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Genetic Polymorphism of 21 Autosomal Short Tandem Repeat Markers in South Khorasan Province of Iran

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ABSTRACT

Background & Objective: Short tandem repeats (STR) are highly polymorphic genetic markers widely used in human identification. This study aimed to investigate the genetic characteristics of 21 autosomal STR loci in individuals from Eastern Iran.

Materials & Methods: Sixty healthy volunteers from twenty families provided buccal samples for analysis using the PowerPlex® 21 System. Various genetic and forensic parameters, including polymorphic information content, random matching probability, allelic discrimination power, paternity index, and exclusion power, were assessed.

Results: Five STR markers (D1S1656, D6S1043, D12S391, Penta D, and Penta E) were identified as prevalent in the Eastern Iranian population, enabling successful parentage verification. All loci were in Hardy-Weinberg equilibrium, with a total of 178 alleles detected. The Penta E locus had the highest number of alleles, while TPOX had the most frequent allele. D12S391 exhibited the highest heterozygosity percentage (96.7%) among the loci analyzed.

Conclusion: The study confirmed the high informativeness of the 20 autosomal STR markers in individuals from Eastern Iran, supporting previous findings. These results contribute to the genetic characterization of the Eastern Iranian population and highlight the utility of these markers in forensic applications.

Keywords: Forensic investigation, Short Tandem Repeat, Eastern Iran, Population genetics, Genetic markers

Introduction

DNA typing plays imperative roles in human identity testing as identification of forensic cases, sexual assault and paternity testing (1). STRs (Short Tandem Repeats or microsatellites) are highly polymorphic loci scattered throughout the eukaryotic genome with 6–10 kb frequency across the human genome. STRs distribution is opposite of variable number tandem repeats (VNTRs) with preferential occurrence through telomeric regions. STRs characteristically consist of sequential, repetitive arrays of two to six nucleotides in lengths identifiable by the STR profiling technique (2, 3). Length and the number of contiguous core repeat units in STRs play a crucial role in their diversity (1, 4). The exquisite sensitivity of the Polymerase Chain Reaction (PCR) approach made STR profiling acquire more widespread acceptance than other DNA typing methods. This is largely attributed to its increased sensitivity, the fact that it is a much quicker process, the capability to analyze multiple STR loci at the same time, and its greater suitability for automation (2). Since 1997 the original thirteen STR loci have been introduced as the National DNA Index System (NDIS) core for human identity and forensic analysis. The Federal Bureau of Investigation (FBI) laboratory announced an expansion of the Combined DNA Index System (CODIS) core loci. It implemented seven additional loci by January 1, 2017 **(5)**. These 20 loci provide the greater potential of discrimination and enhanced kinship analysis for human identification applications typically used in paternity evaluations and missing person inquiries **(6)**. The more DNA markers examined, the lesser the chance of observing two unrelated individuals with identical genotypes **(7)**. There is little evidence regarding paternity indices, allele frequencies, and the heterozygosity rate of STR loci in Iranian populations. The present study assessed twenty STR loci in the Eastern part of Iran using PowerPlex® 21 System. This system allows co-amplification and four-color fluorescent detection of 21 loci (and Amelogenin). They included D1S1656, D2S1338, D3S1358, D5S818, D6S1043, D7S820, D8S1179, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, Amelogenin, CSF1PO, FGA, Penta D, Penta E, TH01, TPOX and vWA in randomly selected sixty normal cases organized as twenty families from different areas of Eastern part of Iran.

Materials and Methods

Volunteers

This survey different Persian-speaking native families from ethnic areas of South Khorasan province (Birjand, Khusf, Ferdows, Tabas, Qaen included sixty normal volunteers grouped into twenty, Sarayan, Sarbisheh, Darmian, Zirkouh, Nehbandan) in the Eastern part of Iran. Their ancestry was confirmed up until the third generation born in the same city (great-grandparents). The research was carried out at Birjand University of Medical Sciences and received approval from the University's Ethics Committee (Ethical code: IR.BUMS.REC.1396.130).

Samples Collection

Following obtaining written consent, buccal swab samples were collected as per the manufacturer's guidelines. Essentially, two swabs were used to gently scrape the inner cheek surface to collect oral epithelial cells from both sides of the mouth. The swabs were then air-dried and kept at room temperature for storage.

Personal Paternity Test

The buccal swabs from each individual were submitted anonymously to Eurofins Company in Germany to establish the genetic profiles of the alleged father, mother, and biological child. Eurofins Medigenomix Forensik GmbH conducted all analyses meticulously and with advanced scientific expertise at specific genetic locations.

DNA Extraction and STR Characterization

DNA isolation was performed individually for each sample using a standard Chelex extraction technique **(8)**. The quantity of recovered DNA was estimated using the Quantifiler Duo DNA Quantification Kit (Life Technologies, Inc.). Genetic characteristics were determined by subsequent PCR-single-locus-technology analysis using Promega PowerPlex 21 kit (WEN ILS 500), which contains the following markers: D3S1358, D1S1656, D6S1043, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, FGA and Amelogenin AM as the gender determinant **(9)**. Positive and negative controls were conducted simultaneously, yielding the anticipated and validated results. The findings were verified by reanalyzing the original samples. All laboratory procedures, the choice of PCR systems, and the biostatistical analysis adhered to the guidelines set forth by the DNA commission of the International Society for Forensic Genetics (ISFG) **(10)**.

Statistical Analysis

Calculations for Chi-square, Hardy-Weinberg equilibrium and allele frequency were performed using online Genepop software. Paternity Index (PI) values for each STR locus, Combined Paternity Index (CPI), Probability of Paternity (POP) and Probability of exclusion were calculated by Powerstats V.12 software (http://www.promega.com/geneticidtools/powerstats).

Results

Personal Paternity Test Data

We genetically determined the STR profiling of 20 loci plus Amelogenin for 60 individual samples related to 20 families, each consisting of three members (alleged father, mother, and biological child). For all samples, the probability of a given person being the alleged father of his child was more significant than 99.9999 %. According to the companies' paternity test certificate, it was proven that the biological fathers and mothers for a given child are the same as nominal persons.

Allele Frequencies

In this report, the allelic frequency of 20 STR loci has been determined in the Eastern population of Iran. Table 1 represents the estimated allele frequencies at each locus. The total number of alleles identified in this study was 178 from 43 different alleles with an average number of 9 per locus. The TPOX's allele 8 was the most frequent one with 0.533898 value of allele frequency **(Table 1)**. The highest and the lowest numbers of alleles belong to Penta E (17 alleles) and TPOX (5 alleles) loci, respectively **(Table 2)**.

Heterozygosity of Alleles

The results have indicated that the D12S391 locus possesses the highest percentage of heterozygosity (96.7%) among other loci in the Eastern population of Iran. In contrast, CSF1PO and D5S818 loci represented the lowest amount of heterozygosity (68.3%) (Table 2).

Genetic Variation and Parameters of Forensic Effectiveness

The maximum and the minimum values for polymorphic information content were related to Penta E and TPOX loci, respectively. TPOX and Penta E loci also represented with the highest and the lowest probability of random matching, respectively, in the present report (by definition, probability of random matching indicates the probability of finding two unrelated individuals in a population that have the same genotype in a locus). Data analysis indicated that the power of allelic discrimination varies between 0.969 and 0.863 for Penta E and CSF1PO loci, respectively. The highest and lowest values related to the typical paternity index were observed in D12S391 and D52818 loci. Finally, the D12S391 locus exhibited the highest value, while both the CSF1PO and D5S818 loci displayed the lowest values of the "power of exclusion" index. This index measures the ability of a genetic marker to rule out an unrelated individual, selected at random from a specific population, as a potential father in paternity testing **(Table 3)**. The Hardy-Weinberg equilibrium was established for all loci, though D18S51 almost represented a deviation from this rule ($p < 0.063749$).

Table 1. Allele frequencies of 20 STR loci in Eastern population of Iran

Alleles	D3S1358	D1S1656	D6S1043	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	THO1	vWA	D21S11	D7S820	D5S818	TPOX	D8S1179	D12S391	D19S433	FGA
5	-	-	-	-	0.041667	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	0.241667	-	-	-	-	-	-	-	-	-
6.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.016667	-
7	-	-	-	-	0.191667	-	-	-	-	-	0.216667	-	-	0.041667	-	-	-	-	-	-
8	-	-	-	0.166667	0.016667	0.033333	-	-	0.008333	0.033333	0.158333	-	-	0.175	0.016667	0.533898	0.033333	-	-	-
9	-	-	-	0.091667	0.008333	0.1	-	-	-	0.175	0.233333	-	-	0.058333	0.008333	0.101695	0.016667	-	-	-
9.1	-	-	-	-	-	-	-	-	-	-	-	-	-	0.016667	-	-	-	-	-	-
9.3	-	-	-	-	-	-	-	-	-	-	0.141667	-	-	-	-	-	-	-	-	-
10	-	-	0.033333	0.025	0.075	0.141667	-	-	0.266667	0.125	0.008333	-	-	0.216667	0.083333	0.067797	0.058333	-	-	-
11	-	0.116667	0.283333	0.275	0.108333	0.241667	0.066667	-	0.391667	0.241667	-	-	-	0.175	0.416667	0.271186	0.058333	-	-	-
12	-	0.15	0.125	0.383333	0.125	0.258333	0.141667	-	0.208333	0.058333	-	-	-	0.258333	0.325	0.025424	0.083333	-	0.025	-
12.4	-	-	-	-	0.016667	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	0.141667	0.15	0.041667	0.05	0.183333	0.1	-	0.1	0.166667	-	-	-	0.058333	0.133333	-	0.325	-	0.3	-
14	0.05	0.108333	0.083333	0.016667	0.066667	0.041667	0.141667	-	0.025	0.108333	-	0.191667	-	-	0.016667	-	0.158333	-	0.266667	-
14.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.033333	-
15	0.225	0.208333	-	-	0.075	-	0.2	-	-	0.05	-	0.108333	-	-	-	-	0.183333	0.041667	0.166667	-
15.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.066667	-
16	0.275	0.1	-	-	0.075	-	0.133333	0.091667	-	0.041667	-	0.216667	-	-	-	-	0.058333	0.025	0.091667	-

16.3	-	0.025	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	0.183333	0.091667	0.025	-	0.041667	-	0.066667	0.066667	-	-	-	0.283333	-	-	-	-	0.025	0.033333	0.025	-
17.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.008333	-
17.3	-	0.033333	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	0.25	0.008333	0.108333	-	0.05	-	0.066667	0.116667	-	-	-	0.1	-	-	-	-	-	0.158333	-	-
18.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.016667	-	-
19	0.016667	-	0.108333	-	0.041667	-	0.05	0.225	-	-	-	0.058333	-	-	-	-	-	0.083333	-	0.041667
19.3	-	0.016667	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	0.083333	-	0.008333	-	-	0.15	-	-	-	0.041667	-	-	-	-	-	0.2	-	0.083333
21	-	-	-	-	0.008333	-	0.033333	0.05	-	-	-	-	-	-	-	-	-	0.116667	-	0.233333
21.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.016667
22	-	-	-	-	-	-	-	0.025	-	-	-	-	-	-	-	-	-	0.116667	-	0.058333
23	-	-	-	-	-	-	-	0.116667	-	-	-	-	-	-	-	-	-	0.108333	-	0.225
24	-	-	-	-	-	-	-	0.066667	-	-	-	-	-	-	-	-	-	0.05	-	0.208333
25	-	-	-	-	-	-	-	0.075	-	-	-	-	0.016667	-	-	-	-	0.05	-	0.083333
26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.041667
27	-	-	-	-	-	-	-	0.016667	-	-	-	-	0.025	-	-	-	-	-	-	0.008333
28	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-	-	-	0.183333	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	0.133333	-	-	-	-	-	-	-
30.2	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-	-	-	-	-	-	0.033333	-	-	-	-	-	-	-
31.2	-	-	-	-	-	-	-	-	-	-	-	-	0.191667	-	-	-	-	-	-	-
32.2	-	-	-	-	-	-	-	-	-	-	-	-	0.158333	-	-	-	-	-	-	-
33.2	-	-	-	-	-	-	-	-	-	-	-	-	0.108333	-	-	-	-	-	-	-

Table 2. **Paternity factors among 60 Eastern Iranian individuals typed at Promega PowerPlex 21 system.**

Locus	No.	%	MP	PD	PIC	PE	TPI	P-value
D3S1358	6	76.7	0.097	0.903	0.74	0.539	2.14	0.998831
D1S1656	11	76.7	0.049	0.951	0.85	0.539	2.14	0.972112

D6S1043	9	81.7	0.052	0.948	0.83	0.63	2.73	0.998744
D13S317	7	75	0.13	0.87	0.7	0.51	2	0.945463
Penta E	17	93.3	0.031	0.969	0.9	0.864	7.5	0.999148
D16S539	7	81.7	0.071	0.929	0.78	0.63	2.73	0.984309
D18S51	10	75	0.047	0.953	0.86	0.51	2	< 0.063749
D2S1338	11	81.7	0.045	0.955	0.86	0.63	2.73	0.994828
CSF1PO	6	68.3	0.137	0.863	0.67	0.403	1.58	0.998151
Penta D	9	78.3	0.052	0.948	0.83	0.568	2.31	1
TH01	6	83.3	0.101	0.899	0.76	0.662	3	0.998482
vWA	7	78.3	0.071	0.929	0.78	0.568	2.31	0.997877
D21S11	10	83.3	0.044	0.956	0.84	0.662	3	1
D7S820	8	76.7	0.066	0.934	0.79	0.539	2.14	0.989125
D5S818	7	68.3	0.133	0.867	0.64	0.403	1.58	0.994861
TPOX	5	71.2	0.217	0.783	0.57	0.447	1.74	1
D8S1179	10	75	0.061	0.939	0.8	0.51	2	0.996646
D12S391	12	96.7	0.048	0.952	0.87	0.932	15	1
D19S433	10	71.7	0.081	0.919	0.77	0.455	1.76	0.946762
FGA	10	80	0.062	0.938	0.81	0.599	2.5	1
Summary	178							
Min	5							
Max	17							
Average/locus	8.9							
CMP	9.416×10–24							

No Number of alleles per locus, **%** percentage of Hetrozygosity, **MP** Matching Probability, **PD** Power of Discrimination, **PIC** Polymorphic Information Content, **PE** Power of Exclusion, **TPI** Typical Pternity Index, **p-value** Probability Hardy Weinberg Equilibrium test, **CMP** Combined Match Probability, **Min** Minimum Number of alleles, **Max** Maximum Number of alleles. 10^{-24}

Discussion

The present study defined heterozygosity and the paternity indices of 20 STR loci plus gender determining markers. This project was undertaken among 60 individuals belonging to twenty unrelated families of different geographic origins of Eastern Iran to evaluate forensic parameters and paternity factors for 21 loci using the PowerPlex® 21 System. In the present evaluation, the gender identification marker produced two peaks for female samples (XX) and a single peak for male samples (XY). To the best of our knowledge, there is no previous report regarding five STR markers (D1S1656, D6S1043, D12S391, Penta D and Penta E) included in PowerPlex® 21 System in Iranian population studies **(11-15)**. Forensic parameters assessed in the present report include combined match probability, allele frequency, and heterozygosity, polymorphic information content (PIC), combined paternity index (CPI), typical paternity index, and the power of exclusion evaluated factor in the present study. Population data must be compiled to estimate the frequency of each possible allele to determine the probability of occurrence of a given genotype at random in a population **(16)**. STRs are among the most prevalent DNA sequence patterns found in mammalian genomes and have been regularly utilized in human genetic profiling for the past twenty years **(17)**. The more STR loci tested for profiling, the greater the discrimination power. The likelihood that a single person has an identical STR profile with another person taken at random in the population becomes extremely rare **(16).** In line with previous reports, the present study's combined match probability (CMP) value was low enough (9.41×10^{-24}). CMP indicates that the likelihood of encountering two identical DNA profiles across the 20 autosomal STR loci among Eastern Iranian individuals is 1 in 1.06×10^{23}. It is statistically extremely low and makes sure the usefulness of the PowerPlex® 21 System for human identification in our population and also strengthens the power of paternity testing **(18)**. Allelic frequencies in our survey were compared to previously Iranian and regional population data. Consistent with previous studies among Iranians and Kuwaiti individuals, the TPOX locus in the present report represented the lowest number of alleles and the second least heterozygous locus **(14,15,18,19)**. On the other hand, the highly variable STR locus, D12S391, represented the highest percentage of heterozygosity among Eastern individuals of Iran **(20)**. While in our limited population, we only found 12 polymorphic alleles at this locus (which still ranked as the second top loci with the highest number of alleles). Other studies even found more alleles in their larger populations, making this STR suitable for forensic and genetic purposes **(20)**. The combined paternity index (CPI) is calculated by multiplying the individual paternity index (PI) values for each locus. The probability of paternity represents the likelihood, based on the CPI that a randomly selected, unrelated man of the same ethnicity is the biological father of a specific individual. This probability is computed using the formula (CPI / (CPI + 1)) × 100 **(21, 22).** This probability was greater than 99.9999 % for all 20 families. Accordingly, it was practically confirmed in our data that nominal parents of a given individual in each family were their biological fathers and mothers. In line with previous studies, our data indicated that most Polymorphic Information Content (PIC) values for 20 loci were greater than 0.7, suggesting that they are highly polymorphic and would be of great value as informative markers **(15, 23)**. Similar to a recent study conducted among the Kuwaiti population. Penta E processed the highest number of alleles after SE33 locus (which was lacking in our study Penta E was the most discriminative locus in the Eastern Iranian population, exhibiting 17 distinct alleles (PIC = 0.9). Conversely, TPOX was the least discriminative locus, presenting only five different alleles (PIC = 0.57) **(14, 18)**. A similar study also conducted in Western and Southwestern Iran confirmed that the lowest PIC values in the Kurdish and Arab populations were 0.63 and 0.65, both belonging to TPOX locus, supporting our data **(19)**. Penta E, D12S291 and D18S51 and D2S1338 loci, respectively represented with the first to third highest PIC values in the present report, further supporting previous data and may be considered promising polymorphic candidates for ethnic group identification **(19).** The forensic factor typical paternity index ranged between 1.58 for CSF1PO and D5S818 and 15 for D12S391. Besides, the calculated combined PE was greater than 99.9999% **(18)**. These high values also confirmed the usefulness of the Power Plex system for paternity testing and individual identification in the Eastern Iranian population. Furthermore, the power of discrimination (PD) in correlation with matching probability (MP) warranties the high degree of polymorphism between Eastern Iranian individuals, supporting previous reports **(24).** In contrast to the study of people in western Iran in which D13S317 locus deviated from Hardy-Weinberg equilibrium, in the present study, D18S51 value showed such disequilibrium **(19)**. Furthermore, Falcone et al. conducted a study in Calabria, located in the southern region of Italy, where individuals were genetically analyzed for D6S1043, Penta D, and Penta E STR markers. The results of the study revealed that Penta E exhibited the highest level of informativeness among the analyzed loci, as indicated by metrics such as heterozygosity, polymorphic information content (PIC), power of discrimination (PD), and typical paternity index (TPI). In contrast, D6S1043 was identified as the least informative marker in Falcone's study. Additionally, it was noted that D6S1043 is less prevalent and has been predominantly utilized in studies focusing on Asian populations **(25)**. High prevalence of consanguineous marriage in Iran and the phenomenon of allele dropout might be the reasons for this situation **(26).** A study conducted in 2023 investigated five rare alleles across different STR loci and discovered that D12S391 exhibited the highest number of alleles, while fewer alleles were found at the D13S317 locus within the Chinese population. In comparison, our research indicated that the Penta E locus had the highest allele count, followed closely by D12S391 in second place. Additionally, the TPOX locus had the lowest number of alleles, with D13S317 ranking third in our findings **(27).**

This study highlights the importance of conducting paternity tests that include as many loci as possible to address complex cases, particularly in instances of criminal activity or harassment. The non-genetic information provided is often incomplete. Understanding the allelic heterozygosity of a specific geographic area provides essential information related to paternity cases prior to drawing any conclusions **(28).**

Conclusion

The results of this study are consistent with previous research and show a significant correlation between the STR marker and the investigated condition. The high levels of heterozygosity and paternity indices further support the suitability of the STR marker for forensic and genetic purposes. Nonetheless, it is crucial to recognize that the sample size in this study could restrict the broader applicability of the findings. Therefore, future research endeavors are necessary to enhance the accuracy and reliability of these findings and to confirm the applicability of the STR marker in forensic and genetic applications. This study serves as a pioneering effort in the south-Eastern region of Iran, shedding light on the prevalence and familial inheritance pattern of the specific STR marker.

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Authors' Contribution

SN: Conceptualization, Methodology, Formal analysis, Resources, Writing – original draft, Writing. **FV**: Writing review & editing. **EMM:** Conceptualization, Investigation, Resources, Data curation, Writing review & editing, Supervision, Project administration.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Ethics Approval and consent to participate

Ethical code: IR.BUMS.REC.1396.130

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