Original Article

Comparison of the Frequency of Y-short Tandem Repeats Markers between Sadat and Non-Sadat Populations in Isfahan Province of Iran

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Abstract

Background: Y chromosome is one of the two sex chromosomes and is male specific. Due to limited genetic exchange, the main part of that is passed virtually unchanged from one generation to next generation. The short tandem repeats (STRs) are almost constant on chromosomes that make them as an appropriate factor for use in population genetic studies. In this study, we used the STRs of Y chromosome markers in Sadat families and comparison with other families was investigated. **Materials and Methods:** In this study, sampling was done from fifty unrelated males of Sadat families and fifty unrelated males of non-Sadat families. After the extraction of DNA from blood samples and primer design, polymerase chain reaction (PCR) was performed for each primer pairs separately. The PCR products were run on agarose gel that followed by running on polyacrylamide gel for better resolution. In addition, some sequenced samples were used as identified markers to determine the length of other alleles in polyacrylamide gel. **Results:** The survey of six STR in two case and control groups was carried out, and analysis revealed that the frequency of some alleles is different in case group compared to control group. Allele frequency of the markers DYS392, DYS393, DYS19, DYS390, DYS388, and DYS437 on the Y chromosome in Sadat families was quite different in comparison with other families. **Conclusions:** The reason for these differences in allele frequencies of the Sadat family in comparison with other families is having a common ancestor.

Keywords: *Sadat, short tandem repeat, Y chromosome*

Introduction

The Y chromosome is one of two sex chromosomes in humans that contains approximately 2% of the total DNA.[1,2] Unlike other chromosomes, the Y chromosome is unique because it has a haploid state and transmitted relatively unchanged from one generation to another.[3] However, during meiosis, recombination between X and Y chromosomes is limited to small regions at the end of the short (PARI) and long arms (PARII) of Y chromosome. These areas called the pseudoautosomal regions. In fact, the most length of Y chromosome (95%) is formed by Nonrecombining Y portion that reserved unchanged from one generation to another, unless mutation occurs.[1] The lack of recombination in these regions has led researchers to understand the evolutionary events such as migrations and existence of a common ancestor linage in a population.[4]

Short tandem repeats (STRs) are repeated DNA sequences of 2–7 base pairs in length,[5] which are abundant in the human genome. According to the length of repeats, their alleles vary from person to person (except for monozygotic twins); therefore, STRs have been widely used as molecular markers for genome analysis.[6] High variability, short length, easily reproducible and low mutation rate, caused it to be considered as reliable and suitable molecular tools in forensic applications, population genetics, and family relationships identification, especially for paternity testing.[7]

Identifying the ancestry of populations is an important issue in understanding the genetic structure of populations. That can help in many aspects, especially in planning future genetic studies.[8]

In Muslim communities, a subgroup of the population is called Sayed, which means, they are descendants of Prophet Mohammad, (peace be upon him and his pure descendants), the great messenger

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of Islam religion. In fact, Sayed people (Sadat) are the descendants of Hashem. Using genetic markers and their frequencies can be used to identify derivation populations from each other. This research has tried to find some genetic characteristics of Hashem family in Muslim communities. Because of the availability of samples in Iran and the lack of access to samples abroad, we chose samples only from Iran. Therefore, this research was conducted on six Y-STR just in one son of fifty unrelated Sadat families in Isfahan city, the center of Isfahan province, Islamic Republic of Iran.

Materials and Methods

Target population

A total number of fifty males were included from unrelated Sadat families whose ancestors have been living at least three generation, in Isfahan, a central city of Islamic Republic of Iran. Chosen individuals were checked through their pedigrees. The control group was formed by fifty unrelated non-Sadat males from Isfahan city. All participants signed consent form. There was no age limitation for the participating population in the study.

DNA extraction

Five milliliters of peripheral blood were taken from each of the case and control individuals. The blood samples were collected in tubes containing EDTA.[9] DNA was extracted with GenetBio kit (Genetbio Inc, Daejeon, South Korea) according to manufacturer's instruction. The quantity and quality of DNA were determined by spectrophotometer. Samples were kept at −20°C after extracting DNA.

Amplification by polymerase chain reaction

In this study, six STRs including DYS19, DYS388, DYS437, DYS390, DYS392, and DYS393 were tested. We considered some characteristics such as high frequency, short length, easily reproducible, and low mutation rate for selecting of STRs. Relevant primers were designed for these loci and then polymerase chain reaction (PCR) was performed for each of these short repeated sequences separately in 35 cycles. Primer sequences and appropriate annealing temperature of the PCR conditions are shown in Table 1.

PCR reactions were carried out in a total volume of 25 μl containing 2.5 μl buffer (×10), 0.75 μl $Mgcl_2$, 0.3 μl Taq-DNA polymerase, 0.5 μl dNTP, 2 μl extracted DNA, 16 μl ddH_2O, and 2.5 μl of STR-specific primers. The annealing temperature for each PCR reaction was determined with the gradient program of the thermal cycler.

Gel electrophoresis condition

All PCR products were visualized by electrophoresis on 1% of agarose gel. To obtain a higher resolution, the PCR products were run on 10% of polyacrylamide gel stained with silver nitrate.

Confirming the accuracy of genotypes by sequencing

Finally, to confirm the PCR products and accuracy of the method, some samples of PCR products including a sample of case group and a sample of control group for each marker were sequenced.

Statistical analysis

All obtained data from case and control samples were analyzed by SPSS software (version 16, IBM SPSS Statistics, New York, USA). The frequency of six Y-STR markers was calculated in each group by descriptive statistics methods. Allele frequencies were compared between case and control groups by parametric (independent-samples *t*-test) and nonparametric (Mann–Whitney) methods. In addition, Y-STR data of our study were compared with other populations.

Results

In this study, the genotype of six Y-STRs loci including DYS392, DYS393, DYS19, DYS390, DYS388, and DYS437 was evaluated by polyacrylamide electrophoresis in case and control groups [Table 2 and Figure 1]. The

Table 1: Characteristics of the six Y-short tandem repeats and the relevant primers used in this study

STR	Primer sequence	Allele repeats (bp)	Annealing temperature (°C)
DYS19	CTACTGAGTTTCTGTTATAGTATG GCATGTAGTGAGGACA	10-19	58
DYS437	GACTATGGGCGTGAGTGCAT GAGACCCTGTCATTCACAGATGA	12-17	61
DYS388	GTGAGTTAGCCGTTTAGCGA CAGATCGCAACCACTGCG	8-18	57
DYS390	TATATTTTACACATTTTTGGGCC TGACAGTAAAATGAACACATTGC	17-28	54
DYS392	AAAAGCCAAGGAAAACAAA AAACCTACCAATCCCATTCCTT	6-18	57
DYS393	GTGGTCTTCTACTTGTGTCAATAC AACTCAAGTCCAAAAAATGAGG	8-17	57

STR: Short tandem repeat

frequency of each allele was also determined separately for each of the six Y-STRs in case and control groups as shown in Tables 3 and 4, respectively.

Allele distribution pattern of the 6 Y-STR markers is illustrated for both case and control groups in Figures 2 and 3. In addition, the results of sequencing of the STRs have shown in Figure 4.

DYS390

There were 20–23 repeat sequences in Sadat families for DYS390. The most frequent allele belonged to an allele with 22 repeats and a frequency of 50% while there were 21–25 repeat sequences in control group. The most frequent allele in the latter belonged to an allele with 23 repeats and a frequency of 40%.

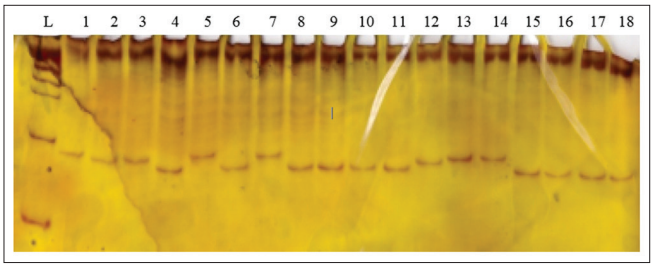

Figure 1: Polyacrylamide gel electrophoresis of DYS388 marker which was genotyped for 18 individuals. The left to right panels are Lader (100 bp) and numbers 1–18 shows the sample 19–36 in case group, respectively

DYS392

DYS392 was the least polymorphic Y-STR with 2 repeats in case group while it was more polymorphic with 6 alleles in control group. The most frequent allele had 11 repeats in both groups, but the frequency of this allele was 90% and 40% in case and control groups, respectively.

DYS393

DYS393 was the most polymorphic Y-STR with 6 alleles in case group. Alleles with 4–9 repeats observed at locus DYS393 in Sadat population while alleles with 10–17 (except 16) repeats observed in control group. The most frequent allele of DYS393 belonged to an allele with 6 repeats and a frequency of 46% in Sadat population while the most frequent allele in non-Sadat population had 12 repeats and a frequency of 40%.

DYS19

Although allele with 14 repeats in DYS19 was the most frequent in the two groups, the percentage frequency of this allele was 36 and 48 in case and control groups, respectively.

DYS388

The analysis showed that the DYS388 allele with 13 repeat alleles was the most common in Sadat population while the

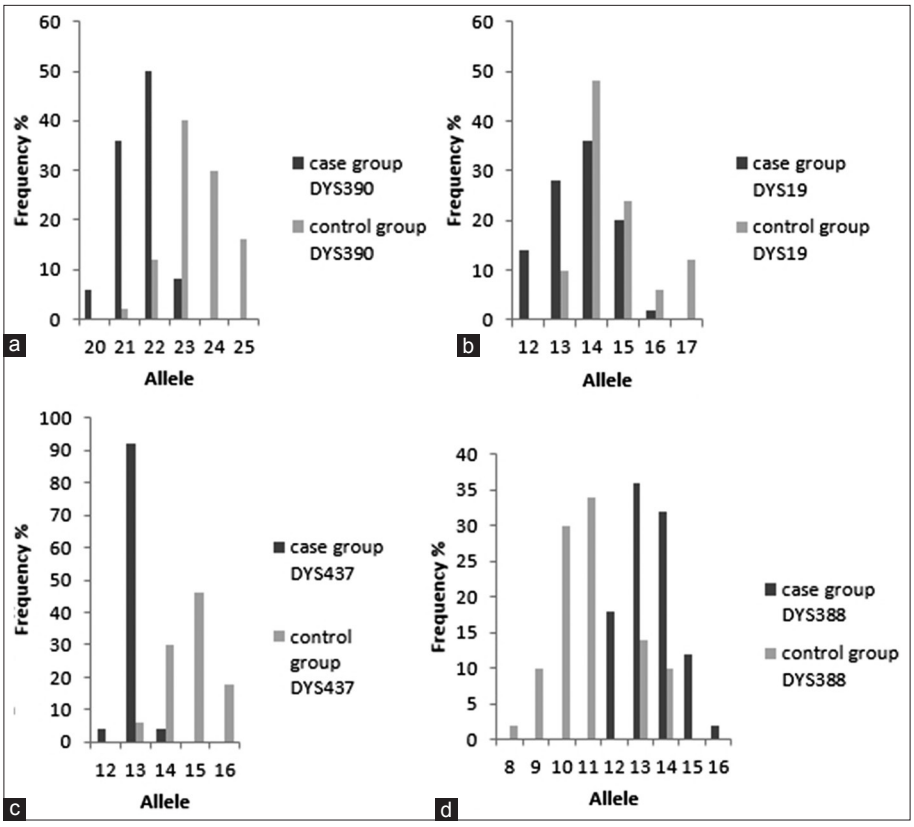

Figure 2: Clustered bar graph of allele frequencies in Sadat and non-Sadat populations. (a) Pattern of allele distribution of DYS390, (b) allele frequencies related to DYS19, (c) DYS437 allele frequencies, and (d) shows DYS388 allele frequencies

Table 2: Allele distribution of the six Y-short tandem repeats among Sadat and non-Sadat populations

STR number	DYS392		DYS393		DYS19		DYS390		DYS388		DYS437	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
1	11	14	6	12	15	14	21	23	14	10	13	15
2	11	14	5	11	14	14	22	24	14	11	13	15
3	11	14	6	12	14	16	22	23	14	10	13	16
4	11	11	5	13	14	16	23	25	14	9	13	14
5	11	11	7	12	15	14	21	23	15	11	13	15
6	11	14	6	11	16	14	22	23	16	9	13	14
7	11	11	7	13	15	14	22	25	15	11	13	14
8	11	13	6	11	13	14	21	24	13	11	13	15
9	11	13	6	12	14	14	21	24	14	11	13	15
10	11	14	6	12	13	14	21	24	13	11	13	14
11	11	11	6	12	13	14	21	22	13	10	13	15
12	11	13	6	13	13	15	22	23	13	14	13	15
13	11	13	6	12	13	15	22	24	13	13	13	15
14	11	11	6	12	13	17	22	25	13	14	13	15
15	11	14	6	17	14	14	22	23	14	11	13	15
16	11	10	6	12	15	17	20	23	14	11	13	14
17	11	11	6	12	15	14	20	23	12	11	13	16
18	11	10	5	14	15	14	21	23	12	9	13	15
19	11	13	5	12	14	14	20	25	14	9	13	14
20	11	11	6	13	15	17	23	25	15	8	13	13
21	11	14	5	13	15	13	21	24	15	10	13	15
22	11	11	7	12	14	15	23	23	14	11	13	16
23	11	11	6	14	14	15	22	20	14	10	13	15
24	12	11	6	10	14	14	22	23	12	11	13	15
25	11	11	5	12	14	14	22	23	12	14	13	14
26	12	12	4	15	14	15	22	23	12	13	13	14
27	12	14	5	10	15	17	22	24	15	11	13	15
28	11	11	6	13	15	14	22	24	15	11	13	14
29	11	13	7	13	14	15	22	24	14	11	13	15
30	11	11	4	13	13	14	21	23	13	10	13	16
31	11	11	4	12	13	13	22	25	13	10	14	15
32	11	11	5	13	14	16	22	24	14	11	12	14
33	11	12	7	13	13	14	22	24	13	13	13	16
34	11	12	7	12	12	14	21	23	14	13	12	14
35	11	11	5	13	12	17	23	25	14	14	13	15
36	12	14	4	12	12	15	21	22	14	13	13	15
37	11	13	4	13	13	14	22	23	13	13	13	16
38	12	11	7	12	12	15	22	24	12	11	13	15
39	11	12	6	14	12	17	22	25	12	10	14	14
40	11	11	6	12	14	14	22	23	13	9	13	15
41	11	12	9	14	14	14	22	24	13	13	13	15
42	11	13	8	12	12	15	22	24	12	14	13	16
43	11	15	6	13	13	13	21	22	13	11	13	14
44	11	15	6	14	14	13	21	22	14	10	13	16
45	11	13	6	10	12	15	21	23	12	10	13	16
46	11	11	7	12	13	14	21	23	13	10	13	15
47	11	14	5	13	14	15	21	22	13	10	13	14
48	11	11	5	13	14	13	21	24	13	10	13	14
49	11	10	6	13	13	14	22	23	13	10	13	13
50	11	14	7	13	13	15	21	22	13	10	13	13

STR: Short tandem repeat

Table 3: Allele frequencies of the six Y-short tandem repeats in Sadat population

Allele	DYS392 (%)	DYS393 (%)	DYS19 (%)	DYS390 (%)	DYS388 (%)	DYS437 (%)
4		10				
5		22				
6		46				
7		18				
8		2				
9		2				
10						
11	90					
12	10		14		18	4
13			28		36	92
14			36		32	4
15			20		12	
16			2		2	
20				6		
21				36		
22				50		
23				8		

Table 4: Allele frequencies of the six Y-short tandem repeat in control group

Allele	DYS392 (%)	DYS393 (%)	DYS19 (%)	DYS390 (%)	DYS388 (%)	DYS437 (%)
8					2	
9					10	
10	6	6			30	
11	40	6			34	
12	10	40				
13	18	34	10		14	6
14	22	10	48		10	30
15	4	2	24			46
16			6			18
17		2	12			
20						
21				2		
22				12		
23				40		
24				30		
25				16		

most frequent allele belonged to allele with 11 repeats in control group.

DYS437

There were alleles with 12–14 repeats in DYS437. The most frequent allele in case group was an allele with 13 repeats and a frequency of 92%. On the other hand, there was allele with 13–16 repeats in control group. In the later, the most frequent one was an allele with 15 repeats and a frequency of 46%. The means and standard deviations of all of the alleles are summarized in Table 5. Comparing the allele frequency of the 6 Y-STRs revealed

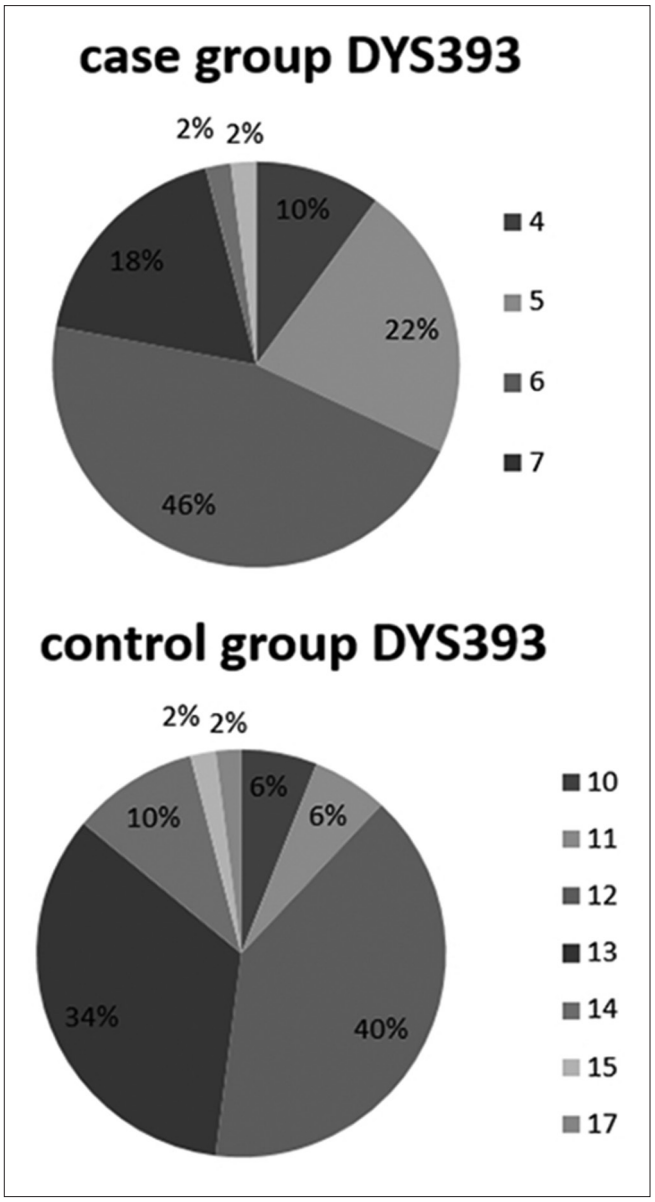

Figure 3: Pie chart of allele frequencies of DYS393 marker in case and control group

significant differences between the case and control groups ($P < 0.001$) [Tables 6 and 7].

Discussion

Several studies have been done on human evolution and ancestral divergences based on which the ancestral situation can be recognized. Y-STRs seem to be a useful tool for anthropological and forensic studies which can be used as a valuable marker in lineage studies.[10]

Based on the previous studies, several differences were identified in the frequency of STRs alleles which may provide valuable information about a common ancestor using STR loci in particular ethnic population. A population study revealed that Y-chromosomal haplotypes have a less variation in a population while variation may be

seen among different ethnic groups.[11] In our study, six different Y-STRs were assessed from the blood samples of Sadats and non-Sadats populations. The results of this study showed that the frequency of STR alleles on the Y chromosome was significantly different between case and control groups. It seems that Y-STR variations can support the hypotheses of a common ancestor in case group (Sadat).

Table 5: Means and standard deviations related to the alleles in case and control groups

Group statistics				
Sample=1, control=2	*n*	Mean	SD	SEM
DYS392				
1	50	11.10	0.303	0.043
2	50	12.22	1.433	0.203
DYS393-S				
1	50	5.86	1.030	0.146
2	50	12.52	1.216	0.172
DYS19-S				
1	50	13.68	1.019	0.144
2	50	14.62	1.141	0.161
DYS390-S				
1	50	21.60	0.728	0.103
2	50	23.44	1.033	0.146
DYS388				
1	50	13.44	0.993	0.140
2	50	11.02	1.545	0.219
DYS437				
1	50	13.00	0.286	0.040
2	50	14.76	0.822	0.116

SD: Standard deviation, SEM: Standard error of mean

In 2009, Farazmand *et al*. studied the frequency of six Y-STR polymorphism in a random sample of males in Iranian Sadat subpopulation. According to this study, the most frequent Y-STR alleles were with 14, 23, 11, and 12 repeats in DYS19, DYS390, DYS392, and DYS393, respectively.[12] In this study, the most frequent alleles were allele with 11, 14, and 22 in DYS392, DYS19, and DYS390 STRs, respectively. Thus, two Y-STRs (DYS392 and DYS19) were found similar in both studies. Comparison of the most frequent alleles between the two studies may show a common linage in Sadat population [Table 8].

Ploski *et al*. investigated nine Y-chromosomal microsatellites on Poland population and they found that there was no difference between Y-STR haplotypes in this population. In contrast, comparison of the Poland with non-Poland European populations showed a statistically significant difference. Thus, this finding was consistent with the assumption of homogeneity of present-day paternal lineages within Poland and their distinctiveness from other parts of Europe.[13]

Based on a study of 17 STRs in the Basque diaspora in the Western USA, it was identified that this population had conserved the Y chromosome lineage characteristic of the autochthonous European Basque population without significant differences.[14] Similarly, the study of some

Table 6: Comparison of the allele frequencies between Sadat and non-Sadat populations

Y-marker	Levene's test for equality of variances		*t*-test for equality of means						
	F	Significant	*t*	df	Significant (two-tailed)	Mean difference	SE difference	95% CI of the difference	
								Lower	Upper
DYS392									
As	144.753	0.000	−5.408	98	0.000	−1.120	0.207	−1.531	−0.709
N-As			−5.408	53.376	0.000	−1.120	0.207	−1.535	−0.705
DYS393-S									
As	0.994	0.321	−29.544	98	0.000	−6.660	0.225	−7.107	−6.213
N-As			−29.544	95.423	0.000	−6.660	0.225	−7.107	−6.213
DYS19-S									
As	0.313	0.577	−4.345	98	0.000	−0.940	0.216	−1.369	−0.511
N-As			−4.345	96.775	0.000	−0.940	0.216	−1.369	−0.511
DYS390-S									
As	4.559	0.035	−10.291	98	0.000	−1.840	0.179	−2.195	−1.485
N-As			−10.291	88.056	0.000	−1.840	0.179	−2.195	−1.485
DYS388									
As	3.788	0.054	9.317	98	0.000	2.420	0.260	1.905	2.935
N-As			9.317	83.580	0.000	2.420	0.260	1.903	2.937
DYS437									
As	58.091	0.000	−14.299	98	0.000	−1.760	0.123	−2.004	−1.516
N-As			−14.299	60.666	0.000	−1.760	0.123	−2.006	−1.514

Independent samples test was used (*P<0.05, **P<0.01, and ***P<0.001). As: Equal variances assumed, N-As: Equal variances not assumed. SE: Standard error, CI: Confidence interval

Table 7: Comparison of the allele frequencies between Sadat and non-Sadat populations

Statistical test	DYS392	DYS393	DYS19	DYS390	DYS388	DYS437
Mann–Whitney U-test	712.500	0.000	733.500	187.500	301.000	90.000
Wilcoxon *W*	1.988E3	1275.000	2.008E3	1462.500	1.576E3	1.365E3
Z	−4.360	−8.738	−3.737	−7.528	−6.657	−8.600
Asymptotic significant (two-tailed)	0.000	0.000	0.000	0.000	0.000	0.000

Nonparametric test (Mann–Whitney U-test) was used (*P<0.05, **P<0.01 and ***P<0.001)

Table 8: Evaluation of Y-short tandem repeat allele frequencies in Sadat families in Tehran

STR	Allele (repeat)	Frequency
DYS19	13	0.1
	14	0.48
	15	0.26
	16	0.16
DYS390	21	0.08
	22	0.52
	23	0/06
	24	0.2
	25	0.12
DYS392	10	0.1
	11	0.8
	12	0.06
	13	0.02
	14	0.02
DYS393	12	0.43
	13	0.29
	14	0/25
	16	0.01
DYS385a	10	0.04
	11	0.08
	12	0.28
	13	0.46
	14	0.06
	15	0.04
	16	0.04
DYS385b	12	0.12
	13	0.14
	14	0/04
	15	0.28
	16	0.12
	17	0.14
	18	0.08
	19	0.06
	20	0.02

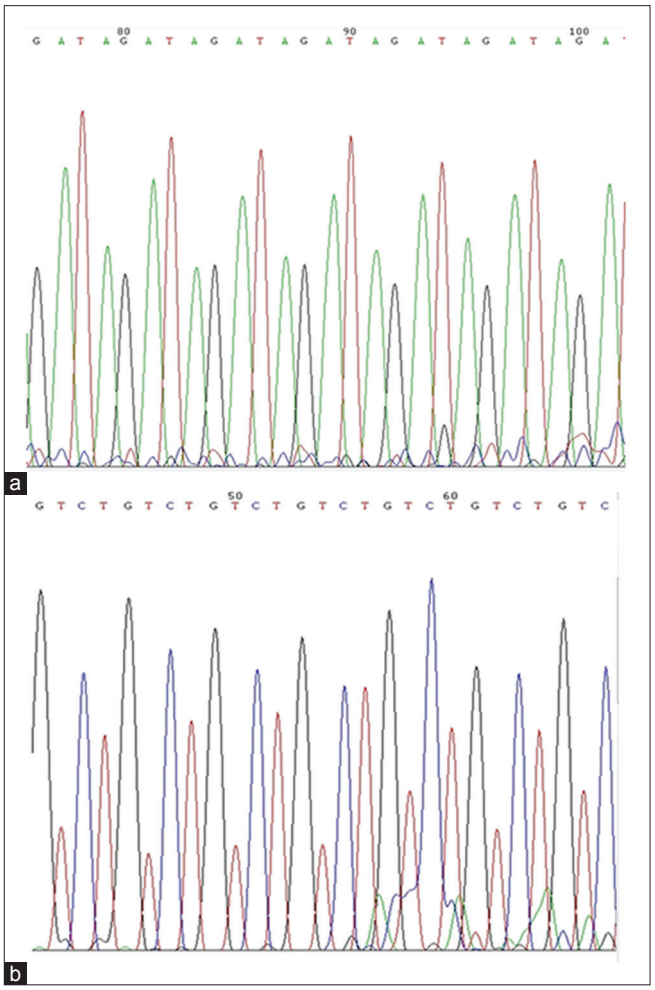

Figure 4: Sequencing result of the two Y chromosome markers. (a) DYS19 and (b) DYS390

autosomal STR variation supports the hypotheses of a common ancestor between North Africa and Southern Europe.[15]

A study performed at 17 Y-STR loci in 249 samples in Cukurova region of Turkey. Comparison of haplotype data with other Turkish populations showed no significant difference between them except for Ankara population. The data also compared with other population and it was revealed that the genetic structure of Y-STR loci was very similar to Lenkoran-Azerbaijani and Iranian Ahvaz populations. This similarity may be due to historical and demographic migrations. The results also showed that Y-STR polymorphisms are a powerful discrimination tool for routine forensic applications and can be used in anthropological investigations.[16]

There are various theories about the origin of the Tai people. An investigation on the frequency of autosomal 10 STRs on this population compared these markers with various hypotheses. It has shown that Southern origin hypotheses have higher probability than the other hypotheses that means Tai people most likely originated from Southern china.[17]

The analysis of six Y-STR polymorphisms in the study population provides a powerful discriminative tool which may serves in anthropological and forensic investigations. This study described the pattern of the frequency distribution of six polymorphic STR of the Y chromosome in the Sadat population in Isfahan. Alleles related to DYS393 were significantly different in the two groups, and thus, this marker can be considered as an important indicator in Sadat population.

Conclusions

According to the statistical analysis, Y-markers can be used as a powerful tool for forensic identification and paternity testing in the populations. In addition, these markers seem to be well used in population differentiation studies. Our data on Y-STR markers support a common ancestor in Sadat population and provided detailed information of the distribution frequency of these markers.

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Conflicts of interest

There are no conflicts of interest.

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