Iranian Lurs Genetic Diversity: An Anthropological View Based on HLA Class II Profiles

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ABSTRACT

Background: HLA genes are highly polymorphic and certain alleles are frequent only in specific populations. Therefore, HLA is a unique tool for studying the genetic relationship between different populations. Iranians are ethnically diverse people and one of the major ethnic groups in Iran is Lur population inhabiting along the central and southern parts of Zagros Chain Mountain. **Objectives:** Genetic relationship among three Lur subpopulations was investigated based on HLA class II profiles. **Methods:** HLA typing was performed using PCR/RFLP and PCR/SSP methods in 154 individuals from three Lur subpopulation living in Luristan, Kohkiloyeh/ Boyerahmad, and Chahar-Mahal/ Bakhtiari. **Results:** The most common DRB1 allele in Lurs of Luristan and Kohkiloyeh/ Boyerahmad was *1103=4 while DRB1*0701 was the most common allele in Bakhtiaris. DQA1*0501 and DQB1*0301 were the most frequent alleles and DRB1*1103=04-DQA1*0501-DQB1*0301 was the predominant haplotype in the three studied subpopulations. Neighbor-joining tree based on Nei's genetic distances and correspondence analysis according to DRB1, DQA1, and DQB1 allele frequencies showed a close genetic relationship between Lurs of Luristan and Lurs of Kohkiloye/ Boyerahmad and they were well separated from Bakhtiaris. The results of AMOVA revealed no significant difference between the three studied groups of Lurs and other major ethnic groups of Iran. **Conclusion:** The results of this study revealed that Bakhtiaris were genetically far from the two other Lur subpopulations. Despite a probable common ancestor, this genetic difference might be explained by Bakhtiaris admixture with other Zagros inhabitants due to their nomadic life style.

Keywords: Anthropology, HLA polymorphism, Lurs, Iran

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INTRODUCTION

In humans, HLA region is extended along 3.6 Mb on chromosome six and contains 239 gene loci which about 40% of them have immunological functions (1). Besides being considered by immunologists for its pivotal role in the immune response, the HLA genes have also attracted the attention of many evolutionary biologists due to the high levels of allelic diversity (2).

Since certain HLA alleles and haplotypes are frequent only in specific populations, these genes are also regarded as useful markers by anthropologists for determination of genetic relationship and interaction among different populations (3,4). Furthermore, knowledge of the HLA allele distributions in various populations is critical for establishing bone marrow donor registries and in studies of HLA associated disease (5,6). On the other hand, to achieve maximum population coverage with peptide vaccines against tumors or infections, information about HLA allele distribution in the target population is necessary (7).

Iran is an ethnically diverse country, consisting different groups including Pars, Turk, Kurd, Arab, Turkmen, Baloch, and Lur. Most of the Iranians are Muslims but Zoroastrians, Jews, and Armenians are also living in this country (8). A long-standing belief of historians is that the most current Iranians are Aryan (9) but during the history, they have been encountered with different foreigners e.g. Macedonians (334 to 331 BC), Arabs (7^{th} century), Turks (10th century), and Mongols (13th to15th centuries) (9, 10). Also, as a country located between Asia and Europe, Iran has played a key role in connecting various populations along the Silk Road (11). Therefore, the population living in this country might be admixed due to encounter with other populations and immigrants from neighboring populations (8). In the present study, genetic relationship among three different Lur subpopulation was investigated based on HLA class II profiles.

MATERIALS AND METHODS

Samples. Blood samples were collected with informed consent from unrelated healthy inhabitants of three provinces of central and southern Zagros including 50 cases from Luristan (Khoramabad), 54 from Kohkiloyeh/ Boyerahmad (Yasouj), and 50 from Chahar-Mahal/ Bakhtiari (Shahrekurd). All participants were third generation natives of the selected areas and none had personal or family history of cancer or autoimmune diseases. DNA was extracted using salting out method (12).

HLA Genotyping. HLA-DQA1, DQB1, and DRB1 typing was performed using PCR/RFLP method (13). In this method, the polymorphic exon 2 domains were amplified and PCR products were digested with appropriate restriction enzymes (13). Then the digested products were subjected to 12% polyacrylamide gel and results were compared with previous reports (13). Samples with DRB1 heterozygous combinations that were not completely distinguishable by the above method were subjected to type by a high resolution DRB kit (Biotest AG, Germany). This kit is based on PCR/SSP method that utilizes allele-specific primer combinations in 73 reactions for DRB typing.

Data Analysis. Allele and haplotype frequencies, as well as Slatkin's implementation of Ewens-Watterson (EW) homozygosity test of neutrality (14), were analysed using PyPop (http://allele5.biol.berkeley.edu/pypop). Significant variation of allele frequencies among the studied ethnic groups was calculated by nonparametric test of Chi-Square using SPSS. Analysis of molecular variance (AMOVA) (15) and Hardy-Weinberg (HW) exact test were performed by ARLEQUINE 2.000 (http://anthro.unige.ch/arlequin). Neighbor-Joining tree was conducted using MEGA2 (http://www.megasoftware.net) based on Nei's genetic distances (16) calculated by DISPAN (http://www.bio.psu.edu/People/faculty/Nei/Laboratory/Programs.html). Correspondence analysis was performed according to HLA class II allele frequencies using MVSP3.1 (http://www.kovcomp.com).

RESULTS

In this study, the results of HLA-DRB1, DQA1 and DQB1 allele frequencies and haplotypes of three different Lur subpopulation of Iran were presented. The results were also compared with Pars (17), Zoroastrian (17), Baloch (18), Arab, Jew, Turk, and Kurd (unpublished data) subpopulations of Iran.

Allele frequencies at the HLA class II loci and the three-locus haplotype frequencies are presented in tables 1 and 2. In this study, DRB1*0401, *0405, *0408, *0410, *0802, *0803, *1304, *1402, *1403, *1503, DQA1*0601, and DQB1*0401 were found in none of the studied populations. The most common DRB1 allele in Lurs of Luristan and Kohkiloyeh/ Boyerahmad was *1103=04 while DRB1*0701 was the most common allele in Bakhtiaris. DQA1*0501 and DQB1*0301 were the most frequent alleles (Table 1) and DRB1*1103=04-DQA1*0501-DQB1*0301 was the predominant haplotype in the three studied subpopulations (Table 2). As shown in Table 1, the frequency of DRB1*1602 in Lurs of Luristan, DRB1*0301 in Lurs of Kohkiloyeh/ Boyerahmad, and DRB1*0701, DQA1*0201, and DQB1*0303 in Bakhtiaris were significantly higher than the other studied subpopulations while DRB1*1103=04, DQA1*0501, and DQB1*0301 alleles were significantly lower in Bakhtiaris.

Significant deviation from HW expectation was observed at HLA-DRB1 locus (excess heterozygosity = 0.03273, $P \approx 0.00$) and DQB1 locus (excess heterozygosity = 0.06828, $P < 0.00041$) in Bakhtiaris as well as HLA-DRB1 locus in Lurs of Luristan (excess heterozygosity = 0.17455, $P \approx 0.00$) (Table 3). The results of homozygosity test of neutrality also revealed significant negative normalized deviations from F at DQA1 (Fnd = -1.3762, $P < 0.0233$) and DQB1 (Fnd = -1.3949, $P < 0.0065$) loci in Bakhtiaris (Table 4).

AMOVA was performed to estimate the distribution of genetic diversity within Lur subpopulation as one group in comparison to Pars, Zoroastrian, Baloch, Arab, Jew, Turk and Kurd subpopulations of Iran based on the allele frequencies in three studied HLA class II loci. The results of AMOVA revealed that the main variation component (95.9%) was contributed to by the within-population level as genetic differentiation (Fst) was 0.04 (Table 5).

Neighbor-joining tree based on Nei's genetic distances and correspondence analysis based on HLA class II allele frequencies show the genetic relationship of Lurs with other Iranian subpopulations (Figure 1). As illustrated, the Lurs of Luristan are close to Lurs of Kohkiloyeh/ Boyerahmad but far from Bakhtiaris.

Table 1. HLA class II allele frequencies in the three Iranian Lur subpopulations.

DRB1	Luristan	Kohkiloyeh/ Boyerahmad	Chahar-Mahal/ Bakhtiari	DQA1	Luristan	Kohkiloyeh / Boyerahmad	Chahar-Mahal/ Bakhtiari
0101	2 (0.020)	–	1 (0.010)	0101=02	23 (0.230)	22 (0.204)	28 (0.280)
0102	–	1 (0.009)	4 (0.040)	0103	12 (0.120)	13 (0.120)	15 (0.150)
0103	–	7 (0.065)	3 (0.030)	0201	2 (0.020)	3 (0.028)	10 (0.100)*
0301	8 (0.080)	25 (0.231)*	9 (0.090)	0301	8 (0.080)	10 (0.093)	13 (0.130)
0302	–	–	1 (0.010)	0401	–	–	2 (0.020)
0402	2 (0.020)	4 (0.037)	6 (0.060)	0501	55 (0.550)	60 (0.556)	32 (0.320)**
0403	2 (0.020)	–	1 (0.010)	Total	100 (1.000)	100 (1.000)	108 (1.000)
0404	1 (0.010)	1 (0.009)	–				
0407	–	–	3 (0.030)				
0409	2 (0.020)	2 (0.019)	–				
0411	–	–	2 (0.020)				
0701	2 (0.020)	3 (0.028)	12 (0.120)*	**DQB1**			
0801	–	–	1 (0.010)	0201	14 (0.140)	25 (0.231)	17 (0.170)
0804	1 (0.010)	–	–	0301	46 (0.460)	38 (0.352)	22 (0.220)**
0901	–	–	1 (0.010)	0302	–	2 (0.019)	–
1001	3 (0.030)	3 (0.028)	5 (0.050)	0303	7 (0.070)	8 (0.074)	17 (0.170)*
1101	10 (0.100)	7 (0.065)	4 (0.040)	0402	1 (0.010)	1 (0.009)	2 (0.020)
1102	–	–	2 (0.020)	0501	6 (0.060)	2 (0.019)	6 (0.060)
1103=04	31 (0.310)	26 (0.241)	10 (0.100)**	0502	11 (0.110)	14 (0.130)	7 (0.070)
1201	2 (0.020)	–	1 (0.010)	0503	1 (0.010)	5 (0.046)	8 (0.080)
1202	–	–	1 (0.010)	0601	6 (0.060)	7 (0.060)	12 (0.120)
1301	6 (0.060)	5 (0.046)	3 (0.030)	0602=03	7 (0.070)	6 (0.056)	6 (0.060)
1302	2 (0.020)	–	1 (0.010)	0604	1 (0.010)	–	3 (0.030)
1303	2 (0.020)	1 (0.009)	3 (0.030)	Total	100 (1.000)	100 (1.000)	108 (1.000)
1305	–	–	3 (0.030)				
1401	1 (0.010)	2 (0.019)	4 (0.040)				
1404	–	–	1 (0.010)				
1501	6 (0.060)	7 (0.065)	7 (0.070)				
1502	6 (0.060)	5 (0.046)	7 (0.070)				
1601	4 (0.040)	6 (0.056)	3 (0.030)				
1602	7 (0.070)*	2 (0.019)	1 (0.010)				
1605	–	1 (0.009)	–				
Total	100 (1.000)	100 (1.000)	108 (1.000)				

The most frequent alleles are bolded
*significantly increased frequency
**significantly decreased frequency

Table 2. Haplotype frequencies (HF) of the most frequent DRB1-DQA1-DQB1 haplotypes in the three Iranian Lur subpopulations.

Luristan		Kohkiloyeh / Boyerahmad		Chahar-Mahal/ Bakhtiari	
DRB1-DQA1-DQB1	**HF**	**DRB1-DQA1-DQB1**	**HF**	**DRB1-DQA1-DQB1**	**HF**
1103=04-0501-0301	0.310	1103=04-0501-0301	0.231	1103=04-0501-0301	0.100
1101-0501-0301	0.100	0301-0501-0201	0.194	0301-0501-0201	0.067
0301-0501-0201	0.080	1101-0501-0301	0.065	1502-0103-0601	0.060
1602-0101=02-0502	0.070	1601-0101=02-0502	0.056	0402-0301-0303	0.060
1502-0103-0601	0.060	1501-0101=02-0502	0.046	0701-0201-0201	0.053
1601-0101=02-0502	0.040	1301-0103-0602=03	0.046	1501-0103-0601	0.030
1301-0103-0602=03	0.040	1502-0103-0601	0.037	0102-0101=02-0501	0.030
1001-0101=02-0501	0.030	0402-0301-0303	0.037	1001-0101=02-0501	0.030
1501-0101=02-0602=03	0.030	0301-0501-0301	0.028	1301-0103-0602=03	0.030
		0701-0201-0201	0.028	1401-0101=02-0503	0.030
				1101-0501-0301	0.030
				1305-0501-0301	0.030
				1501-0101=02-0602=03	0.030
				0407-0301-0303	0.030
				0701-0201-0303	0.027

Haplotype frequencies greater than 0.025 are listed

Table 3. Deviation from Hardy-Weinberg equilibrium in HLA class II loci in the three Iranian Lur subpopulations.

	# Geno.	Obs.Heter.	Exp.Heter.	p-value
Luristan				
DRB1	50	0.70000	0.87455	0.00000*
DQA1	50	0.56000	0.62970	0.56812
DQB1	50	0.72000	0.74687	0.12604
Kohkiloyeh/ Boyerahmad				
DRB1	54	0.83333	0.87227	0.09033
DQA1	54	0.55556	0.69609	0.29476
DQB1	54	0.72222	0.79751	0.10816
Chahar-Mahal/ Bakhtiari				
DRB1	50	0.92000	0.95273	0.00000*
DQA1	50	0.80000	0.77717	0.96880
DQB1	50	0.80000	0.86828	0.00041*

Geno: Number of genotypes
Obs.Heter: Observed heterozygosity
Exp.Heter: Expected heterozygosity
* Statistically significant

Table 4. Slatkin's implementation of EW homozygosity test of neutrality in the three Iranian Lur subpopulations.

	Observed F	Expected F	Fnd	p-value of F
Luristan				
DRB1	0.1342	0.1252	0.2426	0.7020
DQA1	0.3766	0.4907	-0.7154	0.2740
DQB1	0.2606	0.2722	-0.1230	0.5486
Kohkiloyeh/ Boyerahmad				
DRB1	0.1358	0.1463	-0.2286	0.5090
DQA1	0.3740	0.4958	-0.7546	0.2571
DQB1	0.2099	0.2772	-0.6936	0.2612
Chahar-Mahal/ Bakhtiari				
DRB1	0.0604	0.0806	-1.0184	0.0844
DQA1	0.2306	0.4274	-1.3762	0.0233*
DQB1	0.1404	0.2722	-1.3949	0.0065*

Fnd: Normalized deviation of F
* Significant at 5% level

Table 5. AMOVA analysis based on HLA-DRB1, DQA1, and DQB1 allele frequencies when three Lur subpopulation are considered as one group in comparison with Pars, Zoroastrian, Baloch, Arab, Jew, Turk, and Kurd subpopulations of Iran.

Source of Variation	d.f	Sum of Squares	Variance components	Percentage of Variation
Among groups	1	11.743	0.01584	1.24
Among populations within groups	4	23.612	0.03632	2.85
Within populations	776	948.825	1.22271	95.91
Total	781	984.179	1.27487	

Fixation indices
FST: 0.04091*
FSC: 0.02885*
FCT: 0.01242

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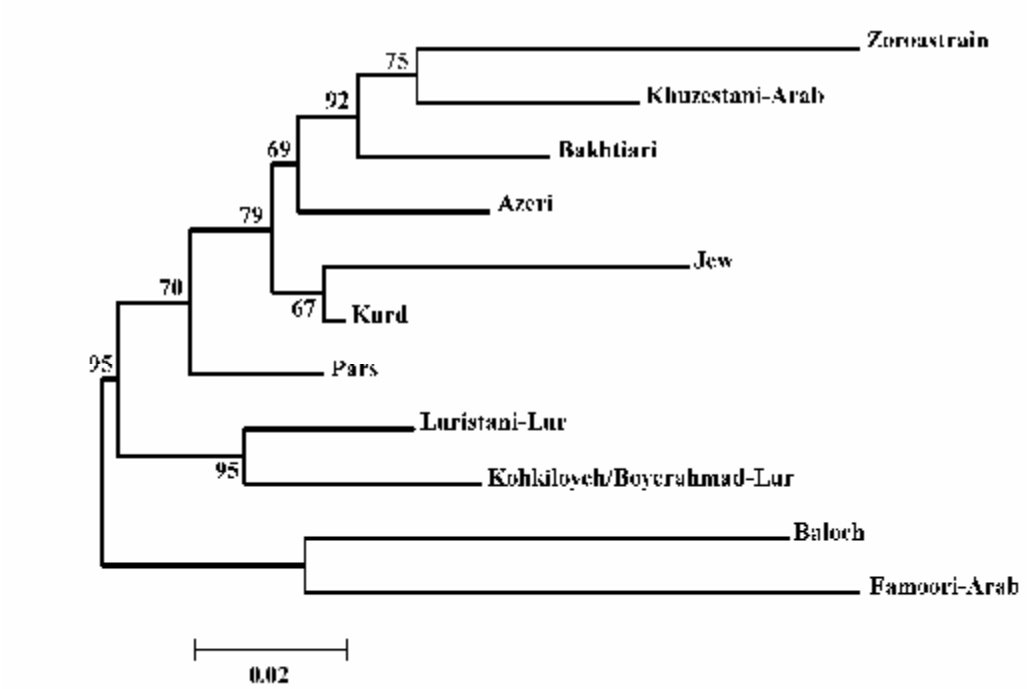
$FCT = \sigma^2a/ \sigma^2T$, $FSC = \sigma^2b/ \sigma^2T$, $FST = \sigma^2a + \sigma^2b/ \sigma^2T$

(a)



(b)

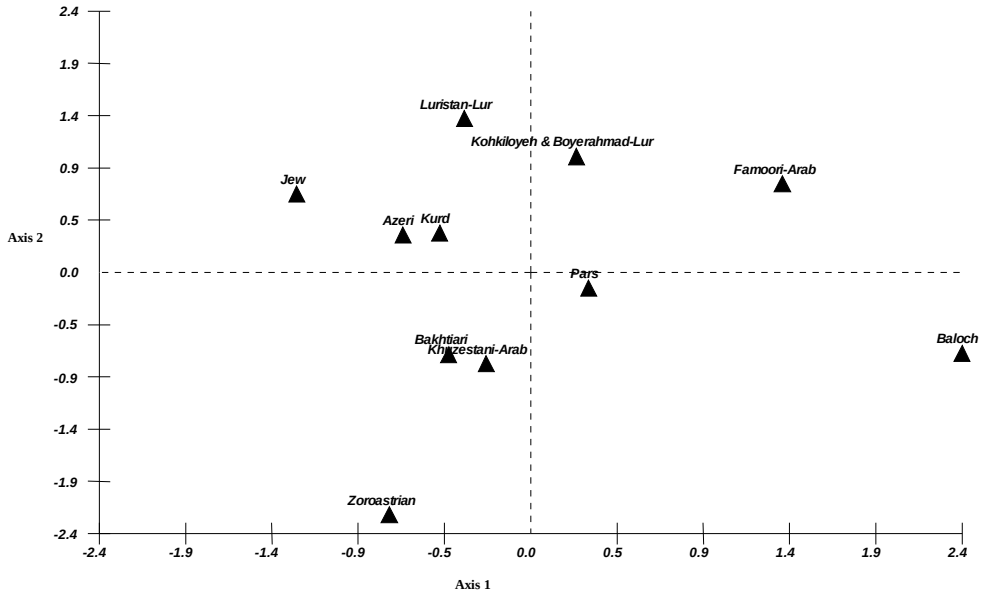


Figure1. (a) *Neighbor-joining tree linking eleven Iranian subpopulations using Nei's genetic distances based on HLA-DRB1, DQA1, and DQB1 allele frequencies. Numbers by internal branches represent bootstrap values (%) based on 1000 replications.* ***(b)*** *Correspondence analysis depicting the genetic relationship among these subpopulations.*

DISCUSSION

It is commonly believed that modern Iranians are Aryans whose ancestors, Indo-Europeans, were originated from southwestern steppes of the present-day Russia. Aryans migrated toward south into Afghanistan and Eastern Iran around 2000 BC. Then, they have been split into Indo-Aryans and Proto-Iranians and the latter group seems to be the direct ancestor of modern Iranians; however, different people with established civilizations inhabited this country long before Indo-Europeans' infiltration (10).

One of the major Iranian ethnic groups is Lur population, inhabiting along the central and southern parts of Zagros Chain Mountain. Their origin might come back to the time before the migration of Indo-Europeans to Iran when other groups called Kassites and Elamites have been living there (19).

The Kassites are said to be the native people of Luristan. Their language was a non-Semitic non-Indo-European language and it differed from the Elamite. The Kassites overthrew the Babylon in 1595 BC and extended their lands to the borders of Egypt and Anatolia. Their last king was defeated by the Elamites in 1180 (19). The remaining of the Kassites subpopulation, who had managed to keep their own identity, retreated back to the high mountains of Luristan, where they became part of the Elamites and eventually the Persian Empire. However, the Kassites stayed independent during the times of the Medes and Achaemenians (19).

In this study, the patterns of HLA class II alleles and haplotypes are almost similar between Lurs of Luristan and Kohkiloyeh/ Boyerahmad but different from Bakhtiaris (Tables 1 and 2). A significant deviation from HW equilibrium with excess of homozygosity at DRB1 locus in Lurs of Luristan as well as in DRB1 and DQB1 loci of Bakhtiaris (Table 3) might be caused by intra-ethnical marriage or pseudo-homozygosity resulted from the resolution of typing methods (20). Homozygote samples might be truly heterozygote when subjected to high resolution typing methods such as SBT analysis.

Significant negative Fnd values in DQA1 and DQB1 loci of Bakhtiaris (Table 4) might also represent a probable direction to balancing selection. Heterozygote advantage as a main cause of balancing selection is very common in HLA genes. It means that natural selection favors HLA heterozygous individuals because they can present more diverse peptides from infectious pathogens than homozygotes (21). This might reflect the Bakhtiaris exposure to more pathogens due to their nomadic life style.

As illustrated in figure 1, Lurs of Luristan and Kohkiloyeh/ Boyerahmad are located in the same cluster which could be explained based on their common genetic background. No strong genetic relationship was observed between them and Bakhtiaris which might be caused by Bakhtiaris genetic admixture with other Iranian ethnic groups due to their life style.

In spite of these differences, the results of AMOVA revealed that about 96% of the variation components among eleven different Iranian subpopulations are contributed to by the within ethnic groups and less than 3% of the variations is related to the Lur subpopulation when considered as a common group (Table 5).

In conclusion, the results of this study revealed that Bakhtiaris were genetically far from the two other Lur subpopulations. Despite a probable common ancestor, this genetic difference might be explained by Bakhtiaris admixture with other Zagros in-

habitants due to their nomadic life style. Complementary data from HLA class I, mitochondrial DNA, Y chromosome, and other nuclear factors will shed more light on the genetic history of Iranian subpopulations.

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