BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2014. Vol. **11**(3), 1785-1789

Allele Frequencies for Six STR Loci with Criminals in Fars Province, Iran

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doi: http://dx.doi.org/10.13005/bbra/1585

(Received: 25 October 2014; accepted: 11 December 2014)

Microsatellites or Short Tandem Repeats (STRs) are loci with alleles composed of tandemly repeated short DNA sequences of 2-7 base pairs in length. In the present study, the polymorphism STR loci CODIS were analyzed in 40 people with a history of crime using multiplex PCR. The 6 STRs loci included TH01, TPOX, D16S539, D18S51, CSF1PO and FGA. The result showed that the highest PD and heterozygosity belongs to allele FGA. The most polymorphism was observed in the D18S51 and FGA loci. Also the highest allele frequency was detected at locus FGA.

Key words: Microsatellites, Short tandem repeats, FGA allele, THO1 allele

Microsatellites or Short Tandem Repeats (STRs) are loci with alleles composed of tandemly repeated short DNA sequences of 2-7 base pairs in length (Weber *et al.* 1989). These sequences are widespread throughout the human genome and show sufficient variability among individuals of population (Santos *et al.* 1996). Autosomal STRs now have become indispensable tools for personal identification and paternity testing in forensics and criminal investigations (Kumar *et al.* 2006). Multiplex PCR amplification and typing system with multi- colored fluorescent labeled primers have made it convenient to genotype STRs using commercially available kits. The FBI in USA adopted the Combined DNA Index System (CODIS) core 13 STRs (D3S1358, Vwa, FGA, D16S539, TH01, TPOX, CSF1PO, D8S1179, D21S11, D18S51, D13S317, and D7S820) to construct a huge database for criminal investigations (Yamamoto *et al.* 2003). These developments together with the recommendations of the National Research Council (NRC) with respect to statistical interpretation of DNA evidence have been instrumental in the worldwide cceptance of DNA evidence in the criminal justice system. However, the database on these 13 loci is largely restricted to broadly defined population groups, such as US Whites, US Blacks, Hispanics (Sun *et al.* 2006). In this study, 6 STRs loci TH01, TPOX, D16S539, D18S51, CSF1PO and FGA are analyzed in a population of 40 Iranian criminals and the genetic relation was assessed within this population.

MATERIALS AND METHODS

Sampling and DNA extraction

Blood samples were collected from a total of 40 murderous criminals. Total genomic DNA was

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extracted from the samples by the standard salting out method. DNA concentration and purity was determined by using UV spectrophotometry. The integrity of the DNA isolates was ascertained on 1% agarose gel (Fermentas, Canada).

Specificity of the primers

To ensure the specificity of the primers, their sequences were checked by the GenBank BLAST database and they were all found to be specific for their respective loci.

PCR amplification

PCR amplification of the CODIS STR loci was carried out using a Genius thermal cycler (Techne, Stafford, UK). PCR reaction mixture (25µL) was composed of 50 ng genomic DNA, 1×PCR buffer, 1.5 mM MgCl2, (except for FGA where 2 mM MgCl2 was used), 0.6 µM each primer, 200 µM each dNTPs (Fermentas, Canada) and 1 unit of Native Taq DNA polymerase (Fermentas, Canada). After an initial denaturation at 95 °C for 3 min,the samples were subjected to 35 cycles of 30 s at 94°C, 45 s at 64°C (For FGAit should be 68 or 72), and a final extension at 72°C for 10 min. The amplified fragments were separated on 14% non-denaturing polyacrylamide gel (Azari *et al.* 2007). The electrophoresis was carried out overnight at maximum running voltage of 110 V. The alleles and molecular size marker were visualized by silver staining.

RESULTS

The results are shown in Fig1and table 2.

Table 1. Characteristics of the 6 CODIS STR loci in this study

STR locus	Chromosomal location	Product size (bp)	PCR primer sequences	Ref.
CSF1PO	5q	291-331	(F) 50-AAC CTG AGT CTG CCA AGG ACT AGC-30 (R) 50-TTC CAC ACA CCA CTG GCC ATC TTC-30	-9
FGA	4q	158-314	(F) 50-GCC CCA TAG GTT TTG AAC TCA-30 (R) 50-TGA TTT GTC TGT AAT TGC CAG C-30	-20
THO1	11p	171-215e	(F) 50-ATT CAA AGG GTA TCT GGG CTC TGG-30 (R) 50-GTG GGC TGA AAA GCT CCC GAT TAT-30	-9
TPOX	2p	220-256	(F) 50-ACT GGC ACA GAA CAG GCA CTT AGG-30 (R) 50-GGA GGA ACT GGG AAC CAC ACA GGT TA-3	-9
D18S51	18q	262e342	(F) 50-CAA ACC CGA CTA CCA GCA AC -30 (R) 50-GAG CCA TGT TCA TGC CAC TG-30	-9
D16S539	16q	264e304	(F) 50-GGG GGT CTA AGA GCT TGT AAA AAG-30 (R) 50-GTT TGT GTG TGC ATC TGT AAG CAT-30	-6

DISCUSSION

In this study, the polymorphism STR loci CODIS were analyzed in criminal record. The loci FGA, D16S539, D18S51, CSF1PO, TPOX and THO1 were shown to have the highest PD, respectively. The locus FGA has been also shown to have the highest PD in other population (Liu *et al.* 2006, Mastana *et al.* 2007, Lim *et al.* 2005, Muro *et al.* 2008, Perez *et al.* 2003, Ashma *et al.* 2002, Ang *et al.* 2005).

In a study by Egyad *et al.* (2006) on eastern Hungary Romanies, alleles 16 and 22.2 were found in a woman in genotype D18S51. The genotype FGA 24.1 with an additional base T in region 5 was found to be in very low frequency. PD was low in CSF1PO sequence like other studied populations. Although, in our study population, allele 16 was not found in the locus D18S51, allele 24.1 of locus FGA was seen.

Heterozygosity is proportional to the amount of genetic variation at the locus. Hence it is the factor that is commonly used to measure genetic variation and loss (Slate *et al.* 2002). The average of genotypic heterozygosity 0.9011 was observed in our population, whereas an average domain of genotypic heterozygosity between 0.768 (Italy) to 0.817 (Spain) has been reported in the European population (Budowle *et al.* 2001).

The highest expected heterozygosity in

Table 2. Allele frequency and forensic efficiency parameters of 6 STR loci in the population (N= 40) in Fars, Iran

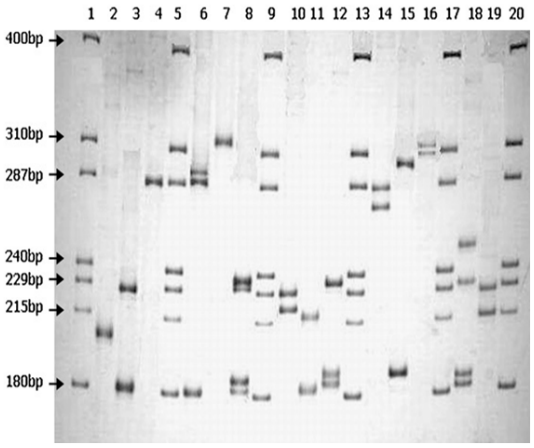
Allele \ Locus	CSF1PO	FGA	THO1	TPOX	D16S539	D18S51
6.3			0.0250			
8				0.0375		
8.1				0.0250		
9			0.0250	0.1000		
9.1			0.0750			
10			0.1125	0.0125		
10.3						
11			0.2000	0.0750		
11.1	0.0125					
12			0.2375	0.2000	0.0250	
12.1			0.0250			
12.3					0.0250	
13			0.1125		0.0250	
13.1				0.0250	0.0250	
14			0.1625	0.0750	0.0375	
14.3					0.0750	
15	0.0250		0.0250	0.1875	0.0875	
15.3						0.0250
16	0.1250			0.1625	0.1625	
16.3						0.0250
17	0.1875				0.1000	
17.3				0.1000		
18	0.1125				0.0750	
18.1						0.0250
19	0.0625	0.0375			0.0125	
19.1						0.0500
19.3		0.0125				
20	0.1750				0.1500	
20.1						0.0250
20.3		0.0250				
21	0.0875	0.0500				0.0625
21.1						0.1125
22	0.0750	0.0250				0.0750
22.1						0.0625
23	0.0750	0.0750			0.0250	
23.1						0.1125
24	0.0250	0.0625			0.1000	0.0375
24.1		0.0750				0.0750
25	0.0250	0.0875				0.1125
25.1		0.0250				
25.3		0.0250				
26	0.0125					0.0750
26.1		0.1000				
26.3		0.1625				
27		0.0500				0.0750
27.1		0.0250				0.0125
28						0.0375
29		0.0750				
29.1						0.0250
29.3		0.0250				0.0500
31		0.0500				
32		0.0125				
H (Exp)	0.8921	0.9339	0.8544	0.8753	0.9133	0.9380
P	P ≤ 0.001	0.002950	P ≤ 0.001	P ≤ 0.001	P ≤ 0.001	0.033564
PD	0.8374	0.9227	0.6718	0.8370	0.8610	0.8604

P: Hardy-Weinberg equilibrium, exact test based, the exact test represents statistically significant difference at the P ≤ 0.05 level; H (Exp): expected heterozygosity; PD: power of discrimination.

the province was belonged to D18S539, FGA, D16S539, CSF1PO, TPOX and THO1, respectively. In Greece, the FGA genotype was also reported with high heterozygosity (Slitsa *et al.* 2003).

The most polymorphism was observed in the D18S51 and FGA loci. Moreover, the highest allele frequency were detected at loci FGA, D16S539, D18S51, CSF1PO, TPOX and THO1 in alleles 26.3, 16 (21.1, 23.1 and 24.1), 17, 12 and 13, respectively. Similar results were observed in other population such as this population (Syn *et al.* 2005, Vecchio *et al.* 2004).

The present study is very similar to another study by Barni *et al* done on fifteen STR loci in southern Iraq in 2007 (Barni *et al.* 2007). In their study, the most PD was observed in locus FGA like ours. They showed the relative similarity between the population of the south of Iraq and the United Arabic Emirates, Oman and Iran. There is not much research on STR loci in Iran. Hadjazi *et al* is one of the most important research conducted in the 2013 (Hedjazi *et al.* 2013). They studied 15 STR loci in southwestern Iran, where the highest PD loci was observed in FGA locus. There was no similarity for allele frequency between their study and the present study. In other research, Lahmi and Valliun investigated on five STR loci for 127 people in Isfahan (Lahmi *et al.* 2009). It was very similar to another study conducted by Valliun and Moeini *et al*. They surveyed 5 STR loci among 220 people in Isfahan in 2006 (Vallian *et al.* 2006). Allelic variation was not observed in these two studies and PD of loci was assessed too low. The present study for allele frequency and PD indicator shows there is no similarity between these criminals and those people who are living in the southwest and center of Iran.



Marker.Lane1: mixture of Roche DNA molecular size markers VI and VIII; Lanes 1, 5, 9, 13, 17, 20. 2:[FGA (204 bp)], 3:[D16S539 (285 bp)], 6:[D18S51 (287 bp, 290 bp)], 7:[CSF1PO (312 bp)], 8:[TH01 (182 bp, 186 bp), TPOX (232 bp, 236 bp)], 11:[FGA (183 bp, 217 bp)], 14:[D16S539 (274 bp, 287 bp)], 15:[D18S51 (301 bp)], 16:[CSF1PO (307 bp, 311 bp)], 18:[TH01 (182 bp, 186 bp), TPOX (233 bp, 253 bp)].

Fig. 1. Electrophoresis of 6 CODIS STR loci

CONCLUSION

In most countries it has been embarked to create a gene bank from the profiles of satellite genes. The results of the present study suggest these sequences as useful genetic markers to identify individuals in a community and the offenders at the crime scene.

ACKNOWLEDGEMENTS

All authors are grateful to the Islamic Azad University, Jahrom branch for their executive support of this project.

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