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Genetic polymorphism of eleven STR loci in Rajput population of Delhi, India

Abstract

This study is an attempt to generate genetic database for endogamous population of Rajputs of Delhi, India. Genetic polymorphism at eleven Short Tandem Repeat (STR) loci (F13A01, FESFPS, vWA, D16S539, D7S820, D13S317, HPRTB, F13B, CSF1PO, TH01 and TPOX) was examined in 87 unrelated Rajputs individuals to evaluate their significance in human identification. There was no evidence for departures from HWE (P>0.001) at all loci except locus HPRTB. All the loci showed high polymorphism with great power of exclusion. The combined matching probability (MP) of the eleven STR system was 1.965 x 10^{-11}, indicating that the system has a much stronger inter-individual discriminating power. The eleven loci showed a combined power of discrimination (PD) of 0.999999. The data suggests that these loci are useful for identity testing, forensics and for solving paternity cases among the Rajput population in Delhi, India.

Keywords: DNA, STR loci, genetic diversity, population data, Rajputs, forensics

Volume 1 Issue 5 - 2015

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Received: October 28, 2015 | **Published:** December 8, 2015

Abbreviations

STR, Short Tandem Repeat; MP, Matching Probability; PD, Power of Discrimination; BDA, Bio Doc Analyze; PIC, Polymorphism Information Content; PE, Power of Exclusion; TPI, Typical Paternity Index

Introduction

Short Tandem Repeat (STR) markers are best choice for the genetic structure assessment of a population due to co-dominant inheritance, high polymorphism, mutation rate and ease of use.[1,2] Polymorphic STR loci have become useful tool for DNA analysis and typing for human identification and paternity testing for forensic purposes in most of the forensic laboratories in the world.[3,4] Determination of the allele frequencies and distribution of genotype are prerequisites for DNA typing of any population. India is a rich country in ethnic, cultural and linguistic variant groups. Human diversity in India is defined by 4693 different and documented population groups that include 2205 major communities, 589 segments and 1900 territorial units spread across the country. Like most other Indians, Rajput is endogamous population.[5] The Rajput clans emerged as a dominant community in northern, central, western India and current eastern Pakistan in the colonial period. They seem to have risen to prominence from the late 6th century C.E. and governed the majority of princely states in Rajasthan and Surashtra. The Rajput population and the former Rajput states are found spread through much of the subcontinent, particularly in north, west and central India. Rajput populations are found in Rajasthan, Gujarat, Delhi, Uttar Pradesh, Himachal Pradesh, Haryana, Jammu, Punjab, Sindh, Uttarakhand, Madhya Pradesh and Bihar. The state of Delhi comprises about 3.8% of the Rajput population.[6] A very few number of genetic studies have been carried out on Rajput population.[7,8] However, there is no published data on the allele frequency of STR loci in Rajput population of Delhi, India. Therefore, the present data would be used in the forensics and individual identification for this population group and this genetic data would enrich the genetic informational resource.

Materials and methods

Sample collection

Blood samples were collected from 87 unrelated healthy donors resident in State of Delhi, India on FTA cards (Whatman technology, Sigma–Aldrich, USA).

STR markers

The selected STR markers are tetra-nucleotide ((F13A01, FESFPS, vWA, D16S539, D7S820, D13S317, HPRTB, F13B, CSF1PO, TH01, TPOX) located on 13 different chromosomes (Table 1).

Table 1 Locus specific information.

STR locus location	Chromosomal definition	Genbank locus and locus definition	Repeat sequence 5'→ 3'	Known alleles	Allele size range (bases)
CSF1PO	5q33.3-34	HUMCSF1PO, Human c-fms proto-oncogene for CSF-1 receptor gene	AGAT	Jun-15	295-323
TH01	11p15.5	HUMTH01, Human Tyrosine hydroxylase gene	AAGT	5-9, 9.3, 10, 11	183-199
TPOX	2p25.1-pter	HUMTH01, Human Thyroid peroxidase gene	AAGT	Jun-13	228-256
D16S539	16q24-qter	NA	AGAT	5, 8-15	276-396

Table continued

STR locus location	Chromosomal definition	Genbank locus and locus definition	Repeat sequence 5'→ 3'	Known alleles	Allele size range (bases)
D7S820	7q11.21-22	NA	AGAT	Jun-14	215-243
D13S317	13q22-q31	NA	AGAT	Jul-15	165-193
FESFPS	15q25-qter	HUMFESFPS, Human c-fes/fps proto-oncogene	AAAT	Jul-14	230-246
F13A01	6p24.3-p25.1	HUMF13A01, Human coagulation factor XIII a subunit gene	AAAG	3.2,4-16	281-331
vWA	12p12-pter	HUMVWA31, Human von Willebr and factor gene	AGAT	11, 13-21	143-171
F13B	1q31-q32.1	HUMBFXIII, Human factor XIII b subunit gene	AAAT	06-Dec	169-189
HPRTB	Xq26	HUMHPRTB, Human hypoxanthinephosphoribosyl-transferase gene	AGAT	11-15, 17	279-303

DNA extraction

A 1.2 mm punch from a dried sample spot on FTA paper was taken in a PCR tube. 200µl FTA purification reagent ((Whatman technology, Sigma –Aldrich, USA) was added to PCR tube, incubated for 5minutes at room temperature and then continuously agitated by using a pipette. This process was repeated thrice with FTA purification reagent and twice with 200µl TE buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0). Finally, the entire unspent TE buffer was removed and discarded by pipetting and the disc was allowed to dry at room temperature for overnight and was directly used for PCR amplification.

PCR amplification

PCR amplification of the 11 STR loci viz. F13A01, FESFPS, vWA, D16S539, D7S820, D13S317, HPRTB, F13B, CSF1PO, TH01, TPOX was performed using Gene Print STR system kit (Promega Corporation, USA) in a 25µl final reaction volume. The PCR reagents were used according to the manufacturer's protocol (Promega Corporation, Madison, US).[9] The samples were amplified on Biometra PCR thermal cycler. Thermal cycling parameters have been standardized in the laboratory for consistency of results (Table 2).

Table 2 Amplification programs for different STR loci.

Program for locus	Initial incubation	Cycling for first 10 cycles	Cycling for last 20 cycles	Extension step	Hold step
CTT Multiplex	96°C for 2 mins.	94°C for 1min. 64°C for 1min. 70°C for 1.5mins.	94°C for 1min. 64°C for 1min. 70°C for 1.5mins.	None	4°C
F13B	96°C for 2 mins.	94°C for 1min. 60°C for 1min. 70°C for 1.5mins.	90°C for 1min. 60°C for 1min. 70°C for 1.5mins.	None	4°C
STRIII Multiplex FFV Multiplex HPRTB	96°C for 2 mins.	94°C for 1min. 60°C for 1min. 70°C for 1.5mins.	90°C for 1min. 60°C for 1min. 70°C for 1.5mins.	60°C for 30mins.	4°C

Genotyping of amplified fragments

Amplified products (2.5µl per sample mixed with an equal volume of STR 2X loading solution) were electrophoresed at 50 Watt for 2-4h on a 40cmX20cm, 0.35mm thick 6% denaturing poly acrylamide gel by manual Geno Sequencer (Atto Corporation, Japan) with 0.5X TBE as gel running buffer. Various alleles were visualized by staining with silver stain (Promega Corporation, USA) described by Bassam et al.,[10] and images were stored in the computer. The allelic ladders were run with the samples to determine the size of the amplified products. Direct comparison between the allelic ladders and amplified samples of the same locus allowed for determination of alleles. Alleles were designated by comparison with appropriate allelic ladders according to the standard nomenclature[11] and calculated the size relative to allelic ladder with Bio Doc Analyze (BDA) system (Biometra GmbH, Germany)

Statistical analysis

The genotype data of the 11 STR loci was compiled for eleven STR markers in Excel sheets to facilitate statistical treatment for generation of allele frequencies. Allele frequencies[12] heterozygosity (observed and expected) values were calculated using software Genetix 4.02.[13] Tests for conformity to Hardy-Weinberg expectations (probability test) were performed through Gene pop 3.3d.[14,15] Statistical parameters of forensic importance like power of discrimination (PD), polymorphism information content (PIC)[16–18] matching probability (MP) and paternity indices viz. power of exclusion (PE) and typical

Citation: Chauhan T, Kushwaha KPS, Chauhan V. Genetic polymorphism of eleven STR loci in Rajput population of Delhi, India. *Forensic Res Criminol Int J.* 2015;1(5):192–196. DOI: 10.15406/frcij.2015.01.00031

paternity index (TPI)[16] were calculated using "Power Stats" Microsoft Excel workbook template provided by Promega Corporation.[19,20]

Results and discussion

Allelic variation at each locus in Rajput population is described in (Table 3). The combined allele distribution for the eleven STR loci in the present study ranged from 5 to 9 at different loci. All STR loci exhibited considerable variation in this population. The distribution of observed allele frequencies, heterozygosity (Observed and expected), P values at eleven STR loci and common forensic efficiency parameters useful for forensic and paternity tests have been tabulated in (Table 4).

Table 3 Variation at each STR locus in Rajput population.

STR Locus	No. of alleles observed at each locus (no. of samples studied=87)	Alleles observed in present study	Alleles observed in Bihar population[7]	Alleles observed in Haryana population [8]
CSF1PO	8	Jul-14	Aug-14	14-Aug
THO1	6	6- 9, 9.3, 10	Same	Same
TPOX	7	7-12, 14	8-13, 15	12-Jul
D16S539	6	Aug-13	Aug-14	Not studied
D7S820	8	Jun-13	Jul-14	Not studied
D13S317	8	Jul-14	Same	Not studied
FESFPS	5	Sep-13	Not studied	14-Aug
F13A01	9	3.2,4-8, 14-16	Not studied	Not studied
vWA	8	14-21	13-19	Not studied
F13B	5	6,8-11	Not studied	Not studied
HPRTB	6	11-15, 17	Not studied	Not studied

Table 4 Allele frequency and other forensic efficiency parameters of Rajput population.

Alleles	CSF1PO	D7S820	D13S317	D16S539	F13A01	F13B	FESFPS	HPRTB	THO1	TPOX	vWA
3.2					0.133						
4					0.084						
5					0.398						
6		0.01			0.169	0.111			0.316		
7	0.006	0.042	0.01		0.139				0.184	0.006	
8	0.006	0.25	0.2	0.053	0.006	0.21			0.109	0.425	
9	0.023	0.104	0.1	0.171		0.309	0.006		0.253	0.155	
9.3									0.121		
10	0.218	0.156	0.15	0.132		0.364	0.217		0.017	0.057	
11	0.339	0.198	0.26	0.276		0.006	0.367	0.11		0.293	
12	0.351	0.167	0.21	0.25			0.319	0.234		0.052	
13	0.046	0.073	0.06	0.118			0.09	0.351			
14	0.011		0.01		0.024			0.221		0.011	0.107
15					0.036			0.078			0.065
16					0.012						0.202
17								0.006			0.315
18											0.22
19											0.065
20											0.018
21											0.006
MP	0.137	0.061	0.076	0.096	0.093	0.149	0.145	0.117	0.093	0.162	0.091
Combined MP					1.965×10^{-11}						

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Table continued

Alleles	CSF1PO	D7S820	D13S317	D16S539	F13A01	F13B	FESFPS	HPRTB	THO1	TPOX	vWA
Expressed as 1 in 7.3		16.5	13.2	10.5	10.7	6.7	6.9	8.5	10.7	6.2	110
PD	0.863	0.939	0.924	0.904	0.907	0.851	0.855	0.883	0.907	0.838	0.909
PIC	0.66	0.81	0.79	0.77	0.74	0.66	0.65	0.72	0.74	0.66	0.76
PE	0.395	0.745	0.637	0.533	0.59	0.581	0.484	0.161	0.586	0.525	0.781
TPI	1.55	4	2.78	2.11	2.44	2.38	1.89	0.94	2.42	2.07	4.64
Ho	0.6782	0.875	0.8333	0.7632	0.7952	0.7901	0.7349	0.4675	0.7931	0.7586	0.8916
He	0.7116	0.8281	0.8121	0.7978	0.7675	0.7157	0.7078	0.7554	0.7755	0.703	0.7882
PHW	0.238	0.9913	0.5795	0.0545	0.2916	0.569	0.9391	0	0.8621	0.4132	0.4446
Total alleles	174	96	100	76	166	162	166	154	174	174	168

MP, Matching Probability, PD, Power of Discrimination, PE, Power of exclusion; TPI, Typical Paternity index; Ho, Homozygotes (%); He, Heterozygotes (%) PHW, Probability value of significant deviation from Hardy Weinberg Equilibrium

None of the alleles in eleven STR loci exceeded 50% frequency reflecting the usefulness and validity of these loci in calculated paternity indices and discriminating individuals.[12] The high level of heterozygosity observed (range 0.468-0.892) for eleven STR system is an indication of that the Rajput population has a high level of genetic variation and there would be successfully utilized in discriminating between individuals. The results indicate that for the analysed population, all the STR loci met Hardy-Weinberg expectations except locus HPRTB. Significant deviation from HW expectations at HPRTB locus was depicted in probability test. The results revealed deficiencies of heterozygotes at this locus. The number of alleles at many loci viz. CSF1PO, TH01, TPOX, FESFPS, vWA, D7S820 and D16S539 are different those reported for Rajput population from other states (Table 3).

However, the heterozygosity is generally within the range of those reported in Rajput population from other states[7,8] PIC values>0.5 for all STR loci (range 0.65-0.81) indicating that the analysed system is informative and useful for identification purpose. The combined matching probability (MP) of the eleven STR systems was 1.965×10^{-11}, indicating that the system has a much stronger inter-individual discriminating power. The eleven loci showed a combined power of discrimination (PD) of 0.999999. Practically this degree of MP and PD means that no other individual with the same profile for all eleven loci could exist in Rajput population, as these two parameters express the probability of two random DNA profile matching at the loci tested: MP expressed as 1 individual for eleven STR loci is ~ one Trillion is a population that is over 1.24 billion population in country.

Conclusion

All eleven loci were found to be informative and useful for forensic identity testing. The expected heterozygosity and the power of discrimination calculated from the gene frequencies obtained in the population reveal that the combination of 11 STR loci has a high forensic efficiency. In addition, the present study involves the development of forensic databases for indigenous population of Delhi, India.

Acknowledgments

Thanks are accorded to Ministry of Homes, Government of India for financial support. Excellent technical assistance provided by Mr. Pawan Kumar and Mr. Bhagat Singh is duly acknowledged. The work was carried out under the XI FYP project, MHA GOI.

Conflicts of interest

The author declares that there are no conflicts of interest.

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Citation: Chauhan T**,** Kushwaha KPS**,** Chauhan V**.** Genetic polymorphism of eleven STR loci in Rajput population of Delhi, India. *Forensic Res Criminol Int J.* 2015;1(5):192–196. DOI: 10.15406/frcij.2015.01.00031