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Genetic analysis of two STR loci (VWA and TPOX) in the Iranian province of Khuzestan

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ABSTRACT

Objective(s): Short tandem repeat (STR) loci are the most informative DNA genetic markers for attempting to individualize biological material for application in paternity and forensic cases.
Materials and Methods: Blood samples were collected and the total genomic DNA was extracted. The DNA samples were used for genotyping VWA and TPOX STR loci using PCR and polyacrylamide gel electrophoresis.
Results: This report presents allele frequency data and parameters of biological or legal interest, such as heterozygosity value, polymorphic information content (PIC), genetic diversity index (GD) and population parameter (θ) in Arab and non-Arab population of Khuzestan province (Iran) for the loci VWA and TPOX. Blood samples (N= 392 for VWA and N=308 for TPOX) were collected from individuals unrelated throughout Khuzestan province. The loci were genotyped using the polymerase chain reaction (PCR) followed by polyacrylamide gel electrophoresis (PAGE) and silver staining. Chi-square test showed that neither STR loci were in agreement with the Hardy-Weinberg equilibrium.
Conclusion: The examined STR loci in this study have proven a relatively high genetic variation in the Iranian population. The data could be used for construction of a forensic genetic database for the Iranian population.

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Introduction

STR (or microsatellite) loci consist of simple tandem repeated sequences of 1–6 bp in length. As with the larger variable number tandem repeat (VNTR or minisatellite) loci, STRs may exhibit a high degree of length polymorphism owing to variation in the number of repeat units displayed (1). However, unlike VNTRs, which occur predominantly in telomeric regions, STRs appear to be abundant throughout the human genome and occur, on average, every 6–10 kb (1). Because of their abundance, polymorphic nature, and amenability to amplification by PCR, STRs are ideal markers for genomic mapping and genetic linkage analysis (2). In addition to their suitability for mapping and linkage analysis, STRs provide a source of highly informative loci for use in the identification of individuals. DNA profiling based on PCR amplification of STRs has the advantage of being more sensitive than conventional techniques. Furthermore, because of their small allele sizes (generally < 300 bp), STR systems are more likely to be successful on old or poorly stored specimens that contain only degraded DNA (3–5). Also, the ability to resolve PCR products differing in size by just 1 base on polyacrylamide gels allows precise allele designation, thus eliminating the need for the continuous allele distribution models currently employed with VNTR systems (6, 7). High mutational rates of microsatellites lead to extensive polymorphism and increase the probability that isolated populations diverge rapidly at these loci. Such characteristics make them particularly useful in the study of population genetics of species showing limited variability at other markers (8). It has been almost a decade since the 13 genetic markers that form the core of the FBI Laboratory's Combined DNA Index System (CODIS) were selected in November 1997 (9–11). The 13 CODIS loci used in the U.S. are CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11. These loci have become the common currency of data exchange for human identity testing

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

Locus	Chromosomal location	Product size (bp)	Primer sequences (forward and reverse) (5′–3′)	Annealing temp
TPOX	2p25.3;	216–256	F5′ACTGGCACAGAACAGGCACTTAGG3′ R5′GGAGGAACTGGGAACCACACAGGT3′	60
VWA	12p13.31	134–166	F5′CCCTAGTGGATAAGAATAATC3′ R5′GGACAGATGATAAATACATAGGATGGATGG3′	60

Determined in the present study; Abbreviations are as follows: TPOX, Human thyroid peroxidase gene; VWA, Human von Willebrand factor gene

both in forensic casework and paternity testing largely because of their ease of use in the form of commercial STR kits (12). Finding suitable and specific markers for ethnic groups or populations would be useful in different studies such as forensic, population diversity, and paternity.

Materials and Methods

Samplin

Blood samples (N = 308 for TPOX and N= 392 for VWA) were collected from unrelated, randomly selected individuals throughout Khuzestan province.

DNA extraction

The total genomic DNA was extracted from the leukocytes using DIAtom DNA Prep extraction kit (Gen Fanavaran, Iran) according to the instructions provided by the manufacturer.

PCR amplification and STR genotyping

The DNA samples were used to genotype VWA and TPOX 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 2 units Taq-DNA polymerase (Cinagen, Iran) in a PCR buffer [50 mM Tris-HCl pH 8.8, 15 mM (NH4)2SO4, 1.5 mM $MgCl_2$, 0.01% gelatin] (13). The reactions were performed in a Gradient Thermal Cycler (BioRad, USA).

The amplification cycles were as: 1 cycle at 94˚C for 4 min; 30 cycles (94˚C 30 sec, 60˚C for 45 sec, 72˚C for 1 min), followed by 1 cycle 72˚C for 5 min.

The motives and respective locations of these loci are as follows:

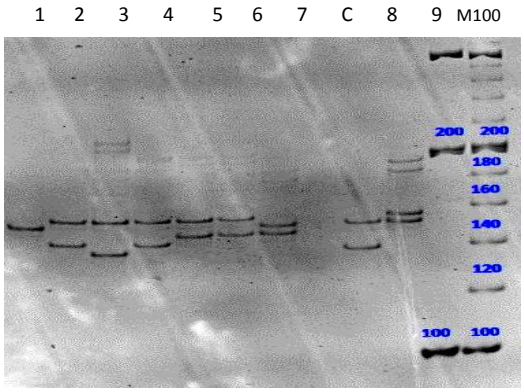

Figure 1. Genotyping of VWA STR marker. The PCR products were analyzed on a 12% polyacrylamide gel and visualized by silver staining. M100 and M20 represent the 100 bp and 20 bp DNA ladder, respectively. The numbers on the left, show the molecular weight for some bands of the marker. The numbers on the top, show the sample number, C: control

TPOX: (AATG)n, intron 10 of the thyroid peroxidase gene (2p23-2pter); (May 2004, NCBI build 35) (GenBank Accession M68651)

VWA: (TCTA)n, intron 40 of the von Willebrand factor gene (12p12-pter); (May 2004, NCBI build 35) (GenBank Accession M25858)

The PCR products (2 µl) were separated on a vertical 15 cm × 25 cm × 0.75 mm polyacrylamide gel electrophoresis (PAGE). Visualization was undertaken by the silver staining method (14) using the UV tec gel documentation apparatus (UV tec, UK). Allele size was determined by comparison with the 20 bp DNA ladder (Fermentase, CA). The 20 bp marker contains DNA fragments in the size range from 20 to 300 base pair (Figure 1, Figure 2). ONE-Dscan 1.3 software (Scanalytics, Billerica, MA) was used to analyze the gel run.

Statistical analysis

Statistical analysis was undertaken using the following statistical packages: ARLEQUIN software version 3.1 was used to assess Hardy-Weinberg equilibrium (HWE) and to determine population parameter (θ) and gene diversity indices (GD) (15); The frequency of each allele for each locus tested was calculated by Power Marker version 3.25 (16). Polymorphic information content (PIC), observed heterozygosity (Ho), expected heterozygosity (He) were performed with Power Marker version 3.25.

Results

Genotyping and allele frequency of TPOX and VWA loci were investigated. In Table 1, the characteristics ofthe STR loci examined are illustrated. The observed allele frequencies of TPOX and VWA loci in Arab and non-Arab populations are shown in Table 2.

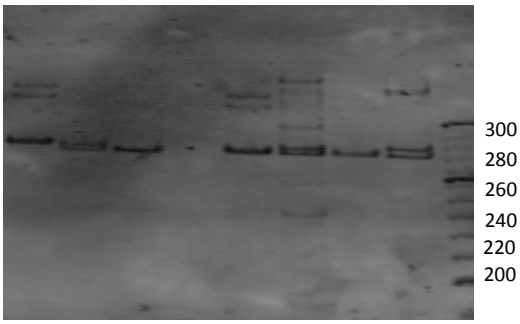

Figure 2. Genotyping of TOPX STR marker. The PCR products were analyzed on an 8% polyacrylamide gel and visualized by silver staining. M20 represents the 20 bp DNA ladder (Fermentase, CA). The numbers on the left, show the molecular weight for some bands of the marker. The numbers on the top, show the sample number, C: control

Table 2. Allele frequency of two STR loci in the two Arab and non-Arab populations

Allele	TPOX (n=308) Non-Arab (Freq %)	Arab (Freq %)	VWA (n=392) Non-Arab (Freq %)	Arab (Freq %)
4	2	1.7		
5	2.7	4		
6	8	7.2		
7	10	11		
8	13	15.8		
9	21	15.8		
10	14	11.3		
11	11.5	8.2	0.4	0
12	7.3	10.3	0	0.5
13	4.2	8.6	1.2	1
14	3	3.7	5	4.3
15	1.2	0.6	10	14.5
16	0.3	1	15.2	17.5
17			19	21.4
18			18.3	19.7
19			20.2	9.8
20			7.8	6.3
21			1.2	1.3
22			0.4	0.5
23			0.5	1
24			0	0.8
25			0.2	0.5

Moreover, the expected (*He*) and observed (*Ho*) heterozygosity, PIC, *GD*, *θ,* and *P*-values were calculated and presented in Table 3. The results obtained revealed that neither of the markers studied were in agreement with the Hardy-Weinberg expectations in this population. The differences between observed and expected heterozygosis were significant, which demonstrates an excess of homozygotes in our sample. This disequilibrium can be due to sub-population and too many consanguineous marriages (17, 18).

Heterozygosity value is estimated as 0.78 and 0.53 for VWA and TPOX, respectively.

For VWA, the 17 and 18 alleles in Arab population and the 19 and 17 alleles in non-Arab population had the highest frequency (Table 2). For TPOX, the 9 allele in non-Arab population and the 8 and 9 alleles in Arab population had the highest frequency (Table 2). Both STR loci showed a high degree of *PIC* values (0.83 for VWA and 0.87 for TPOX).

Discussion

The high level of heterozygosity (0.78) for VWA marker indicated that this locus potentially can be a very promising marker and could be used in individual and ethnic group identification and also for identity testing in Khuzestan province. Moreover, at the TPOX locus the fairly even allele distribution within the examined population makes it particularly informative for forensic and paternity testing purposes. Polymorphic information content (*PIC*) analysis for both STRs (VWA and TPOX) showed relatively similar values for forensic and individual identification purposes (see Table 3). These loci were very informative based on observation of a high degree of *PIC* values, 0.83 for VWA and 0.87 for TPOX. These data were also compared with other reports from Iranian and non-Iranian populations (19, 20). In our study *PIC* values for VWA and TPOX were higher than those reported for the Iranian population (19). In addition, the VWA and the TPOX markers showed a high level of heterozygosity (0.78) and a lower level of heterozygosity (0.53), respectively. The *PIC* values and *Ho* for VWA locus were closer compared with the TPOX locus.

Furthermore, our results for *Ho* and *PIC* measures of VWA and TPOX loci were compared with other populations (21–24). The highest *Ho* for VWA and TPOX was seen in Portugal (21). although the lowest values of *Ho* for VWA and TPOX were reported in China (24). However our study had the highest PIC for both loci. Neither loci in our populations, Arab

Table 3. Statistical parameters for the 2 STR loci

Locus	Ho	He	*P*-value	GD	θ	PIC
VWA	0.78	0.87	0	0.85	0.006	0.83
TPOX	0.53	0.88	0	0.86	0.002	0.87

Ho: observed heterozygosity; He: expected heterozygosity; *P*-value: HWE, exact test *P*-values; GD: gene diversity; θ: population parameter; PIC: polymorphic information content

and non-Arab, were in the Hardy-Weinberg equilibrium. This is not unexpected, due to many sub-populations and too many consanguineous marriages (17, 18, 23).

As reported previously, these loci have been found highly polymorphic with a large degree of variability in different populations (19, 21, 25–29). Reports from other populations indicated a high degree of variation in allele numbers of these STR loci, which makes them particularly informative for forensics and paternity testing purposes (22, 30–32).

Conclusion

Our results strongly support the application of VWA and TPOX genetic markers for personal identity testing in the Iranian population

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Maryam Jari collected samples, carried out the experimental studies, performed the statistical analysis and wrote the paper. Ali Mohammad Froughmand was the supervisor and edited the manuscript. Seyed Reza Kazeminez was co-supervisor. Arezu Abdollahi and Leila Ahmadi collected samples, carried out the experimental studies, and Maryam Heidari helped to carry out the experimental studies. All authors read and approved the final manuscript.

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