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

Research Article

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.

***Corresponding Author**:
Tanya Chauhan,
LNJN National Institute of Criminology and Forensic Science, Sector-3, Rohini, Delhi, India.
E-mail: tanyachauhan@yahoo.com

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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 Ramgaria-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-

lelic 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 Ramgaria-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 Ramgaria-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 Africans-Americans with a frequency of 0.002. This variant also been reported in Germens, Moroccans, Japanese and Papuans with a frequency of 0.001, 0.004, 0.004 and 0.009, respectively [9]. Vari-

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.

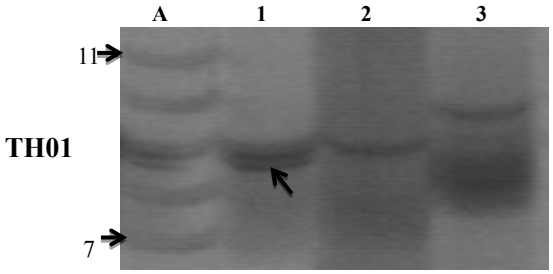

Figure 2: Variant $TPOX_{14}$ paired with allele$TPOX_{9}$ (sample 4 and 5) at TPOX locus. 1-8 are the samples amplified and A is the Allelic ladder of respective locus.

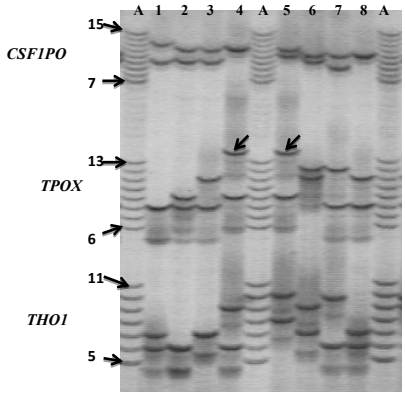

Figure 3: Variant vWA_{21} paired with vWA_{18} allele (sample 5) at vWA locus. 1-8 are the samples amplified and A is the Allelic ladder of respective locus.

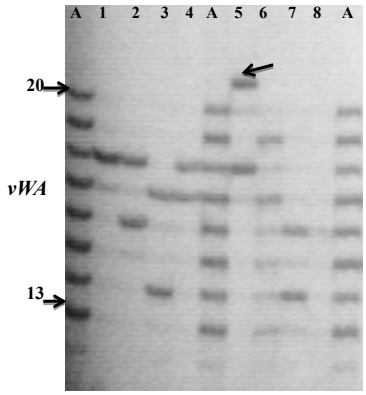

Table 2. Comparison of Allele frequencies of observed variant alleles in different populations.

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
				Frequency	Population	Reference	Frequency	Population	Reference	
TH01	8.3	1(Ramgaria Sikhs, Delhi)	0.003 (n=157)	-	-	-	0.001 0.003 0.05	Germans European-Americans Bosnia	[7] [12] [13]	$(AATG)_5$ ATG $(AATG)_3$
TPOX	14	2(Rajputs Delhi)	0.011 (n=87)	0.008 0.011 0.008	Khandayat, Orissa Marathas, Maharashtra Brahmins Maharashtra	[16] [17] [17]	0.004 0.007	Chinese Argentina	[14] [15]	$(AATG)_{14}$
vWA	21	1(Rajputs, Delhi)	0.006 (n=87)	0.01 0.016 0.019	Rajput, U. P Reddy, A. P. Brahmins, A. P.	[18] [19] [20]	0.004 0.006 0.001 0.009 0.004 0.004 0.002 0.004 0.004 0.005	Turks Chinese Germans Papuans Moroccans Japanese Africans-Americans Caucasians Africans-Americans Hispanics African- Jordanian	[7] [9] [8] [10] [11]	TCTA $(TCTG)_4$ $(TCTA)_{16}$ TCCAT CTTA

ant vWA_{21} also been reported in Caucasian, African-Americans as well as Hispanics [10] with a frequency of 0.002, 0.004 and 0.004, respectively. African-Jordanians population has a frequency 0.005 of vWA_{21} variant allele [11]. Variant allele $TH01_{8.3}$ has been reported with frequency of 0.001 in German population [7]. This variant also been reported in European-Americans and Bosnia population with a frequency of 0.003 and 0.050, respectively [12, 13]. $TPOX_{14}$ has previously been reported in Chinese population with a frequency of 0.004 [14]. Berardi et al. [15] reported this variant allele in Argentina population with a frequency of 0.0071 (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.

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