Journal of Sciences, Islamic Republic of Iran 17(2): 113-117 (2006) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Genotyping of Five Polymorphic STR Loci in Iranian Province of Isfahan

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Abstract

Genotyping for five short tandem repeat (STR) loci HUMvWA, HUMFES, HUMTPO, HUMTH01 and D3S1359 was done in 220 unrelated individuals from the population of Isfahan province of IR Iran. The loci were genotyped using the polymerase chain reaction (PCR) followed by polyacrylamide gel electrophoresis (PAGE) and silver staining. The data demonstrated that the STR markers were all found informative in the population examined. The markers were also found to have a relatively high degree of heterozygosity. Forensic and paternity indices including power of discrimination (PD) and exclusion (PE) as well as polymorphism information content (PIC) and typical paternity index were determined for the examined STR alleles. Together, the examined STR loci in this study could be considered for paternity testing and individual identification in Iranian population. Moreover, the data could be used in construction of the first Iranian STR genetic database.

Keywords: Genotyping; Iranian population; Paternity testing; Short tandem repeat

Introduction

The human genome contains a large amount of polymorphic sequences, which are known as polymorphic markers. These markers were found useful in genetic linkage and association analysis, especially in determination of disease genes by linkage disequilibrium [1-3]. The polymorphic markers were also used in investigations of transmission of normal alleles from generation to generation in paternity and forensic studies. The first generation of these markers were restriction fragment length polymorphism (RFLP) [4,5]. The large size and very low number of variable alleles of RFLPs put a limit on their usefulness as genetic markers in forensic studies. The second generation of the polymorphic markers was those with a tandem core sequence (repeat unit), known as mini- and macro-satellites, such as short tandem repeats (STRs) and variable number of tandem repeats (VNTRs) [6]. Since the variation in the number of repeat units, both STR and VNTR exhibit a high degree of length polymorphism. However, due to the smaller size of the repeat unit of STR markers (2-7 base pair) compare to VNTRs (15-35 base pair), the former encompasses more allele frequency in different human populations studied [7].

In recent years, as a result of advances in human genome technologies, a third generation of polymorphic markers named single nucleotide polymorphism (SNP)

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has been identified [8]. These markers seem to be the most polymorphic sites in the human genome with approximately in every 1000 base pair, with great genetic stability, which is important in paternity testing. Therefore, it has been proposed these markers to be replaced by STRs in paternity and individual investtigations. However, in a comprehensive study on the effectiveness in relationship analysis of SNPs compare to STRs, unexpectedly it was found that the possibility of inconclusive results is much higher with SNPs [9,10]. This study puts under question the validity of SNP markers for use in routine paternity investigations, raising the necessity for further assessment of these markers before any kind of these applications.

At the moment STRs are used as markers of choice in most forensic, paternity testing and individual identification studies. Usually, a battery of 5-10 STR markers is used in each study. The degree of allele frequency for each STR marker has been found to be basically population dependent [11,12]. The selection of each marker for forensic investigations is based on the previous studies performed on the situation of the allele frequency of STRs in the population of interest. This makes the analysis of allele frequency of known STR markers a prerequisite to forensic study in each population.

In this study, five tetra-nucleotide repeat STR markers including HUMvWA, HUMFES, HUMTPO, HUMTH01, and D3S1359, which have been frequently used for forensic and paternity testing in different populations were genotyped for 220 unrelated individuals from Iranian population of the province of Isfahan [12-16]. To our knowledge, this is the first study on the analysis of the allele frequency and genotyping of the STR loci in Iranian population. Therefore, the obtained data could be used to initiate an Iranian genetic database for the STR polymorphic markers. Moreover, the results of this study could show that the STR markers were informative for paternity testing and individual identifications.

Materials and Methods

DNA Samples

Blood samples were collected from 220 unrelated volunteer individuals. The total genomic DNA was extracted from the leukocytes using standard salting out procedure [17].

PCR Amplification and STR Genotyping

The DNA samples were used to genotype HUMvWA, HUMFES, HUMTPO HUMTH01 and D3S1359 STR loci using polymerase chain reaction (PCR). The PCR reactions were carried out in a 25 µl total volume containing 0.2 mM of dNTP and Taq-DNA polymerase (Cinagen, IR Iran) in a PCR buffer [50 mM Tris-HCl pH 8.8, 15 mM (NH4)2SO4, 1.5 mM MgCl2, 0.01% gelatin]. The reactions were performed in a Gradient Thermal Cycler (Eppendorf, Germany). The amplification cycles were as: 1 cycle at 94°C for 4 min; 30 cycles (94°C 1 min, annealing for 1 min, 72°C for 1 min), followed by 1 cycle 72°C for 5 min. The annealing temperature for each set of primers, as determined with the gradient program of the Thermal Cycler, and their sequences are shown in Table 1 [18].

Table 1. Characteristics of the STR loci and primers used in this study

Locus Accession	Chromosome	Allele number*	Product size (bp)	Primer sequences (forward and reverse) (5′-3′)	Annealing Temp
HUMvWA	12	9	134-166	CCC TAG TGG ATG ATA AGA ATA ATC GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG	54
HUMTPO	2	7	106-130	CAC TAG CAC CCA GAA CCG TC CCT TGT CAG CGT TTA TTT GCC	54
HUMTH01	11	6	155-179	GTG ATT CCC ATT GGC CTG TTC CTC GTG GGC TGA AAA GCT CCC GAT TAT	55
HUMFES/FPS	15 r	6	211-234	GGG ATT TCC CTA TGG ATT GG GCG AAA GAA TGA GAC TAC AT	54
D3S1359	3	6	197-217	ATG CTA AGT GCT AAG TCA ACT GTT GCC TCT GAC ATG GCT TT	56

*Determined in the present study; Abbreviations are as follows: HUMTPO, Human thyroid peroxidase gene; HUMvWA, Human von Willebrand factor gene; HUMTH01, Human Tyrosine hydroxylase; HUMFES/FPS, Human C-fes/fps proto-oncogene

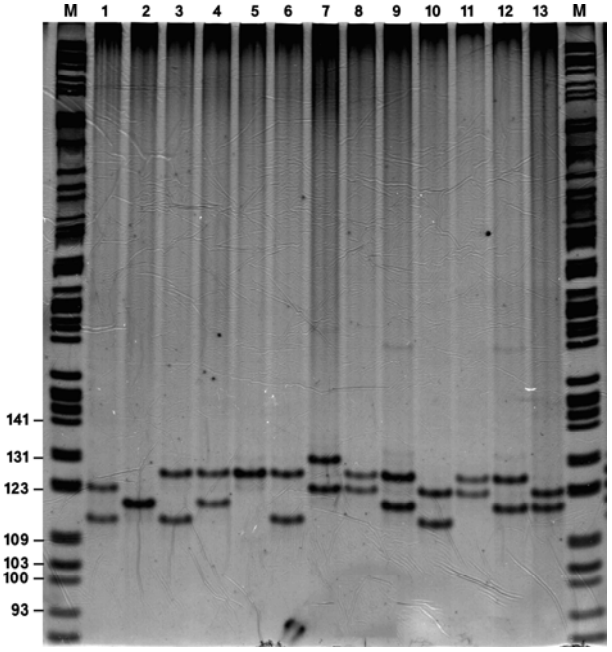

Figure 1. Genotyping of HUMTPO STR marker. A typical polyacrylamide gel electrophoresis of HUMTPO STR marker, which was genotyped for 13 individuals, is presented. Genotyping was performed on a sample of total genomic DNA from each individual using PCR with primers for HUMTPO genomic sequence. The PCR products were analyzed on a 12% polyacrylamide gel and visualized by silver staining. M represents the M3 DNA size ladder. The numbers on the left, show the molecular weight for some bands of the M3 marker. See Materials and Methods for details.

The PCR products (1.5 µl) were resolved in a vertical 15 cm × 25 cm × 0.75 mm polyacryl amide gel electrophoresis (PAGE). The separated alleles were silver stained and visualized using the Biometra gel documentation apparatus (Biometra, Germany). The size of the alleles was determined by comparison with the M3 DNA size marker (Elchrom Scientifics AG, Switzerland) using the fragment analyzer software accompanied with the gel documentation apparatus. The M3 marker contains over 50 DNA fragments in the size range from 75 to 622 base pair.

Statistical Analysis

The allele frequency of the five STR loci examined in this study were calculated based on the number of the detected alleles for specific locus and the total alleles present for that locus in the population tested. Evaluation for forensic purposes was carried out by calculating the power of exclusion (PE), for paternity testing and power of discrimination (PD) for identity testing using the PowerStatsV12.xls software, which is freely available on the net [19]. Also, the polymorphism information content (PIC), typical paternity index and matching probability were calculated using similar software [19].

Results and Discussion

In the present study the genotyping and allele frequency of five STR loci including HUMvWA, HUMFES, HUMTPO, HUMTH01 and D3S1359 were investigated. As reported previously, these loci have been found highly polymorphic with a large degree of variability as examined in different populations [12-16]. In Table 1, the characteristics of the STR loci examined are illustrated. In a sample of 220 unrelated Iranians, for HUMvWA, HUMTPO, HUMTH01, HUMFES and D3S1359 loci 9, 7, 6, 6, and 6 different alleles were found, respectively. As shown in Table 1, for each locus the alleles found were evenly spaced and resided between 134-166 base pair for HUMvWA, 106-130 base pair for HUMTPO, 155-179 base pair for HUMTH01, 211-234 base pair for HUMFES and 197-217 base pair for D3S1359.

Based on the position of the primers on the genomic sequence of the loci and the number of repeats and the size of the alleles, the core four-nucleotide repeat was calculated with the aid of DNA analyzer using the M3 DNA size marker as standard (see Material and Methods). Figure 1 represents a typical genotyping analysis for HUMTPO STR using a 12% polyacrylamide gel electrophoresis. As shown in Table 2, within the population examined, the core four-nucleotide repeat in HUMvWA locus was in the range of 13-21 base pair, among them the 17 repeats had the highest frequency of 30.9%. In loci HUMTPO, HUMTH01, HUMFES and D3S1359 the core four-nucleotide repeats were as 6-12, 5-10, 8-13, 4-9, respectively. For HUMTPO, the eight and 11 core repeat alleles had the highest (34.1%) and lowest (2.3%) frequency, respectively. However, the degree of allele variability for HUMTH01 and HUMFES was lower than HUMvWA and HUMTPO within the population examined (Table 2). The data reported from other populations indicated a high degree of variation in allele number of these STR loci, which makes them particularly informative for forensics and paternity testing purposes [12,18].

Forensic parameters including matching probability and power of discrimination as well as polymorphic information content (PIC) for the STR loci HUMvWA, HUMTPO, HUMTH01, HUMFES and D3S1359 were

Table 2. Allele frequency of five STR loci in the population of Isfahan

HUMvWA			HUMTPO			HUMTH01			HUMFES/FPS			D3S1359		
Allele*	Length (bp)	Freq (%)	Allele*	Length (bp)	Freq (%)	Allele*	Length (bp)	Freq (%)	Allele*	Length (bp)	Freq (%)	Allele*	Length (bp)	Freq (%)
13	134	5.0	6	106	20.0	5	155	35.5	8	211	27.7	4	197	7.0
14	138	6.4	7	110	11.4	6	159	19.1	9	215	28.2	5	201	6.8
15	142	6.4	8	114	34.1	7	163	10.5	10	219	19.5	6	205	9.3
16	146	13.6	9	118	15.9	8	167	10.9	11	223	10.0	7	209	12.0
17	150	30.9	10	122	12.7	9	171	12.3	12	227	12.3	8	213	11.5
18	154	22.7	11	126	2.3	10	175	11.8	13	231	2.3	9	217	14.8
19	158	5.5	12	130	3.6									
20	162	3.2												
21	166	6.4												

*Represents the core four-nucleotide repeat

Table 3. Analysis of forensic, paternity and allele frequency values for HUMvWA, HUMTPO, HUMTH01, HUMFES/FPS and D3S1359 in Isfahan

	HUMvWA	HUMTPO	HUMTH01	HUMFES/FPS	D3S1359
Forensic					
Matching Probability	0.079	0.082	0.075	0.089	0.075
Power of Discrimination	0.921	0.918	0.925	0.911	0.925
Polymorphic information content	0.790	0.760	0.760	0.750	0.760
Paternity					
Power of Exclusion	0.502	0.549	0.549	0.457	0.549
Typical Paternity Index	1.960	2.200	2.200	1.770	2.200
Allele Frequencies					
Homozygotes	25.5%	22.7%	22.7%	28.2%	24.7%
Heterozygotes	74.5%	77.3%	77.3%	71.8%	75.3%
Total Alleles	440	440	440	440	440

shown in Table 3. These parameters were calculated based on the alleles for each individual for each STR marker with the aid of PowerStatsV12.xls computer software [19]. Analysis of the data indicated that all the STRs showed relatively similar values for forensic and individual identification purposes. All the five STR loci showed a high degree of PIC values (above 0.5), suggesting that the loci were very informative. The paternity indices such as power of exclusion and typical paternity index were also determined using the similar software [19] (Table 3). The data showed that all five STR loci had essentially similar power of exclusion. However, the HUMTPO, HUMTH01 and D3S1359 showed higher typical paternity index compare to HUMvWA and HUMFES. The distribution of observed heterozygosity for STRs was calculated. All five loci were found highly heterozygous within the population studied (Table 3).

In conclusion the STR system appeared to be highly discriminating and could result in reliable typing of five specific loci. Thus this STR system can be suggested as useful tool for individual human identification and forensic applications in Iranian population.

Acknowledgments

We are thankful to all the individuals participated in this study. This work was supported by the internal research grant number 820308 from the department of research of the University of Isfahan, Isfahan IR Iran.

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