International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2019; 7 (1):61-69
ISSN:2320-7817(p) | 2320-964X(o)

Original Article Open Access

Genomic diversity in Madhya Pradesh, India: Genetic relationship among three ethnic populations from Madhya Pradesh based on STR polymorphism

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Manuscript details:

Received : 14.01.2019
Accepted : 01.02.2019
Published : 28.03.2019

Editor: Dr. Arvind Chavhan

Cite this article as:
Chaudhary Ruchira and Batav Nitin (2019) Genomic diversity in Madhya Pradesh, India: Genetic relationship among three ethnic populations from Madhya Pradesh based on STR polymorphism, *Int. J. of. Life Sciences*, Volume 7(1): 61-69.

Available online on
http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

Genomic diversity based on 4 STR loci, CSF1P0, TPOX , TH01 and FGA is studied in Gond, Bhil and Brahmin populations of the Madhya Pradesh, India. The 161 blood samples analysed for the present study. For this study samples was collected from Betul (Gond), Bhil and Brahmin (Neemach and Mandsaur). The population wise average H_o/H_e ratio at four STR loci amongst the three studied population was calculated as 0.979 for Gond, 1.016 for Bhil and 1.029 for Brahmin population of Madhya Pradesh. All loci fall under Hardy-Weinberg equilibrium except TPOX. These STR loci were highly 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 and Hardy-Weinberg equilibrium.

INTRODUCTION

Indian populations having a unique population structure based on the caste system and because of its variation considered a natural laboratory for population genetics research, hence the primary focus of such studies. The most unique and fundamental to the Indian population structure is the existence of endogamous sub castes within many of these castes within any region or linguistic area. These sub castes are usually characterized by high degree of isolation, small effective population size, and high degree of inbreeding, the conditions that are both conducive and prerequisite for the process of rapid micro-differentiation. There are indications that, in most cases, these sub castes may have evolved from the common parental stock, involving different processes of fission (Basu, 1969 and Malhotra, 1978 a,b). The evolutionary history of these subpopulations might have been relatively short albeit it is at this level of Mendelian units that the forces of evolution basically operate (Reddy et al., 2001). Indian populations are known for their unique cultural and linguistic diversity (Chaubey, 2010). They vary in size from a few hundred to a few million and speak four major language families belonging to Austroasiatic (AA), Dravidian (DRA),

Indo-European (IE) and Tibeto-Burman (TB). Madhya Pradesh (MP) is the second largest Indian state by area, is located in the central part and is homeland of several caste and tribal groups. It is bordered by the states of Uttar Pradesh in the north, Chhattisgarh in the east, Maharashtra in south, Gujarat in the west and Rajasthan in the northwest. Except for the valleys of the Narmada and the Tapti, Madhya Pradesh consists of a plateau, straddled by the river Narmada and interspersed with the mountains of the Vindhya and the Satpura ranges. It is one of the largest states of India inhabited by the bulk of tribal populations of the country constituting 20.3% of the total tribal populations. There are 46 Scheduled Tribes (ST) (Sharma et al., 2012). The tribal groups of MP are mainly hunter gatherers, labours and farmers and belong to IE, DRA and AA families, which are widely spread language families in India (Chaubey, 2010).

Due to ease of use due to multiplexing, these markers are routinely used in forensic, anthropological and medical studies. With the growing number of laboratories using STR analysis technology, more and more population STR data have been reported (Tandon et al., 2002; Sarkar and Kashyap 2002; Sahoo and Kashyap 2002; Gaikwad and Kashyap 2002; Rajkumar and Kashyap 2004; Narkuti et al., 2008; Dubey et al. 2009; Ghosh et al., 2011; Chaudhari and Dahiya 2014; Giroti and Talwar 2010; Shrivastava et al., 2015a; b).

MATERIAL AND METHODS

Blood Sample Collection

The population sample consisted of 161 healthy, unrelated individuals (Gond-50 from Betul, Bhil-56 from Neemach and Mandsour, Brahmin- 55 Neemach and Mandsour) originating from different geographical regions of Madhya Pradesh. All blood samples were collected after written consent of all subjects.

DNA extraction

A 1.2 mm punch from a dried sample spot on FTA paper was taken in a PCR tube. FTA purification reagent (200 µl) was added to PCR tube, incubated for 5 minutes at room temperature and then continuously agitated by using a pipette. This process was repeated thrice with FTA purification reagent and twice with 100 µl TE-buffer. Finally the entire unspent TE buffer was removed and discarded by pipetting and the disc was allowed to dry at room temp for overnight and was 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 consistency of results. PCR was performed by taking the ½ reaction volume of the manufacturer's recommended protocol (Shrivastava et al., 2013) 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 PCR 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 according to the laboratory internal standards and respective kit controls.

Analysis of data

Allele frequency of the 4 STR loci was calculated by GenAlEx 6.5 software (Peakall and Smouse, 2006). 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 spreadsheet program (Tereba, 1999). Observed heterozygosity (Hobs), Expected Heterozygosity (Hexp) and Hardy–Weinberg equilibrium (HWE) using exact test was calculated using Arlequin v3.5 (Excoffier et al., 2007). Allele frequencies of studied population were compared with other published populations using Fst pair wise distance by Arlequin v3.5 software (Excoffier et al., 2007). Nei's genetic distances (Nei, 1972) among compared populations were derived and subsequently used to generate a Neighbour joining (NJ) dendrogram using POPTREE2 program (Takezaki et al., 2010).

RESULT AND DISCUSSION

The allele frequency distribution observed in studied autosomal STR loci for the Gond, Bhil and Brahmin population and statistical analysis of forensic parameters are shown is summarized in Table-1, Table-2 and Table-3 respectively. A total of 27 alleles were observed in Gond population. The corresponding allele frequency ranging from 0.020 to 0.390 in (Table 1) in which CSF1PO locus, from 0.020 to 0.530 for locus TH01, from 0.010 to 0.480 for locus TPOX, and 0.010 to 0.210 for locus FGA. TH01 showed maximum allele frequency with allele 9 (0.530) and FGA showed minimum allele frequency at allele 23 (0.210) in Gond population.

A total of 26 alleles were observed in Bhil population within the corresponding allele frequency ranging from 0.009 to 0.411 for locus CSF1PO, from 0.000 to 0.500 for locus TH01, from 0.009 to 0.527 for locus TPOX, and from 0.009 to 0.188 for locus FGA (Table 2). Locus CSF1PO, showed the maximum frequency of allele 12 (0.411) and minimum of allele 14 (0.009); locus TH01, showed maximum frequency of allele 9 (0.500) and minimum of allele 7 (0.054); TPOX showed the maximum frequency of allele 11 (0.527) and minimum of allele 12 and 13 (0.009); total 9 alleles ranging from 19 to 27 were observed on locus FGA, with the maximum frequency of allele 24 (0.188) and the minimum of allele 27 (0.009).

Table 1: An allele frequency distribution for 4 autosomal STR Loci investigated in an 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

Table 2: An allele frequency distribution for 4 autosomal STR Loci investigated in Bhil population of M.P.

Allele/n	CSF1PO	TH01	TPOX	FGA
N	**56**	**56**	**56**	**56**
6		0.295		
7		0.054		
8		0.152	0.268	
9	0.027	**0.500**	0.143	
10	0.161	0.000	0.045	
11	0.339		**0.527**	
12	**0.411**		0.009	
13	0.054		0.009	
14	0.009			
18				
19				0.089
20				0.161
21				0.179
22				0.107
23				0.179
24				**0.188**
25				0.054
26				0.036
27				0.009
PM	0.172	0.124	0.190	0.048
PD	0.828	0.876	0.810	0.810
PIC	0.631	0.700	0.570	0.837
PE	0.345	0.709	0.322	0.745
Ho	0.693	0.643	0.634	0.859
He	0.643	0.750	0.625	0.857
P-value	0.255	0.403	0.551	0.906

A total of 31 alleles were observed in Brahmin population within the corresponding allele frequency ranging from 0.009 to 0.409 for locus CSF1PO, from 0.009 to 0.300 for locus TH01, from 0.027 to 0.391 for locus TPOX, and from 0.009 to 0.182 for locus FGA (Table 3). Locus CSF1PO, showed maximum frequency of allele 12 (0.409) and minimum of allele 9.2 (0.009); on locus TH01 the maximum frequency of allele 6 (0.300) and minimum of allele 10 (0.009); on locus TPOX with the maximum frequency of allele 8 (0.391) and minimum of allele 12 (0.027); on locus FGA, with the maximum frequency of allele 24 (0.182) and the minimum of allele 23.2 & 27 (0.009). The observed variations in the allele frequency of studied populations are may be due to random genetic drift or admixture (Namita Mukherje et al., 2000).

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 1,2 and 3. 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.)

The population wise average H_o/H_e ratio at four STR loci amongst the three studied population was calculated as 0.976 for Gond, 1.016 for Bhil and 1.029 for Brahmin population of Madhya Pradesh (Table 5) Observed heterozygosity of present study is further compared with previously published population data to get a clear image of inter population diversity (Table 6). These values are not significantly varied from the expected ratio of 1.

Table 3: An allele frequency distribution for 4 autosomal STR Loci investigated in Brahmin population of M.P.

Allele/n	CSF1PO	TH01	TPOX	FGA
N	**55**	**55**	**55**	**55**
6		**0.300**		
7		0.173		
8		0.145	**0.391**	
9	0.018	0.255	0.118	
9.2	0.009			
9.3		0.118		
10	0.218	0.009	0.118	
11	0.209		0.345	
12	**0.409**		0.027	
13	0.091			
14	0.045			
18				0.018
19				0.091
20				0.064
21				0.136
21.2				0.018
22				0.155
22.2				0.018
23				0.127
23.2				0.009
24				**0.182**
25				0.136
26				0.036
27				0.009
PM	0.123	0.089	0.164	0.047
PD	0.877	0.911	0.836	0.836
PIC	0.690	0.750	0.650	0.860
PE	0.502	0.566	0.566	0.777
Ho	0.737	0.787	0.705	0.882
He	0.571	0.816	0.946	0.363
P-value	0.571	0.816	0.946	0.363

Table 4: The estimates of pairwise F_{ST} distance and corresponding P- value of the present Brahmin, Gond and Bhil populations of M.P.

	Brahmin	Gond	Bhil
Brahmin	*	**0.0076**	0.0000
Gond	0.0020	*	**0.0131**
Bhil	0.0039	0.0035	*

Table 5: Locus and population wise estimates of heterozygosity of the studied population of M.P.

Observed Heterozygosity (H_o) and expected Heterozygosity (H_e) at four loci					
Locus	**CSF1PO**	**TH01**	**TPOX**	**FGA**	**Average (H_o/H_e)**
Gond Population					
H_o	0.700	0.660	0.640	0.860	0.715
H_e	0.730	0.650	0.673	0.869	0.730
H_o/H_e	0.959	1.015	0.951	0.990	0.979
Bhil Population					
H_o	0.643	0.750	0.625	0.857	0.718
H_e	0.693	0.643	0.634	0.859	0.707
H_o/H_e	0.928	1.166	0.986	0.998	1.016
Brahmin Population					
H_o	0.745	0.782	0.782	0.891	0.800
H_e	0.737	0.787	0.705	0.882	0.777
H_o/H_e	1.011	0.994	1.109	1.010	1.029

Table 6: Comparison of observed heterozygosity for the studied population with earlier reports.

Sample location	Group	CSF1PO	TH01	TPOX	FGA
M.P. (Present study)	Gond	0.700	0.660	0.640	0.860
	Bhil	0.643	0.750	0.625	0.857
	Brahmin	0.745	0.782	0.782	0.891
M. P. (Dubey *et al*.,2009)	Gond	0.703	0.742	0.707	0.904
	Brahmin	0.766	0.791	0.729	0.873
Gujarat (Chaudhari *et al*., 2014)	Bhil	0.723	0.667	0.678	0.865
South West India (Revathi, *et al* .,2004)	Brahmin	0.723	0.815	0.707	0.861
	Lingayat	0.734	0.785	0.581	0.894
	Gowda	0.745	0.678	0.542	0.803
	Muslim	0.733	0.688	0.555	0.911
Bengal (Singh *et al*., 2006)	Lodha	0.768	0.243	0.002	0.020
	Kora	0.678	0.610	0.780	0.932
	Kamali	0.745	0.588	0.647	0.784
	Maheli	0.755	0.898	0.714	0.878
North India (Tandon *et al*., 2004)	Jat	0.792	0.76	0.691	0.833
	Kurmi	0.801	0.681	0.52	0.966
Sikkim (Guha *et al*., 2005)	Nepali	0.742	0.715	0.634	0.800
	Bhuti	0.750	0.657	0.656	0.563
	Lepcha	0.660	0.432	0.546	0.523

Table 6: Continued…

Sample location	Group	CSF1PO	TH01	TPOX	FGA
Arunachal Pradesh (Krithika *et al*., 2005)	Adi Pasi	0.670	0.621	0.555	0.795
Eastern India (Kashyap *et al*., 2004)	Kayastha	NA	NA	NA	0.095
	Brahmin	NA	NA	NA	0.188
	Garo	NA	NA	NA	0.136
	Meitei	NA	NA	NA	0.496
	Naga	NA	NA	NA	0.140
	Kuki	NA	NA	NA	0.054
	Hmar	NA	NA	NA	0.049
	Muslim	NA	NA	NA	0.552
M.P. (Kashyap , *et al* .,2004)	Caucasoid	0.723	0.749	0.666	0.821
	Mangolid	0.739	0.680	0.601	0.735
	Australoid	0.639	0.694	0.656	0.794
North West Punjab (Kaur *et al*., 2014)	Jat Sikh	0.5893	0.6518	0.6429	NA
	Majbi Sikh	0.6961	0.7941	0.7451	NA
	Brahmin	0.6522	0.7463	0.7246	NA
	Ramdasia Sikh	0.7692	0.8462	0.7692	NA

*Indicates the studied population.

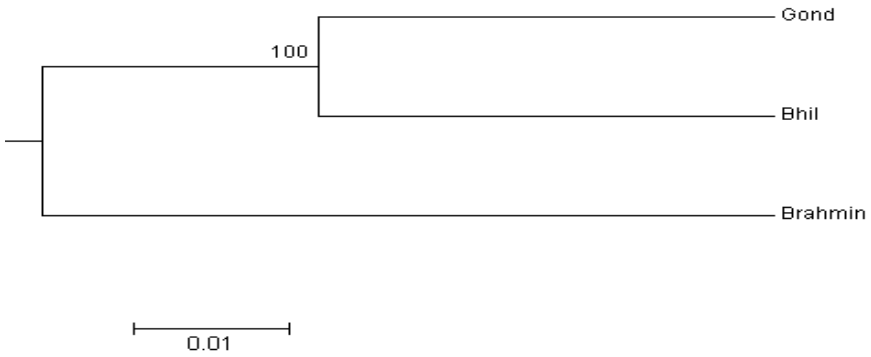

Figure 1: Dendogram based on genetic distance D showing a genetic relationship among the Gond, Bhil and Brahmin population of M.P. based on four autosomal STR loci

Eaaswarkhanth et al., 2009 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). Kashyap *et al*., 2004 reported observed heterozygosity (H_o) values ranging between 0.729 (central) and 0.784 (west) and analyzed five geographical regions of India. They reported variation

between 0.739 (Dravidian) and 0.797 (Austro-Asiatic) in the linguistic groups. In the same study the range of average heterozygosity was found in the three ethnic groups varied between 0.750 (Mongoloid) and 0.773 (Indo-Caucasoid). Highest average heterozygosity was observed amongst the population of Caucasoid origin, occupying the north and west regions, this confirms high allelic diversity in north Indian population of Caucasoid ethnic origin and Indo-European linguistic affiliation (Kashyap *et al*., 2004). Inter population variation has already been reported from the other central Indian populations (Gaikwad *et al*., 2002; Dubey *et al*., 2009; Sharma *et al*., 2012). Lanchbury, *et al*., 1996 provided (H) average heterozygosities estimates for various Hindu and Muslim groups of Uttar Pradesh in the range of 0.303-0.319. Kashyap, *et al*., 2004 reported lowest average heterozygosity among the population of central India (0.729) and highest among the population from north (0.777) and west (0.784) region of the country. The average heterozygosity value reported for Bharia, Bhil and Saharia of M.P. (range 0.2325-0.3374) by Sharma *et al*., (2012) were found to be very low in comparison to the four tribal population from adjoining state Gujarat (range 0.305-0.309) in a study by Bhasin *et al*., 1985. The average heterozygosity values in the present study for Gond, Bhil and Brahmin of Madhya Pradesh were 0.715, 0.718 and 0.8 respectively. Which is slightly low then reported earlier in tribal populations of M.P. As well as for other reported populations from north and central India (Kashyap *et al*., 2002; Kashyap *et al*., 2004; Dubey *et al*., 2009). But higher than reported by Sharma *et al*., 2012. Bhil population in the present study show less average Heterozygosity in compare to Bhil population of Gujarat (Chaudhari *et al*., 2014). Brahmin population of Madhya Pradesh showed high value of average heterozygosity in comparison with the earlier report of Dubey *et al*., 2009 and Kaur *et al*., 2014. Heterozygosity value of Brahmin population is observed comparatively higher than the Gond and Bhil of Madhya Pradesh, suggesting that the Brahmin population is more out breeding than Gond and Bhil population of Madhya Pradesh.

CONCLUSION

Fst (Table 4) value between Brahmin and Gond population is 0.0076. Fst value between Gond and Bhil is 0.0131 which indicate Gond and Bhil populations are genetically close as compare to Brahmin population. The clustering of Bhil & Gond as one shows close affinity between them.

Acknowledgement

Authors are thankful to Director and Dr. Pankaj Shrivastava State Forensic Science Laboratory, Sagour, MP, India for providing genotyping facility. Authors also thankful for all subjects who gave blood samples for this study.

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