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Molecular analysis of HLA allele frequencies and haplotypes in Baloch of Iran compared with related populations of Pakistan

Key words:
anthropology; Baloch; HLA polymorphism; Iran; population genetics

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Abstract: The extreme polymorphism in different loci of the human leukocyte antigen (HLA) system has been used as an invaluable tool for anthropological studies. Determination of HLA allele and haplotype frequencies in different ethnic groups is useful for population genetic analyses and the study of genetic relationships among them. In the present study, molecular analysis of *HLA-A*, *-B*, *-C*, *-DQA1*, *-DQB1*, and *-DRB1* genes has been used to assign HLA allele and haplotype frequencies in 100 unrelated healthy individuals from the Baloch ethnic group of Iran. The results were compared with Baloch and other ethnic groups in the neighboring Pakistan. The results of this study showed that the most frequent HLA class I alleles were A*02011 (20.2%), B*4006 (11.1%), and C*04011 (28.6%). The most common HLA class II alleles were DQA1*0101/2 (42.5%), DQB1*0201 (32%), and DRB1*0301 (29%). Three-locus haplotype analysis revealed that A*11011-B*4006-C*15021 (5.8%) and DQA1*0501-DQB1*0201-DRB1*0301 (22.1%) were the most common HLA class I and II haplotypes, respectively, in this population. Neighbor-joining tree based on DA genetic distances and correspondence analysis according to HLA-A, -B, -DQB1, and -DRB1 allele frequencies showed that Baloch of Iran are genetically very close to Baloch and Brahui of Pakistan. This may reflect an admixture of Brahui and Baloch ethnic groups of Pakistan in the Balochistan province of Iran.

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Iran with a population of over 60 million individuals and an area of about 1.6 million square kilometers is situated in south-western Asia and borders the Republic of Armenia, Azerbaijan, and Turkmenistan, as well as the Caspian Sea to the north; Turkey and Iraq to the west; the Persian Gulf and the Gulf of Oman to the south; and Pakistan and Afghanistan to the east. The oldest known civilization in Iran is Elam in the 10th century BC, but Arab and subsequent Mongol and Tatar invasions, each left its own impression on the subsequent development of different populations in this country (1). Iran also, as a country located between China and Europe, played a key role in connecting various cultures and civilizations that existed along the Silk Road (2).

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Iranian populations are divided into several ethnic groups such as Persian, Turk, Kurd, Arab, Turkmen, Baloch, and Lur. Most of Iranians are Muslims but Zoroastrians, Jews, Armenians, and Nestorians are also living in this country (3).

Although it seems that most Iranian people are of Aryan origin whose ancestors migrated from Central Asia, the populations living in this country are highly admixed because of its invasion by other populations and immigration of some people from neighboring countries.

Polymorphic genes combined with their unique haplotypic inheritance pattern have proven invaluable for genetic analyses and anthropological studies (4–6). However and despite the wide use of various kinds of DNA markers, e.g., mitochondrial DNA, Y-chromosome, and microsatellites, polymorphic human leukocyte antigen (HLA) loci continue to be regarded as useful genetic markers for determination of genetic relatedness and the degree of admixture between different populations (7, 8). In the present study, relatedness of Baloch of Iran and Pakistan and finding a probable genetic relation between these populations and other major ethnic groups in Pakistan were our main objectives. This work is part of a larger effort aimed at analysis of the genetic profile of diverse Iranian ethnics.

Materials and methods

Samples

Blood samples were collected with informed consent from 100 unrelated healthy Baloch individuals residing in the Balochistan province of Iran. DNA was isolated from fresh blood using salting out extraction procedure as described by Miller et al. (9).

HLA class I genotyping

HLA-A, -B, and -C alleles were determined by sequence-based typing (SBT) method using AlleleSEQR kits (Forensic Analytical, Amersham Biosciences, NJ, USA). In this method, amplification of exons 2, 3, and 4 for HLA-A and -B and exons 2 and 3 for HLA-C was performed in one polymerase chain reaction (PCR) reaction for each locus. Subsequently, forward and reverse fluorescent products were produced using nested primers for each exon separately. Fluorescent products were then precipitated by ethanol and mixed with loading dye containing 5 : 1 deionized formamid : blue dextran (50 mg/ml) and ethylenediaminetetraacetic acid (25 mM). Each mixture was heated to 97°C for 2 min prior to loading on a 0.4-mm thick 4% acrylamide−7 M urea gel. Electrophoresis was run on an ABI PRISM 377 DNA sequencer (Applied Biosystems, CA, USA). Gel-running conditions were set on 1.68 kV, 50 mA, and 150 W at 51°C for 7 h and fluorescent emission was measured every 2 s. Finally, HLA alleles were assigned using MATCH TOOLS and MT-NAVIGATOR softwares (Applied Biosystems).

HLA class II genotyping

HLA-DQA1, -DQB1, and -DRB1 typing was performed by PCR/restriction fragment length polymorphism (RFLP) method as described previously (10). In this method, the polymorphic exon 2 domains in aforementioned genes were amplified and PCR products were digested with appropriate restriction enzymes. Digested products were then subjected to 12% polyacrylamide gel, and results were compared with previously reported patterns (10). The nomenclature for HLA-class II typing was in accordance with Marsh et al. (11).

Statistical analyses

Allele and haplotype frequencies were calculated by ARLEQUIN version 2.000 (http://anthro.unige.ch/arlequin). Nei's genetic distances – DA distances – (12) were calculated based on HLA-A, -B, -DQB1, and -DRB1 allele frequencies using DISPAN (http://www.bio.psu.edu/People/faculty/Nei/Laboratory/Programs.html). Phylogenetic tree was constructed using neighbor-joining method (13) by MEGA version 2.1 (http://www.megasoftware.net). 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 or other allele frequencies. This type of analysis tends to give results similar to those of dendrograms but is more informative and accurate than dendrograms especially when there is considerable genetic exchange between close geographic neighbors (14). Correspondence analysis in three dimensions and its bi-dimensional representation was performed according to HLA-A, -B, -DQB1, and -DRB1 allele frequencies using VISTA version 6.4 (15) (http://forest.psyh.unc.edu).

Results

In this study, the results of HLA class I and class II typing of 100 unrelated healthy Baloch individuals of Iran are reported. The results were also compared with Baloch and other major ethnic groups of Pakistan.

A total of 44, 45, and 25 alleles were detected in HLA-A, -B, and -C loci, respectively. As summarized in Table 1, the two most frequent HLA class I alleles in each locus were A*02011 (20.2%) and *11011 (14.6%); B*4006 (11.1%) and *5301 (9.1%); and C*04011 (28.6%) and *15021 (15.6%).

HLA-A, -B, -C, -DQA1, -DQB1, and -DRB1 allele frequencies (F%) in Baloch of Iran

HLA-A	F (%)	HLA-B	F (%)	HLA-C	F (%)	DQA-1	F (%)	DQB-1	F (%)	DRB-1	F (%)
01011	1.7	07021	1.0	0102	3.10	0101/2	42.5	0201	32.0	0101	15.0
0103	0.6	07023	0.5	02022	0.5	0103	8	0301	10.5	0102	2.5
02011	20.2	0705	1.5	02024	0.5	0201	3.5	0302	1.5	0103	2.0
0204	0.6	0801	7.6	0302	4.7	0301	6	0303	2.5	0301	29.0
0205	0.6	1302	0.5	03031	0.5	0401	4.5	0402	3.0	0302	8.5
0206	1.1	1402	1.5	04011	28.6	0501	35.5	0501	10.5	0403	1.0
0209	1.1	14062	1.5	0404	0.5			0502	19.0	0405	1.5
0211	1.1	1503	1.0	0407	0.5			0503	8.5	0701	3.0
0212	0.6	1510	1.0	0501	0.5			0601	8.0	080 (2,4)	0.5
0224	0.6	1801	7.1	0602	4.2			0602 = 3	3.5	1101	1.5
0225	0.6	1803	1.0	07011	7.8			0604	1.0	110 (1,3 = 4)	2.0
0236	0.6	1806	1.5	0702	9.4					1102	1.5
03011	5.6	1807	1.5	0710	0.5					110 (3 = 4)	3.5
0302	0.6	1811	0.5	0802	2.6					1301	1.0
11011	14.6	27052	0.5	0804	2.6					130 (1,2)	2.5
2301	1.1	27054	0.5	12021	3.1					130 (3,4)	0.5
24021	3.4	2707	0.5	12031	4.2					1305	0.5
24031	0.6	35011	8.1	14021	2.1					1404	1.0
2413	0.6	3502	2.0	15021	15.6					1501	4.5
2416	0.6	3503	1.0	1504	0.5					1502	8.0
2423	0.6	3508	1.5	15051	1.6					1601	4.5
2427	0.6	3527	4.0	1601	1.0					1602	6.0
2601	4.5	39062	1.0	1602	2.6						
2610	0.6	4006	11.1	1701	1.6						
2901	2.8	4201	1.5	1801	1.0						
2904	0.6	44031	2.5								
3001	2.2	4418	0.5								
3002	2.8	4501	1.5								
3004	0.6	4901	0.5								
3009	0.6	51011	5.6								
31012	2.2	51012	2.5								
3201	6.7	5104	0.5								
3301	2.8	5105	0.5								
3303	5.6	5108	0.5								
51012	0.6	5109	0.5								
5801	0.6	52011	2.5								
6601	0.6	5301	9.1								
68011	1.1	5304	0.5								
68012	1.7	5501	2.5								

continued

HLA-A	F (%)	HLA-B	F (%)	HLA-C	F (%)	DQA-1	F (%)	DQB-1	F (%)	DRB-1	F (%)
6802	1.1	5503	0.5								
6901	0.6	5701	1.0								
7401	2.8	5801	4.0								
7402	1.1	5802	1.0								
7403	0.6	7301	0.5								
		8101	3.5								

Table 1

Three-locus haplotype analysis showed 473 different HLA class I haplotypes in which A*11011-B*4006-C*15021 (5.8%); A*02011-B*3527-C*04011 (3.5%); and A*3303-B*5801-C*0302 (3.5%) were the most common (Table 2).

There were 6, 11, and 22 alleles in HLA-DQA1, -DQB1, and -DRB1 loci, respectively. As summarized in Table 1, the two most frequent HLA class II alleles in each locus were DQA1*0101/2 (42.5%), *0501 (35.5%); DQB1*0201 (32%), *0502 (19%); and DRB1*0301 (29%) and *0101 (15%).

There were also 209 different three-locus HLA-II haplotypes (Table 2). DQA1*0501-DQB1*0201-DRB1*0301 (22.1%); QA1*0101/2-DQB1*0501-DRB1*0101 (7%); and DQA1*0101/2-DQB1*0502-DRB1*1602 (6%) were three most common haplotypes.

HLA-A-B-C, HLA-DQA1-DQB1-DRB1, and HLA-A-B-DRB1 three-locus haplotype frequencies (HF%) in Baloch of Iran

A	B	C	HF (%)	DQA1	DQB1	DRB1	HF (%)	A	B	DRB1	HF (%)
11011	4006	15021	5.8	0501	0201	0301	22.1	11011	4006	1502	2.3
02011	3527	04011	3.5	0101/2	0501	0101	7.0	2601	0801	0301	2.3
3303	5801	0302	3.5	0101/2	0502	1602	6.0	02011	1801	0301	1.7
11011	5301	04011	2.7	0101/2	0503	0101	4.4	02011	3527	0301	1.7
03011	35011	04011	2.3	0101/2	0502	1601	4.0	03011	35011	0101	1.7
3002	5301	04011	2.3	0103	0601	1502	3.5	3002	5301	0301	1.7
02011	1801	07011	1.7	0103	0601	1501	2.5	3303	5801	0301	1.7
11011	35011	04011	1.7	0201	0201	0701	2.5	7401	8101	0302	1.7
2601	0801	0702	1.7	0401	0402	0302	2.5	02011	0801	0301	1.1
7401	8101	0804	1.7	0501	0301	110 (3 = 4)	2.5	02011	1806	0101	1.1
02011	0801	0702	1.6	0501	0201	0302	2.4	02011	35011	0101	1.1
02011	5301	04011	1.3	0101/2	0502	0301	2.2	02011	4006	0301	1.1
11011	0801	0702	1.3	0101/2	0502	0101	1.6	02011	4201	1501	1.1
02011	4006	15021	1.2	0101/2	0501	0102	1.5	02011	5301	0301	1.1
03011	4006	15021	1.2	0501	0301	110 (1,3 = 4)	1.5	11011	0801	0302	1.1
11011	52011	12021	1.2	0501	0301	1102	1.5	11011	1801	1602	1.1
2601	51011	04011	1.2	0101/2	0502	0103	1.4	2601	51011	0101	1.1
2901	0705	15051	1.2	0301	0201	0301	1.1	3201	51011	0101	1.1
3201	51011	15021	1.2	0501	0201	0101	1.0	3303	51011	0301	1.1
3301	1402	0802	1.2	0101/2	0501	130 (1,2)	1.0	3303	5801	1602	1.1
3301	8101	1801	1.2	0101/2	0502	1502	1.0				
3303	51011	15021	1.2	0101/2	0602 = 3	1101	1.0				
68012	51011	15021	1.2								

Haplotypes with frequencies higher than 0.6% are shown.

Table 2

HLA-A-B-C-DRB1 four-locus haplotype frequencies (HF%) in Baloch of Iran

A	B	C	DRBI	HF (%)
3303	5801	0302	0301	2.9
11011	4006	15021	1502	2.3
02011	3527	04011	0301	1.7
03011	35011	04011	0101	1.7
3002	5301	04011	0301	1.7
7401	8101	0804	0302	1.7
02011	0801	0702	0301	1.1
02011	3527	04011	0101	1.1
02011	4201	1701	1501	1.1
02011	5301	04011	0301	1.1
11011	0801	0702	0302	1.1
11011	35011	04011	0101	1.1
11011	4006	15021	1602	1.1
2601	4006	0702	0301	1.1
3201	51011	15021	0101	1.1
3303	51011	15021	0301	1.1

Haplotypes with frequencies higher than 0.6% are shown.

Table 3

Four-locus haplotype analysis showed that A*3303-B*5801-Cw*0302-DRB1*0301 haplotype with a population frequency of 2.9% was predominant in this population (Table 3).

Significant deviation from Hardy–Wienberg expectation was observed in HLA-DQA1 (excess of homozygosity = 0.1), HLA-DQB1 (excess of homozygosity = 0.07), and HLA-DRB1 (excess of homozygosity = 0.24) (Table 4).

Neighbor-joining tree was constructed according to DA distances for HLA-A, -B, -DQB1, and -DRB1 allele frequencies (Fig. 1). The relationship between Baloch of Iran and the six ethnic groups of Pakistan (19) has been shown in this figure. As shown, Baloch of Iran is very close to Baloch and Brahui of Pakistan, and these three ethnic groups are well separated from other ethnic groups of Pakistan. This result was confirmed by correspondence analysis (Fig. 2).

Discussion

Tribal groups known as Baloch are people who probably share a common origin and history as well as language, traditions, and religion. They mostly populated Iran, Pakistan, and Afghanistan; however, small Baloch communities are also living in Turkmenistan, India, East Africa, and Oman (16). The origin of Baloch people is still obscure, but some historians believe that they belong to Aryans who inhabited the northern regions of Elborz and the east of Caspian Sea. Some scholars state that they are Semites and originated from Halab in Syria. It is also believed that they belong to the old stock of Sumerians of Mesopotamia, while others regard the Baloch as the remnants of indigenous population of the area (1, 17, 18).

Our study was designed to investigate the genetic relatedness between Baloch of Iran and Baloch of Pakistan, using allele and haplotype frequencies in six HLA loci.

The results of HLA class I and II allele frequencies showed that most frequent alleles in Baloch of Iran were one of the three most common alleles in Baloch of Pakistan but in some loci, these frequencies were more similar to those of other ethnic groups in Pakistan. There are six major ethnic groups in Pakistan. Baloch, Brahui, and Sindhi are distributed in the south and Brusho, Kalash, and Pathan are living in the north of Pakistan (19).

The most frequent HLA-A alleles in Baloch of Iran are *02011 and *11011. A*02 and *11 are also the most frequent alleles in Sindhi, Brahui, and Baloch of Pakistan, but in two latter subpopulations, A*11 is more frequent than A*02.

The HLA-A*02 repertoire in Baloch population of Iran displays a high prevalence of A*0201 which is typically associated with western Caucasoid, while the two alleles which are typically Oriental, A*0206 and A*0207, occurred with low frequencies (1.1 and 0%, respectively)

Deviation from Hardy–Wienberg (HW) equilibrium in six HLA loci in Baloch population of Iran

Locus	Number of alleles	Observed heterozygosity	Expected heterozygosity	Deviation from HW
HLA-A	44	0.85057	0.92167	0.08774
HLA-B	45	0.88506	0.95156	0.05976
HLA-C	25	0.80460	0.87363	0.05213
DQA1	7	0.60000	0.70010	0.00000
DQB1	11	0.76000	0.82688	0.00036
DRB1	22	0.63000	0.87136	0.00000

Table 4

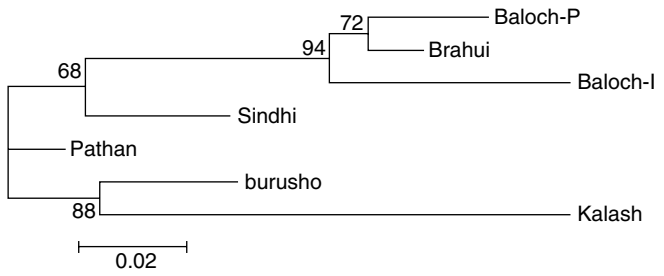

Fig. 1. Neighbor-joining tree showing relationship between Baloch of Iran (Baloch-I) and six ethnic groups of Pakistan (19) (Baloch-P; Baloch of Pakistan) based on HLA-A, -B, -DQB1, -DRB1 allele frequencies. Bootstrap values from 1000 replicates are shown.

in this population. A*0205, predominant African allele, and A*0211, unique North Indian allele, were also observed with low frequencies of 0.6 and 1.1%, respectively (20).

The frequencies of six serological splits of HLA-A19, i.e., A29, A30, A31, A32, A33, and A74 alleles occurred at similar frequencies in Baloch of Iran as in Caucasians (21).

HLA-B*4006 is the most popular allele in Baloch of Iran. B*40 is also the most frequent among Baloch of Pakistan whereas it is the second common allele in Brahui and Sindhi. HLA-C*04011, *15021 are the most common alleles in Baloch of Iran. C*04 and *15 are also the most frequent in Brahui and Baloch of Pakistan while C*15 is the most common in Sindhi.

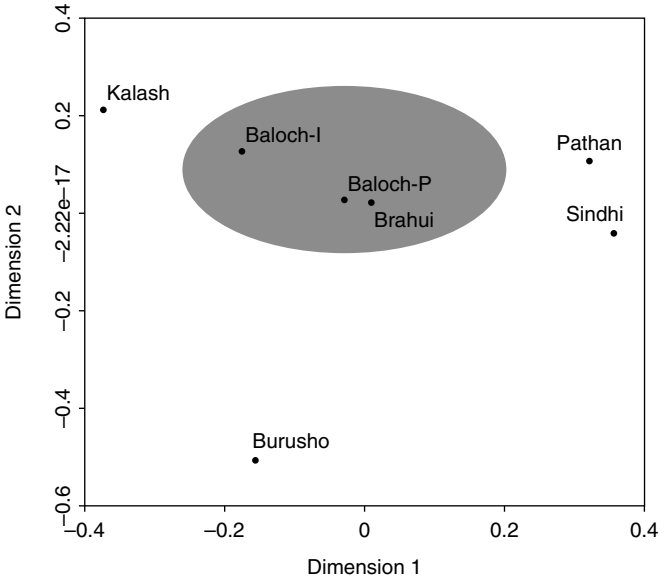

Fig. 2. Corresponding analysis showing the relationship between Baloch of Iran (BALOCH-I) and six ethnic groups of Pakistan (19) (Baloch-P; Baloch of Pakistan) according to HLA-A, -B, -DQB1, -DRB1 allele frequencies in three dimensions (bi-dimensional representation).

HLA-DRB1*0301 and *0101 are the most common alleles in Baloch of Iran. DRB1*03 and *01 are also the most frequent in Brahui and Baloch of Pakistan (19). The results of three-locus haplotype analysis showed that the three most frequent HLA class I haplotypes in Baloch of Iran (Table 2) are also the most common in Brahui while A*02-B*35-C*04 is not observed in Baloch of Pakistan and the frequency of A*33-B*58-C*03 haplotype is also more than A*11-B*40-C*15 in this group.

A*11-B*40-C*15, which has been reported in Australian and Pacific populations, is the most common HLA class I haplotype in the south of Pakistan (19). This haplotype (A*11011-B*4006-C*15021) is also the most common haplotype in Baloch people of Iran.

Interestingly, the most frequent DR3-associated haplotype in this population is A*2601-B*0801-DRB1*0301 which is an 8.2 ancestral haplotype (A26-B8-DR3) rather than 8.1AH (HLA-A1, B8, DR3) that occurs predominantly among Caucasoid. These haplotypes are associated with many immunopathological diseases and differ by several repeat units at microsatellites and the subtypes of HLA alleles in other related loci such as HLA-C and HLA-DQB1. HLA*B8-DR3 haplotype in India is also associated with HLA-A26 instead of HLA-A1 (22). The frequencies of such a haplotype in four- and five-locus analyses are very limited. It shows that the presence of special HLA and non-HLA alleles might be necessary for predisposition to some diseases.

Same as Mongolian population, A*3303-B*5801-Cw*0302-DRB1*0301 haplotype is predominant in Baloch of Iran (Table 3), which may be considered as the remnant of Mongolian invasion to this country. A*3303-B*5801-Cw*0302-DRB1*1302, which is a common haplotype in East Asian populations such as South Korean, Chinese-Korean, Buryat, and Japanese populations, was not observed in Baloch of Iran (23).

In contrast to HLA class I loci, deviation from Hardy–Weinberg equilibrium was observed in HLA class II loci in Baloch population of Iran. Because Baloch people constitute only about 2% of the

Fig. 3. Map of Iran and Pakistan showing the geographic locations of Balochistan provinces.

population of Iran with their special culture and language, inbreeding can be considered as one of the possible cause of deviation from Hardy–Weinberg equilibrium. Local infections with specific major histocompatibility complex class II-restricted antigens and some sort of environmental selection pressures might be other causes of this deviation just in class II but not in class I loci.

These results show that Baloch people of Iran are very close to Baloch and Brahui of Pakistan. As shown in Fig. 1, these three groups are located in the same cluster of the phylogenetic tree. These results were also confirmed by correspondence analysis (Fig. 2). As previously reported (19), Brahui and Baloch of Pakistan are very close to each other and both of them are living in Balochistan province in south-west of Pakistan which has an extended common frontier with Balochistan province of Iran (Fig. 3).

Although these results show a close relatedness between Baloch of Iran and Pakistan, there are also some differences, which may have been caused by admixture of each one of these groups with other subpopulations. Determination of the influence of other specific ethnic groups in Iran, which now is in progress, will help to shed further light on these populations.

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