Identification of Variant Alleles at CODIS STR loci in Different Populations of India

Tanya Chauhan

LNJN National Institute of Criminology and Forensic Science, Sector-3, Rohini, Delhi, India.

Abstract

Two population’s DNA profiling was screened for variant alleles not included within the allelic ladder provided by the manufacturer of GenePrint STR system (Promega Corporation, Madison, US). A total of 3 variant alleles were identified at 3 out of 12 STR loci tested. This study identified one variant allele at TH01 locus (allele 8.3), one at TPOX locus (allele 14) and one at vWA locus (allele 21). All these alleles have been reported previously in different populations. Allele 8.3 found at TH01 fell within the allelic range while allele 14 found at TPOX and allele 21 found at vWA fell outside the allelic range.

Keywords: Variant Alleles; CODIS STR; Forensics; TPOX; TH01; vWA.

Introduction

Polymorphic Short Tandem Repeat (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 [1, 2]. STR alleles of a questioned individual are designated by comparing with alleles of allelic ladder, provided by manufacturer of commercial kit [3]. Allelic ladder is the combination of alleles based on population genetic variations observed in developmental process. STR alleles of most of the DNA profiles represented with in allelic ladder are found in a small number of individuals.

For proper casework interpretations, the forensic scientists should be aware of the existence of rare alleles so that the variants can be recognised. Correctly designated alleles can sometimes greatly increased power of discrimination of STR analysis. It is, therefore, important that forensic scientists should share information on the occurrence of these variants in different populations.

India is culturally and geographically a highly diverse country. Human Diversity in India is defined by 4693 different documented population groups that include 2205 major communities, 589 segments and 1900 territorial units spread across the country [4]. This diversity is due to different cultural heritage (linguistic, ethnic etc.), history of different waves of migration and impact of deep seated caste systems (or practice of strict endogamy/marriage rule). In this study, 3 variant alleles were found at 3 GenePrint STR loci, viz. TPOX, TH01 and vWA (Promega Corporation, Madison, US) during Indian population studies.

Materials and Methods

Samples and DNA extraction

A total of 244 blood samples were collected from unrelated individuals of two populations namely Rajputs and Ramgarhia-Sikhs from Delhi, India. Genomic DNA from each sample was extracted by Phenol: Chloroform extraction method [5]. Whole blood sample was used as a DNA source. Extracted DNA samples were quantified by using NanoDrop spectrophotometer.

PCR Amplification and STR typing

About 2-5 ng of total DNA was used as template DNA in a total reaction volume of 25 µl for each amplification process. Initially, 244 samples were screened for 12 STR loci namely CSF1PO, D16S539, D7S820, D13S317, F13A01, F13B, FESFPS, HPRTB, LPL, TPOX, TH01 and vWA using GenePrint STR system (Promega Corporation, Madison, US) and polyacrylamide gel electrophoresis.

Thermal cycling parameters were setup according to the manufacturer’s protocol (Promega Corporation, Madison, US). Amplification products (2.5 µl per sample mixed with an equal volume of STR 2X loading solution) were electrophoresed at 50 Watt for 2-4 h on a 40cmX20cm, 0.35mm thick 6% denaturing polyacrylamide gel by manual GenoSequencer (Atto Corporation, Japan) with 0.5X TBE as gel running buffer. The bands were visualized by staining with silver stain [6]. Direct comparison between the al-
allel ladders and amplified samples of the same locus allowed for determination of alleles. Variant alleles were designated according to PCR product size, calculated relative to a molecular marker (pBR322 DNA/MspI digest) with Image master 1D Elite v3.01 (Amersham Biosciences, USA).

The genotype data was compiled for twelve STR markers in Excel sheets to facilitate for statistical treatment for generation of allele frequencies of the 12 STR loci. The Allele frequencies and statistical population parameters were calculated using “PowerStats” Microsoft Excel workbook template provided by Promega Corporation (http://www.promega.com/geneticidtools/).

Results

This study screened a total of 244 individuals of two different Indian populations namely Rajputs and Ramgarh-Sikhs from Delhi, for variant alleles not included in the allelic ladder provided by the manufacturer. DNA samples were initially screened for 12 STR loci by silver stain detection method. A total of 3 variant alleles were recognized at 3 STR loci viz. TPOX, TH01 and vWA and determined by a visual comparison of sample band position relative to those of known to allelic ladder. Each variant allele was confirmed by re-extraction and amplification of each locus. Allelic ladders from kit contain alleles 5-11 at TH01, 6-13 at TPOX, whereas at vWA alleles ranging from 13 to 20 (Table 1). All these variants were not included in the allelic ladder provided by GenePrint STR system amplification kit. All three variants were heterozygotes, paired with well characterized sister allele represented in allelic ladder in each case. The TH01 variant TH01_8.3 (allele 8.3) was found with TH01_9 allele (Figure 1) as heterozygous condition in Ramgarh-Sikh population. While variants TPOX_14 (allele 14 at TPOX) (Figure 2) and vWA_21 (allele 21 at vWA) (Figure 3) were paired with TPOX_9 and vWA_18 alleles, respectively in Rajput population. Variant of TPOX and vWA loci contains an allele that was larger than 4 repeats which was largest in their respective ladder (Table 2).

Discussion

The STRBase website (http://www.cstl.nist.gov/biotech/strbase) lists only two variants TH01_8.3 and TPOX_14. Alleles TH01_8.3 and TPOX_14 have been separately reported 26 and 7 times respectively in STRBase. Allele vWA_21 was not reported previously in STRBase. Variant vWA_21 has previously been reported in Turks and Chinese population with a frequency of 0.004 and 0.006, respectively [7]. While Lins et al. [8] reported this variant in African-Americans with a frequency of 0.002. This variant also been reported in Germans, Moroccans, Japanese and Papuans with a frequency of 0.001, 0.004, 0.004 and 0.009, respectively [9].

### Table 1. Characteristics of STR loci that showed variant alleles.

| STR locus | Chromosome location | Repeat motif | Alleles present on locus | Alleles included in kit | Allele size range |
|-----------|---------------------|--------------|------------------------|------------------------|------------------|
| TH01      | 11p15.5             | AATG         | 5-11                   | 5, 6, 7, 8, 9, 9.3, 10, 11 | 179-203          |
| TPOX      | 2p25.1-pter         | AATG         | 6-13                   | 6, 7, 8, 9, 10, 11, 12, 13 | 224-252          |
| vWA       | 12p12-pter          | AGAT         | 13-20                  | 13, 14, 15, 16, 17, 18, 19, 20 | 139-167          |

Figure 1. Variant TH0 8.3 paired with allele TH0 9 (sample 1) at TH01 locus. 1-3 are the samples amplified and A is the Allelic ladder of respective locus.

Figure 2: Variant TPOX 14 paired with allele TPOX 9 (sample 4 and 5) at TPOX locus. 1-8 are the samples amplified and A is the Allelic ladder of respective locus.
A literature search shows that variant alleles vWA\(_{21}\) and TPOX\(_{14}\) have been reported in different Indian populations also (Table 2).

A literature search shows that variant alleles vWA\(_{21}\) and TPOX\(_{14}\) have been reported in different Indian populations also (Table 2). Variant allele TPOX\(_{14}\) has been reported in a Khandayat (Orissa), Marathas and Brahmins (Maharashtra) populations with a frequency of 0.008, 0.11, 0.008 respectively [16, 17]. Variant allele vWA has been reported in Rajputs (U. P), Reddy and Brahmins (A. P) populations of with a frequency of 0.010, 0.016 and 0.019, respectively [18-20].

Conclusion

This study identified 3 unusual variant alleles at 3 loci that are not represented in commercially available allelic ladder in a database of nuclear STR profiles from India. Variant alleles TH01\(_{8.3}\) and TPOX\(_{14}\) have been separately reported in STRBase. While allele vWA\(_{21}\) was not reported previously in STRBase. The variant allele TH01\(_{8.3}\) was not previously reported in any population of India. Dissemination of the information will assist Forensic DNA analysis and other human identification laboratories in an Awareness of anomalies due to variants off-ladder alleles encountered during analysis.

Table 2. Comparison of Allele frequencies of observed variant alleles in different populations.

| STR locus | Variant alleles (VA) Observed (Population) | No. of VA Observed in Present Study | Frequency of VA observed in Present study (n=no. of samples) | Frequency of VA observed in different populations of India | Frequency of VA observed in other populations | Published sequence of variant alleles |
|-----------|------------------------------------------|----------------------------------|----------------------------------------------------------|----------------------------------------------------------|------------------------------------------|-----------------------------------|
| TH01      | 8.3                                      | 1(Ramgarh Sikhs, Delhi)          | 0.003 (n=157)                                            | -                                                       | -                                          | (AATG)\(_k\), ATG (AATG)\(_k\) |
| TPOX      | 14                                       | 2(Rajputs Delhi)                 | 0.011 (n=87)                                              | 0.008 Khandayat, Orissa [16]                            | 0.004 Chinese [14]                      | (AATG)\(_k\)                       |
|           |                                          |                                  |                                                          | 0.011 Marathas, Maharashtra [17]                         | 0.007 Argentina [15]                    |                                    |
| vWA       | 21                                       | 1(Rajputs, Delhi)                | 0.006 (n=87)                                              | 0.01 Rajput, U. P [18]                                  | 0.004 Turks [7]                         |                                    |
|           |                                          |                                  |                                                          | 0.016 Reddy, A. P [19]                                  | 0.001 Germans [9]                       | TCTA (TCTG)\(_k\), TCTA, TCCAT |
|           |                                          |                                  |                                                          | 0.019 Brahmins, A. P [20]                               | 0.002 Africans-Americans [8]            |                                    |
|           |                                          |                                  |                                                          |                                                          | 0.002 Caucasians [10]                    |                                    |
|           |                                          |                                  |                                                          |                                                          | 0.004 Africans [11]                     |                                    |

Figure 3: Variant vWA\(_{21}\) paired with vWA\(_{18}\) allele (sample 5) at vWA locus. 1-8 are the samples amplified and A is the Allelic ladder of respective locus.

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Acknowledgements

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