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^-9, 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

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.1-3 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.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 and linguistic variant groups. Human diversity in India is defined by 4693 different and documented population groups that include 2205 and linguistic variant groups. 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 and linguistic variant groups. There are 6222 different segments and 1900 territorial units spread across the country. Like most other Indians, Rajput is endogamous particularly in north, west and central India. Rajput populations are found in Rajasthan, Gujarat, Delhi, Uttar Pradesh, Himachal Pradesh, Haryana, Jammu, Punjab, Sindh, Uttarakhando, Madhya Pradesh and Bihar. The state of Delhi comprises about 3.8% of the Rajput population.4 A very few number of genetic studies have been carried out on Rajput population.5 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).

| 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                | S-9, 9.3, 10, 11 | 183-199                  |
| TPOX               | 2p25.1-pter            | HUMTH01, Human Thyroid peroxidase gene | AAGT                | Jun-13       | 228-256                  |
| D16S539            | 16q24-pter             | NA                                | AGAT                | 5, 8-15      | 276-396                  |
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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.             | 94°C for 1min.              | None           | 4°C      |
|                   |                    | 64°C for 1min.             | 64°C for 1min.              |                |          |
|                   |                    | 70°C for 1.5mins.          | 70°C for 1.5mins.           |                |          |
| F13B              | 96°C for 2 mins.  | 94°C for 1min.             | 90°C for 1min.              | None           | 4°C      |
|                   |                    | 60°C for 1min.             | 60°C for 1min.              |                |          |
|                   |                    | 70°C for 1.5mins.          | 70°C for 1.5mins.           |                |          |
| STR III Multiplex FFV Multiplex | 96°C for 2 mins. | 94°C for 1min.             | 90°C for 1min.              | 60°C for 30mins. | 4°C      |
| HPRTB             |                    | 60°C for 1min.             | 60°C for 1min.              |                |          |
|                   |                    | 70°C for 1.5mins.          | 70°C for 1.5mins.           |                |          |

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.,\(^\text{10}\) 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\(^\text{11}\) 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\(^\text{12}\) heterozygosity (observed and expected) values were calculated using software Genetix 4.02.\(^\text{13}\) Tests for conformity to Hardy-Weinberg expectations (probability test) were performed through Gene pop 3.3d.\(^\text{14,15}\) Statistical parameters of forensic importance like power of discrimination (PD), polymorphism information content (PIC)\(^\text{16-18}\) matching probability (MP) and paternity indices viz. power of exclusion (PE) and typical

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Results and discussion

Alloleic 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 | Alleles observed in Haryana population
|------------|---------------------------------------------------------------|-----------------------------------|--------------------------------------|-----------------------------------------|
| 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|        | 0.425 |      |      |     |
| 8       | 0.006  | 0.25   | 0.2     | 0.053   | 0.006  | 0.21 | 0.109  |       |      |      |     |
| 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|        |        |         |         | 1.965 x 10^-11 |       |       |       |      |      |     |
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Table continued

| Alleles  | CSF1PO | D7S820 | D13S317 | D16S539 | F13A01 | F13B | FESFPS | HPRTB | THO1 | TPOX | vWA |
|----------|--------|--------|---------|---------|--------|------|--------|--------|-------|------|-----|
| Expressed as 1 in 73 | 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  | 6.44  |
| 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     | 0.8621 | 0.4132 | 0.4446 |
| Total alleles | 174 | 96       | 100 | 76 | 166 | 162 | 166 | 154 | 174 | 168 |

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, THO1, 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 states12PIC 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 1.965x10^-14, 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.

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Conflicts of interest

The author declares that there are no conflicts of interest.

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