Gond Population Genetic Database on Four STR loci

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Abstract

Allelic scattering of four Short Tandem Repeats loci, CSF1P0, TPOX, TH01 and FGA was studied in Gond populations of the Madhya Pradesh, India. The forty seven blood samples studied for the present work. For this research samples was collected from Betul. Tribal populations are culturally homogenous were generally tribes practices endogamy. The average homozygosity values for Gond are 0.28. All four STR loci chase Hardy-Weinberg equilibrium except TPOX. STR loci used in this research are very informative and discriminating with Power of discrimination values of all tested loci was above 80%. Highest PD values were obtained by FGA locus in all studied populations about 95%.

Keywords *- CSF1P0, TPOX, TH01, FGA, homozygosity and Hardy-Weinberg equilibrium.*

Introduction

Short tandem repeats (STR) are highly polymorphic repeat sequences of nucleotides, which are plentiful in eukaryotic DNA (Weber and May 1989) [1], (Edwards *et al* 1992) [2]. Indian populations represent a distinct population structure based on the caste system. The most distinctive and elementary character of the Indian population structure is the existence of endogamous sub-castes within many of these castes within any region or linguistic region. The tribal populations are generally marked by high degree of isolation, small effective population size, and high degree of inbreeding, the conditions that are both good and prerequisite for the process of rapid micro-differentiation. They mark that, in most cases, these sub-castes may have developed from the common parental stock, involving different processes of fission (Basu 1969; Malhotra 1978 a, b) [3,4,5]. The caste structure is about 3000 years older (Thapar 1977) [6], the growing history of these subpopulations might have been relatively short even if it is at this level of Mendelian units that the forces of progression mostly operate (Reddy et al., 2001) [7]. There are currently about 530 tribal groups in India (Census of India 2011). Madhya Pradesh (MP) is the second richest Indian state by area, is located in the central part and is homeland of several caste and tribal groups. The tribal groups of are mainly trapper, labours and agronomist. STR locus are normally used in forensic, anthropological and medical research.

Methods

Sampling

The population sample consisted of forty seven healthy, unrelated individuals from Betul. All blood samples were collected after written consent of all subjects.

DNA Isolation

A 1.2 mm punch from a dried sample spot on FTA paper was taken in a PCR tube. 200 µl of FTA purification reagent were added to PCR tube, incubated for 5 minutes at room temperature and then continuously agitated by using a pipette. This process was repeated three times with FTA purification reagent and two times with 100 µl TE-buffer. Finally the entire left TE buffer was removed and discarded. FTA discs were allowed to dry at normal temperature for overnight and were directly used for PCR amplification.

PCR amplification

Multiplexed PCR amplifications of the 5 STR loci: CSF1PO, TH01, TPOX, FGA and Amelogenin was performed using AmpFlSTR® MiniFilerTM PCR amplification kit (Applied Biosystem, Foster city, CA, USA). The PCR reagents have been standardized in the laboratory for uniformity of results. PCR was performed by taking the ½ reaction volume of the manufacturer's recommended protocol (Shrivastava et.al 2013) [8] by using 9700 thermal cycler (Applied Biosystems, USA). For one 1.2 mm washed punch of FTA paper the PCR mix was comprised of Reaction Buffer - 5.0 µL, Primers - 2.5 µL, MQ water - 5.0 µL to make final volume 12.5 µL.

Genotyping of amplified fragments

The Polymerase chain reaction products were genotyped using multicapillary electrophoresis with POP-4 polymer in ABI Prism Avant 3100 Genetic Analyzer (Applied Biosystem, Foster city, CA, USA) according to the manufacturer's protocol provided with the kit and the data was analyzed using Gene Mapper Software v3.5 (Applied Biosystem, Foster city, CA, USA) to designate alleles by comparison with the allelic ladder supplied with the kit. Peak detection threshold was set to 50 RFUs for allele designation. All steps were followed according to protocol of respective kit.

Analysis of data

Allele frequency of the 4 STR loci was calculated by GenAlEx 6.5 software (Peakall and Smouse 2006) [9]. Several forensic parameters, i.e., polymorphism information content (PIC), power of discrimination (PD), power of exclusion (PE), matching probability (Pm) and paternity index (PI) was calculated using the PowerStatsV1.2 worksheet program (Tereba 1999) [10]. Observed heterozygosity (Hobs), Expected Heterozygosity (Hexp) and Hardy–Weinberg equilibrium (HWE) using exact test was calculated using Arlequin v3.5 (Excoffier et al. 2007) [11].

Results and Discussion

The allele frequency distribution observed in studied autosomal STR loci for the Gond population is summarized in Table-1. A total of 27 alleles were observed in Gond population. The range of allele frequency is from 0.020 to 0.390 (Table 1) in which CSF1PO locus from 0.020 to 0.530, for locus TH01 from 0.010 to 0.480, for locus TPOX from 0.010 to 0.210 for locus and for FGA locus from 0.010 to 0.030. TH01 showed maximum allele frequency with allele 9 (0.530) and FGA showed minimum allele frequency at allele 23 (0.210) in Gond population. Forensic parameters including Matching Probability (PM), Power of Discrimination (PD) and Polymorphism Information Content (PIC) for the STR loci CSF1PO, TH01, TPOx and FGA were show in Table-1. All the four STR loci show high degree of PIC value (above 0.5). The high PIC value of selected loci confirmed their usefulness for genetic polymorphism (Imad Hadi *et al*. 2014) [12].

The population wise average H_o/H_e ratio at four STR loci of studied population was calculated as 0.976 for Gond. Eaaswarkhanth *et al*., 2009 [13] reported lower observed heterozygosity values than the

expected heterozygosity for Shia, Sunni Muslims of Uttar Pradesh. This observation was predominantly noticeable for FGA locus, which departed from Hardy-Weinberg expectations with H_o/H_e ratios of 0.70 for Shia and 0.79 for Sunni Muslims, respectively. This indicates the scientific fact that homozygosity at genetic loci increases distinctly in populations practicing consanguinity seems to be reflected in the form of comparatively low observed heterozygosity values for most of the STR Loci and the departure from HWE detected in the Shia and Sunni Muslim population is also due to an excess of homozygous over heterozygous as a result high consanguinity rates reported for these populations (Afzal.1984; Bittle & Hussain, 2000) [14,15]

Table 1: An allele frequency distribution for 4 autosomal STR Loci investigated in a Gond population of M.P.

Allele/n	CSF1PO	TH01	TPOX	FGA
N	50	50	50	50
6		0.180		
7		0.080		
8		0.190	0.260	
9	0.030	0.530	0.170	
10	0.210	0.020	0.080	
11	0.270		0.480	
12	0.390		0.010	
13	0.080			
14	0.020			
18				0.030
19				0.030
20				0.120
21				0.150
22				0.110
23				0.210
24				0.160
25				0.130
26				0.040
27				0.010
28				0.010
PM	0.136	0.116	0.186	0.045
PD	0.864	0.884	0.814	0.814
PIC	0.680	0.700	0.610	0.860
PE	0.428	0.460	0.342	0.755
Ho	0.700	0.660	0.640	0.860
He	0.730	0.650	0.640	0.860
P-value	0.268	0.379	0.003	0.427

Conclusion

In conclusion, a population database has been established for the Gond population of Madhya Pradesh for the four STR loci CSF1PO, TH01, TPOX and FGA. High combined power of discrimination for these loci shows their usefulness for forensic purposes.

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