA* genes provide useful information for bone marrow donor registry, forensic medicine, disease association studies, as well as designing peptide vaccines against tumors, and infectious or autoimmune diseases. The aim of this study was to determine the *HLA-DRB1* and *HLA-DQB1* allele frequencies in 100 unrelated Lak individuals from Lorestan province of Iran. Finally, we compared the results with those previously described in four other Iranian populations. Commercial HLA-Type kits were used for determination of the *HLA-DRB1* and *HLA-DQB1* allele frequencies. Differences between populations in the distribution of *HLA-DRB1* and *HLA-DQB1* alleles were estimated by $\chi 2$ test with Yate's correction and Fisher's exact test. The most frequent *HLA-DRB1* alleles were *1103 = 4 (23%), *1502 (9.5%), *0701 (9%), *0301 (8.5%), *1101 (7.5%) and *1501 (6%) while *HLA-DQB1*0301 (40%), *0201 (15%), *0502 (10.5%), *0303 (10%), *0602 = 3 (9.5%), and *0501 (7.5%) were the most frequent alleles in Lak population. *HLA-DRB1*0409, *0804, *1102, *1112, *1405, and *HLA-DQB1*0503, *0604 were the least observed frequencies in Lak population. Our results based on *HLA-DRB1* and *HLA-DQB1* allele frequencies showed that the Lak population possesses the previously reported general features of the Lur and Kurd populations but still with unique, decreased or increased frequencies of several alleles. In other words, the Lak population is close to Lurs Khorramabadi and Kurd but far from Lurs Kohkiloyeh/Boyerahmad and Bakhtiari.

1. Introduction

Human leukocyte antigen (*HLA*) genes are the most polymorphic loci in the human genome. They express on the surface of the T lymphocytes and, therefore, play a major role in the regulation of the immune system [1]. Studies of *HLA* gene diversity in different populations [2] can regard as useful markers by anthropologists for determination of genetic relationship and interaction among different populations [3,4]. Also, anthropological studies based on highly polymorphic *HLA* genes provide useful information for bone marrow donor registry [5], forensic medicine [6], disease association studies [7], as well as designing peptide vaccines against tumors [8], and infectious or autoimmune diseases [9].

Iranians are ethnically diverse people. Most of the Iranians are Muslims. The population living in Iran might be admixed due to encounter with other populations and immigrants from neighboring populations. In previous studies, the frequencies of *HLA* class II alleles in most Iranian populations have been determined and compared with each other [10–15].

In the present study, due to the lack of this study in Lak population, initially frequency of *HLA-DRB1* and *HLA-DQB1* alleles was determined in Lak population of Iran. Finally, we compared the results with those previously described in Lur of Khorramabadi [10], Lur of Kohkiluyeh/Buyerahmad [10], Lur of Bakhtiari [10], and Kurd [14] populations. Indeed, in this study, genetic relationship among Laks, Lurs, and Kurds of Iran was investigated based on *HLA-DQB1 and HLA-DRB1* allele frequencies.

2. Materials and methods

2.1. Study subjects

Laks dwell in the northwest and west of Lorestan province, southern Kermanshah province and east Ilam provincel (Fig. 1)

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Fig. 1. The map that shows the geographical location of all studied Iranian populations.

and are speaking Laki, a dialect of Kurdish language. The vast majority of this population lives in the cities of Aleshtar, Nurabad and Kuhdasht from Lorestan, Harsin and Kangavar from Kermanshah and Chardavol and Darreh Shahr from Ilam. Also, there are some other minority groups from Laks reside in Iraq. Some call them Lur, some call them Kurd, and some call them an ethnic between Lur and Kurd. Some also know the Laks an independent division of Iranian ethnic groups. Lurs are living in three provinces of central and southern Zagros including Lorestan, Kohkiloyeh/Boyerahmad, and Chahar-Mahal/Bakhtiari (Fig. 1) and are speaking Luri, a dialect of Persian language. Kurds of Iran are living in West Azerbaijan, Kurdistan, Kermanshah and Ilam provinces (Fig. 1). Their language, Kurdish, is related to Persian and belongs to the Indo-European languages [10].

Table 1
Distribution of *HLA-DRB1* and *HLA-DQB1* alleles in Lak population compared with four other Iranian populations[10,14].

	Lak (n = 100)	Lur of Khorramabadi (n = 50)	Lur of Kohkiluyeh/Buyerahmad (n = 54)	Lur of Bakhtiari (n = 50)	Kurd (n = 100)
DRB1					
0101	**3 (0.015)**	2 (0.020)	–	1 (0.010)	5 (0.025)
0102	**–**	–	1 (0.009)	4 (0.040)	3 (0.015)
0103	**3 (0.015)**[a]	–	**7 (0.065)**	3 (0.030)	–
0301	**17 (0.085)**[a]	8 (0.080)	**25 (0.230)**	9 (0.090)	16 (0.080)
0302	–	–	–	1 (0.010)	1 (0.005)
0402	**6 (0.030)**	2 (0.020)	4 (0.037)	6 (0.060)	11 (0.055)
0403	**6 (0.030)**	2 (0.020)	–	1 (0.010)	14 (0.070)
0404	–	1 (0.010)	1 (0.009)	–	1 (0.005)
0407	–	–	–	3 (0.030)	–
0409	**1 (0.005)**	2 (0.020)	2 (0.019)	–	2 (0.010)
0411	–	–	–	2 (0.020)	–
0701	**18 (0.090)**[a]	**2 (0.020)**	3 (0.028)	12 (0.120)	18 (0.090)
0801	–	–	–	1 (0.010)	–
0804	**1 (0.005)**	1 (0.010)	–	–	–
0901	**–**	–	–	1 (0.010)	–
1001	**4 (0.020)**	3 (0.030)	3 (0.028)	5 (0.050)	1 (0.005)
1101	**15 (0.075)**	10 (0.100)	7 (0.065)	4 (0.040)	14 (0.070)
1102	**1 (0.005)**	–	–	2 (0.020)	1 (0.005)
1103 = 4	**46 (0.230)**[a]	31 (0.310)	26 (0.241)	**10 (0.100)**	41 (0.205)
1112	**1 (0.005)**	–	–	–	1 (0.005)
1201	**2 (0.010)**	1 (0.010)	–	–	1 (0.005)
1202	–	–	–	1 (0.010)	–
1301	**10 (0.050)**	6 (0.060)	5 (0.046)	3 (0.030)	13 (0.065)
1302	**4 (0.020)**	2 (0.020)	–	1 (0.010)	2 (0.010)
1303	**4 (0.020)**	2 (0.020)	1 (0.009)	3 (0.030)	2 (0.010)
1305	**2 (0.010)**	–	–	3 (0.030)	1 (0.005)
1401	**5 (0.025)**	1 (0.010)	2 (0.019)	4 (0.040)	8 (0.040)
1404	–	–	–	1 (0.010)	–
1405	**1 (0.005)**	–	–	–	2 (0.010)
1501	**12 (0.060)**	6 (0.060)	7 (0.065)	7 (0.070)	11 (0.055)
1502	**19 (0.095)**	6 (0.060)	5 (0.046)	7 (0.070)	17 (0.085)
1601	**9 (0.045)**	4 (0.040)	6 (0.056)	3 (0.030)	7 (0.035)
1602	**10 (0.050)**	7 (0.070)	2 (0.019)	1 (0.010)	7 (0.035)
1605	–	–	1 (0.009)	–	–
Total	**200 (1.000)**	100 (1.000)	108 (1.000)	100 (1.000)	200 (1.000)
DQB1					
0201	**30 (0.150)**	14 (0.140)	25 (0.231)	17 (0.170)	33 (0.165)
0301	**80 (0.400)**[a]	46 (0.460)	38 (0.351)	**22 (0.220)**	64 (0.320)
0302	–	–	2 (0.019)	–	–
0303	**20 (0.100)**	7 (0.070)	8 (0.074)	17 (0.170)	24 (0.120)
0402	**3 (0.015)**	1 (0.010)	1 (0.009)	2 (0.020)	3 (0.015)
0501	**15 (0.075)**[b]	6 (0.060)	**2 (0.019)**	6 (0.060)	8 (0.040)
0502	**21 (0.105)**	11 (0.110)	14 (0.130)	7 (0.070)	13 (0.065)
0503	**1 (0.005)**[a]	1 (0.010)	5 (0.046)	**8 (0.080)**	**14 (0.070)**
0601	**10 (0.050)** [b]	6 (0.060)	7 (0.065)	**12 (0.120)**	18 (0.090)
0602 = 3	**19 (0.095)**	7 (0.070)	6 (0.056)	6 (0.060)	18 (0.090)
0604	**1 (0.005)**	1 (0.010)	–	3 (0.030)	5 (0.025)
Total	**200 (1.0000)**	100 (1.000)	108 (1.000)	100 (1.000)	200 (1.000)

Frequencies of Lak population of Iran and those statistically different to Iranians in other populations are shown in bold.
[a] Significant after correction.
[b] Significant before correction.

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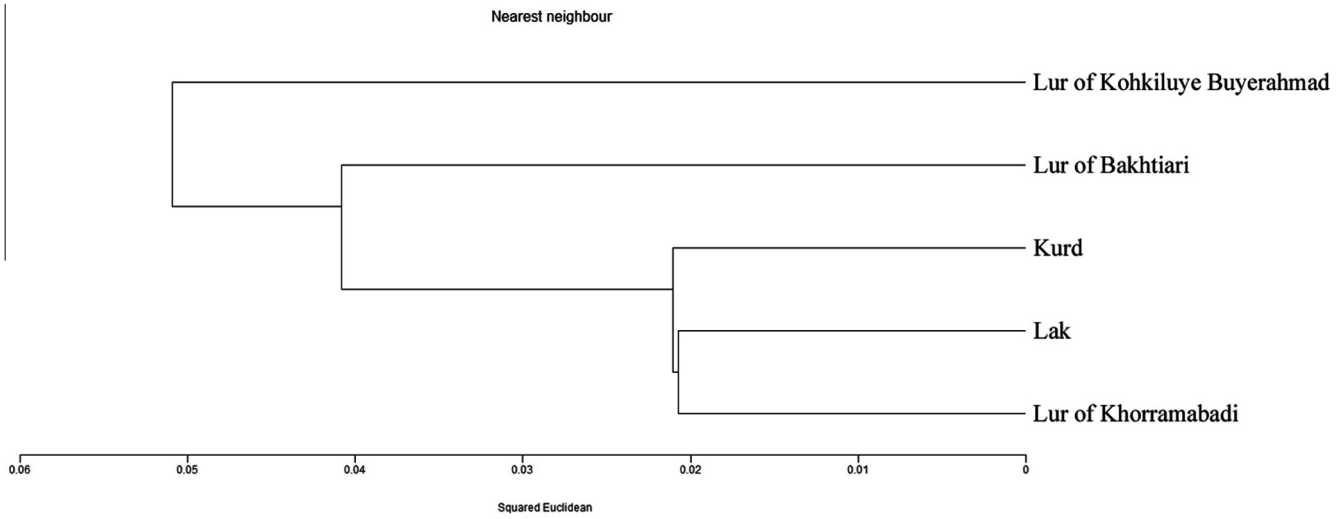

Fig. 2. Neighbor-joining tree linking our study population and 4 populations previously described elsewhere [10,14].

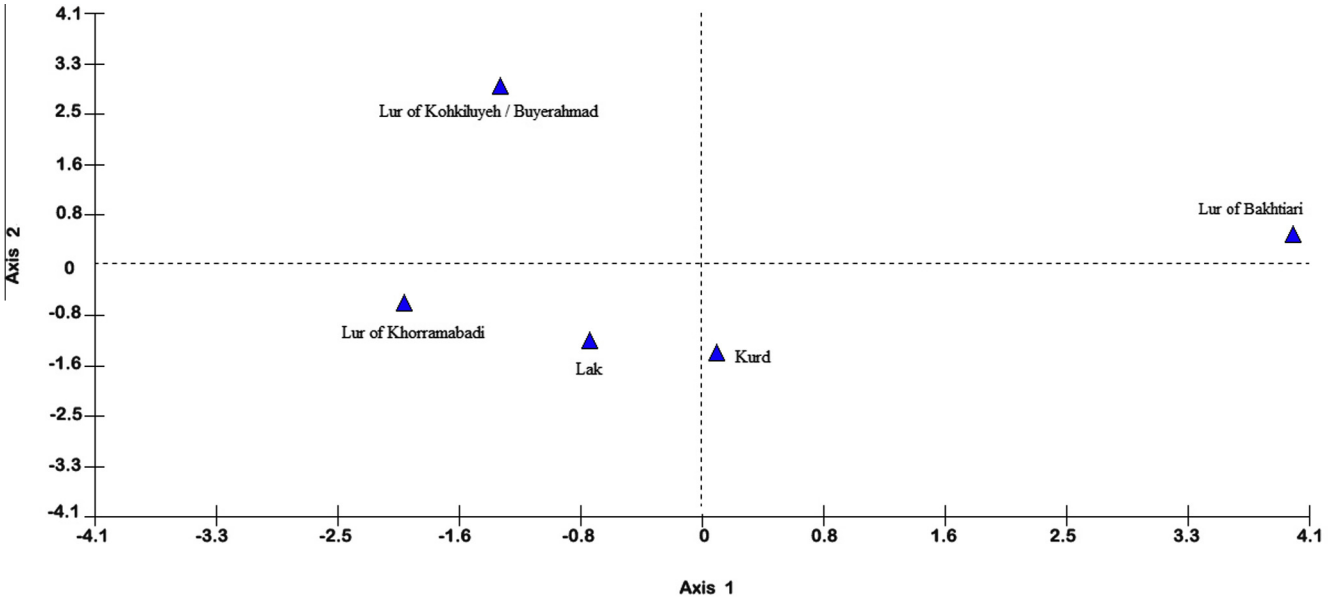

Fig. 3. Correspondence analysis using *HLA-DRB1* and *HLA-DQB1* allele data from our study population and 4 populations previously described elsewhere [10,14]. A total of 45 *HLA-DRB1* and *HLA-DQB1* allele frequencies were considered: 34 *HLA-DRB1* alleles and 11 *HLA-DQB1* alleles. The relative map position of each point representing 1 of the 5 populations (Table 1) is indicative of genetic difference.

2.2. Samples

In this study, blood samples were collected with informed consent from 100 unrelated Lak individuals 20–30 years old, male and female each 50, inhabitant of Lorestan province of Iran. All participants were third generation natives of the selected area and none had personal or family history of cancer or autoimmune diseases. Their genomic DNA was extracted from peripheral blood leukocytes using EXTRA GENE I kit (BAG Health Care GmbH, Lich, Germany).

2.3. HLA genotyping

Commercial HLA-Type kits from BAG Health Care GmbH (Lich, Germany) company were used for determination of the *HLA-DQB1 and HLA-DRB1* allele frequencies in genomic DNA, based on polymerase chain reaction-sequence specific primers (PCR-SSP) assay. The PCR products were analyzed in 2% agarose gel stained with ethidium bromide. The amplification was checked on an ultraviolet transilluminator and photographed.

2.4. Data analysis and statistical methods

Estimation of frequencies for each particular *HLA-DRB1* and *HLA-DQB1* allele was determined by direct counting. Differences between populations in the distribution of *HLA-DRB1* and *HLA-DQB1* alleles were estimated by $\chi 2$ test with Yate's correction and Fisher's exact test when relevant. All presented p values are uncorrected and labeling with ((a)) indicates values which remain significant after correction ($P < 0.05$). For assessing the consistency of genotype distribution with Hardy-Weinberg equilibrium, $\chi 2$ test was used. Neighbor-Joining tree was conducted using MEGA2 (http://www.megasoftware.net) based on Nei's genetic distances. Correspondence analysis was performed using MVSP3.1

(http://www.kovcomp.com) to determine genetic affinities according to *HLA-DRB1* and *HLA-DQB1* alleles data from 5 different Iranian populations, i.e. the Lak Iranian population described in this study and 4 other populations described elsewhere.

3. Results

The frequencies of *HLA-DRB1* and *HLA-DQB1* alleles in Lak population of Iran are showed in Table 1. We identified 24 alleles for *HLA-DRB1* and 10 alleles for *HLA-DQB1* in our population sample of 100 Lak individuals.

The most frequent *HLA-DRB1* alleles were *1103 = 4 (23%), *1502 (9.5%), *0701 (9%), *0301 (8.5%), *1101 (7.5%) and *1501 (6%) while *HLA-DQB1**0301 (40%), *0201 (15%), *0502 (10.5%), *0303 (10%), *0602 = 3 (9.5%), and *0501 (7.5%) were the most frequent alleles in Lak population. *HLA-DRB1**0409, *0804, *1102, *1112, *1405, and *HLA-DQB1**0503, *0604 were the least observed frequencies in Lak population.

Neighbor-joining tree based on Nei's genetic distances (Fig. 2) and correspondence analysis (Fig. 3) based on *HLA-DRB1* and *HLA-DQB1* allele frequencies show the genetic relationship of Laks with other Iranian subpopulations. As illustrated, the Laks are close to Lurs of Khorramabadi and Kurd but far from Lurs of Kohkiloyeh/Boyerahmad and Bakhtiari.

4. Discussion

In the present study, *HLA-DRB1* and *HLA-DQB1* allele frequencies were tested in 100 unrelated Lak individuals by a PCR-SSP typing method. The *HLA-DRB1* and *HLA-DQB1* allele frequencies in Lak, Lur of Khorramabadi [10], Lur of Kohkiluyeh/Buyerahmad [10], Lur of Bakhtiari [10], and Kurd [14] populations of Iran are indicated in Table 1, which also shows any significant differences.

In this study, *HLA-DRB1**0401, *0405, *0408, *0410, *0802, *0803, *1304, *1402, *1403, *1503, and *HLA-DQB1**0401 were found in none of the studied populations. The most common *HLA-DRB1* allele in Lak, Lurs of Khorramabadi and Kohkiloyeh/Boyerahmad and Kurd was *1103 = 04 while *HLA-DRB1**0701 was the most common allele in Lur of Bakhtiari. The frequency of *HLA-DQB1**0301 was the most common allele in all studied populations, including ours (Table 1).

Comparing our results with those of the studies in the certain other populations of Iran [10,14], we found high similarity of the *HLA-DRB1* and *HLA-DQB1* allele frequencies for the Lak, Lur of Khorramabadi and Kurd populations. On the other hand, considerable differences with previous studies were found for certain *HLA-DRB1* and *HLA-DQB1* alleles (Table 1): *HLA-DRB1*0701 (Lur of Khorramabadi), *HLA-DRB1**0103, *0301 and *HLA-DQB1**0501 (Lur of Kohkiloyeh/Boyerahmad), *HLA-DRB1**1103 = 4 and *HLA-DQB1**0301, *0503, *0601 (Lur of Bakhtiari), and *HLA-DQB1**0503 (Kurd).

As shown in Table 1, the frequencies of *HLA-DRB1**0103, *0301, and *HLA-DQB1**0503, *0601 in Lak population were significantly lower than the other studied populations while *HLA-DRB1**0701, *1103 = 04, and *HLA-DQB1**0301, *0501 alleles were significantly higher than the other studied populations.

Neighbor-joining tree based on Nei's genetic distances (Fig. 2) and correspondence analysis (Fig. 3) using HLA-DRB1 and HLA-DQB1 allele data from Lak population and a total of 4 previously described populations [10,14] illustrates relationship of Lak population with Lur of Khorramabadi [10] and Kurd [14] populations and at most HLA-DRB1 and HLA-DQB1 alleles difference with Lur of Kohkiloyeh/Boyerahmad and Bakhtiari [10] populations. Despite a probable common ancestor [10], this genetic difference might be explained by their admixture with other Iranian ethnic groups due to their life style. HLA genes are still considered as useful markers for study of correlation between genetic and the degree of admixture in different populations, however the extensive use of various kinds of DNA markers, for example, mitochondrial DNA and Y chromosome. As a result, to assess the genetic relationships among populations from different geographic areas in anthropological studies, analysis of HLA-DRB1 and HLA-DQB1 allele data could be used as an exclusive means.

Moreover, establishment of positive percentage for HLA-DRB1 and HLA-DQB1 alleles in the Lak population of Iran may be applicable for establishing of bone marrow donor registries as well as in studies of HLA-associated diseases. In addition, to achieve highest population coverage with peptide vaccines against tumors and infectious agents, the information about HLA-DRB1 and HLA-DQB1 alleles distribution in the Lak population is a necessity.

In conclusion, our results based on *HLA-DRB1* and *HLA-DQB1* allele frequencies showed that the Lak population possesses the previously reported general features of the Lur and Kurd populations, with some additional interesting differences. In other words, the Lak population is close to Lurs of Khorramabadi and Kurd but far from Lurs of Kohkiloyeh/Boyerahmad and Bakhtiari.

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