Genetic polymorphism and phylogenetic analysis of 15 autosomal STR markers in the Santal indigenous population of Bangladesh

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ABSTRACT: Fifteen autosomal STR markers, namely D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA were typed using AmpF*l*STR® Identifiler® Plus PCR amplification systems in 132 unrelated Santal individuals of Bangladesh. Forensic efficiency parameters like, matching probability (MP), power of discrimination (PD), polymorphism information content (PIC), power of exclusion (PE), typical paternity index (TPI), observed heterozygosity (H_{obs}), and expected heterozygosity (H_{exp}) were calculated for all the loci. No deviations from Hardy-Weinberg equilibrium were detected for the loci after Bonferroni correction. The combined matching probability (MP), combined power of discrimination (PD) and combined power of exclusion (PE) for the 15 tested STR markers were 8.38 x 10^{-17}, 0.999999998 and 0.0.999993866, respectively. A comparison of the locus wise allele frequencies of autosomal STR data of the Santal population with the published geographically close population data based on Nei's genetic distance revealed that the Santal population is closely related to Munda population from Jharkhand, India.

KEYWORDS: Autosomal STRs, forensic parameters, polymorphism, phylogenetic tree, matching probability, power of discrimination.

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Introduction

Bangladesh is a South Asian country and inhibited by more than 45 indigenous populations. Being ethnically homogeneous the mainstream Bengalis comprise 98% of the population. The remainder is mostly indigenous ethnic minorities living in different pockets of the hilly zone as well as some plain lands of the country and covers about 2% of the population. The Santal is one of the major aborigines that belong to Austric-speaking Proto-Australoid race[1]. They predominantly live in different districts of Rajshahi and Rangpur division bordering with West Bengal of India.

Shot tandem repeats (STRs) or microsatellites are tandemly repeated DNA sequences involving 2-7 bp in length and spread over the entire human genome. Because of their polymorphic nature and high mutation rates STRs are widely used in population genetic study to infer population structure, migration pattern and genetic affinities among continental as well as between contiguous populations[2,3]. Besides, autosomal STRs have now become an indispensable tool in forensic applications such as, personal identification, parentage testing and complex kinship studies. In this study we report allele frequencies of 15 autosomal STR loci in the Santal population of Bangladesh and evaluation of different forensic efficiency parameters. The genetic distance or genetic affinity of this population with 28 other geographically close population was also studied by comparing the genetic data published elsewhere.

Materials and methods

Sample collection

Buccal swab samples were collected from randomly selected unrelated individuals from the Santal tribal population in Dinajpur district of Bangladesh with written informed consent of the donors and following procedures that are in accordance with Helsinki declaration of 1975, revised in 1983[4].

Genomic DNA extraction and quantitation

The genomic DNA was extracted using the Chelex® 100 method[5]. Concentrations and purity of the extracted genomic DNA samples were quantified by using NanoDrop-1000 Spectrophotometer (NanoDrop Technologies, Inc, Washington DE 19810, USA). Due to high concentration of DNA obtained from the saliva, about 1.0 µL of genomic DNA was diluted 100 times with ddH_2O (1:99 µL ddH_2O) prior to the spectrophotometric measurement.

Multiplex PCR amplification and genotyping

About 1.0 ng of genomic DNA was used for multiplex PCR amplification of 15 autosomal STR markers using AmpF*l*STR® Identifiler® Plus Kit (Applied Biosystems, Foster City, CA, USA). The reaction was carried out in a Veriti® Thermal Cycler. The thermal cycling parameters were employed according to the protocol provided by the manufacturer (Applied Biosystems, Foster City, CA, USA). About 1.0 µL of each amplicon was mixed with 8.5 µL of Hi-Di™ Formamide and 0.5 µL of GeneScan™ 600 LIZ® Size Standard. Then amplicons were separated and typed in a 3500

Genetic Analyzer using POP-4 polymer and Data Collection Software v1.0 (Applied Biosystems). A peak detection threshold of 50 RFU was used for allele designation using GeneMapper ID-X™ software v1.2. Allele call at all STR markers was determined by comparing to AmpF*l*STR® Identifiler® Plus Allelic Ladder.

Population samples

A Neighbor-Joining (N-J) tree was illustrated using pairwise Nei's genetic distance by comparing the allele frequencies for the 15 autosomal microsatellite markers of the studied Santal population with 28 previously published populations (Table 3). For inter-population comparison, four reference population were selected from Bangladesh, twenty from India, one from Pakistan, one from Nepal, one from China, and one from Uganda. The selected populations and sample size were as follows: Santal (n= 132), Dinajpur-Bangladesh; Bengali (n=595), Dhaka-Bangladesh[6]; Chakma (n=113), Tripura (n=83), and Marma (n=83), Chittagong Hill Tracts-Bangladesh[7]; Karmali (n=102), Kora (n=118), Maheli (n=98) and Lodha (n=198), Mahishya (n=120), Bauri (n=108) and Namasudra (n=110), Rajbanshi (n=91), Paliya (n=107) and Dhimal (n=66), West-Bengal India8-[8-10]; Munda (n=68), Jharkhand-India[11]; Bhil (n=297), Gujarat-India[12]; Gond (n=89) and Brahmin (n=110), Madhya Pradesh-India[13]; Adi Pasi (n=406), Aarunachal Pradesh-India[14]; Balmiki (n=62), Punjab-India[15]; Mahadev Koli (n=65), Maharashtra-India[15]; Iyengar (n=67), Kurumans, (n=67) and Tamil (n=272), Tamilnadu-India[15-16]; Nepalese (n=233), Nepal[17]; Sindhi (n=181), Sindh-Pakistan[18]; Han (n=208), Henan-central China[19]; and Karimojong (n=218), Karamoja-Uganda[20].

Statistical analysis

Allele frequencies and forensic efficiency parameters at all microsatellite markers were calculated by using Microsoft Excel spreadsheet PowerStats Workbook v1.2[21]. The Hardy-Weinberg equilibrium analysis, calculation of observed and expected heterozygosity, and *p*-values for all loci were calculated using Arlequin Software v3.5[22]. A Neighbor-Joining (N-J) tree was constructed based on Nei's genetic distance by using POPTREE2 software[23].

Quality control

AmpF*l*STR® Control DNA 9947A (0.1 ng/µL) was used in every run as positive controls and deionized water instead of template DNA was used as a negative control to check for any DNA contamination.

Results and discussions

The allele frequency distribution at 15 STR loci and forensic efficiency parameters for the studied population are presented in Table 1 & 2.

Table 1. Allele frequencies of 15 autosomal STR markers for Santal population in Bangladesh (n = 132).

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
6	-	-	-	-	-	0.102	-	-	-	-	-	-	-	-	-
7	-	-	0.008	-	-	0.136	0.011	-	-	-	-	-	-	-	-
8	-	-	0.345	-	-	0.091	0.409	0.064	-	-	-	0.299	-	0.004	-
8.3	-	-	-	-	-	0.004	-	-	-	-	-	-	-	-	-
9	-	-	0.045	0.011	-	0.576	0.072	0.136	-	0.011	-	0.155	-	0.087	-
9.3	-	-	-	-	-	0.083	-	-	-	-	-	-	-	-	-
10	0.136	-	0.106	0.129	-	-	0.083	0.083	-	0.008	-	0.121	0.011	0.121	-
11	0.091	-	0.326	0.303	-	0.004	0.273	0.356	-	0.034	-	0.405	-	0.341	-
11.2	-	-	-	-	-	-	-	-	-	0.004	-	-	-	-	-
12	0.049	-	0.140	0.473	-	-	0.114	0.197	-	0.091	-	0.015	0.053	0.295	-
13	0.053	-	0.030	0.068	-	-	0.023	0.117	-	0.318	-	0.004	0.152	0.140	-
13.2	-	-	-	-	-	-	-	-	-	0.011	-	-	-	-	-
14	0.205	-	-	0.011	0.049	-	0.015	0.045	-	0.242	0.163	-	0.242	0.011	-
14.2	-	-	-	-	-	-	-	-	-	0.027	-	-	-	-	-
15	0.337	-	-	-	0.337	-	-	-	-	0.148	0.068	-	0.242	-	-
15.2	-	-	-	-	-	-	-	-	-	0.087	-	-	-	-	-
16	0.106	-	-	0.004	0.269	-	-	-	-	0.015	0.136	-	0.201	-	-
16.2	-	-	-	-	-	-	-	-	-	0.004	-	-	-	-	-
17	0.023	-	-	-	0.239	-	-	-	0.087	-	0.205	-	0.061	-	-
18	-	-	-	-	0.098	-	-	-	0.182	-	0.330	-	0.015	-	0.030
19	-	-	-	-	0.008	0.004	-	-	0.193	-	0.091	-	-	-	0.068
20	-	-	-	-	-	-	-	-	0.110	-	0.008	-	0.004	-	0.170
21	-	-	-	-	-	-	-	-	0.087	-	-	-	0.011	-	0.064
21.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.019

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
22	-	-	-	-	-	-	-	-	0.042	-	-	-	0.008	-	0.110
22.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.038
23	-	-	-	-	-	-	-	-	0.197	-	-	-	-	-	0.235
23.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.004
24	-	-	-	-	-	-	-	-	0.064	-	-	-	-	-	0.152
24.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.011
25	-	-	-	-	-	-	-	-	0.027	-	-	-	-	-	0.072
26	-	-	-	-	-	-	-	-	0.011	-	-	-	-	-	0.019
27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.008
28	-	0.102	-	-	-	-	-	-	-	-	-	-	-	-	-
28.2	-	0.004	-	-	-	-	-	-	-	-	-	-	-	-	-
29	-	0.246	-	-	-	-	-	-	-	-	-	-	-	-	-
29.2	-	0.004	-	-	-	-	-	-	-	-	-	-	-	-	-
30	-	0.091	-	-	-	-	-	-	-	-	-	-	-	-	-
30.2	-	0.023	-	-	-	-	-	-	-	-	-	-	-	-	-
31	-	0.053	-	-	-	-	-	-	-	-	-	-	-	-	-
31.2	-	0.193	-	-	-	-	-	-	-	-	-	-	-	-	-
32	-	0.015	-	-	-	-	-	-	-	-	-	-	-	-	-
32.2	-	0.189	-	-	-	-	-	-	-	-	-	-	-	-	-
33.2	-	0.080	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2. Forensic efficiency parameters of 15 autosomal STR markers for Santal population in Bangladesh (n = 132).

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
MP	0.071	0.049	0.100	0.183	0.117	0.172	0.114	0.074	0.042	0.069	0.073	0.137	0.064	0.113	0.037
PD	0.929	0.951	0.900	0.817	0.883	0.828	0.886	0.926	0.958	0.931	0.927	0.863	0.936	0.887	0.963
PIC	0.780	0.820	0.700	0.610	0.700	0.590	0.690	0.760	0.840	0.770	0.760	0.660	0.790	0.720	0.850
PE	0.563	0.536	0.390	0.497	0.563	0.347	0.484	0.549	0.563	0.662	0.549	0.447	0.605	0.633	0.722
TPI	2.280	2.130	1.530	1.940	2.280	1.400	1.890	2.200	2.280	3.000	2.200	1.740	2.540	2.750	3.670
H_{obs}	0.780	0.758	0.667	0.735	0.795	0.644	0.735	0.773	0.780	0.833	0.773	0.712	0.803	0.818	0.864
H_{exp}	0.804	0.840	0.743	0.666	0.747	0.627	0.735	0.789	0.861	0.803	0.794	0.708	0.815	0.759	0.866
P_{HWE}	0.269	0.007	0.028	0.973	0.921	0.753	0.518	0.332	0.004	0.873	0.274	0.607	0.396	0.980	0.533

MP: Matching probability; ***PD***: Power of discrimination; ***PIC***: Polymorphic information content; ***PE***: Power of exclusion; ***TPI*:** Typical paternity index; H_{obs}: Observed heterozygosity; H_{exp}: Expected heterozygosity; P_{HWE}: *p* values of the exact test for the Hardy Weinberg equilibrium after Bonferroni correction (P<0.05/15=0.0033).

A total of 44 alleles were observed in the Santal population with corresponding allele frequencies ranging from 0.004 to 0.576 (Table 1). Allele 9 at TH01 locus was found to be the most frequent allele followed by allele 12 at CSF1P0. A total of six microvariants and rare alleles were observed in loci TH01 (8.3), D19S433 (11.2) and FGA (21.2, 22.2, 23.2, and 24.2) in the studied Santal population. The overall sample size although was not very high (n=132), we checked the Hardy-Weinberg equilibrium (HWE) by Fisher's exact test. Among the studied loci, no significant deviation from Hardy-Weinberg expectations were observed after Bonferrroni correction[24]. Due to limited access to the specific tribal population it extremely difficult to improve the sample size. A high degree of heterozygosity (>7.0) was observed in all loci except for the locus D7S820 (0.6666) and TH01 (0.6439). The most informative locus was FGA (PIC = 0.850), while the least informative locus was CSF1PO (PIC = 0.610). The combined power exclusion (PE) and combined power of discrimination (PD) for all 15 STR loci was 0.999993866 and 0.999999998 respectively. The combined matching probability (PD) was found to be 8.38×10^{-17}. The obtained MP and PE values demonstrated that the combination of 15 autosomal STR have high forensic efficiency in kinship analysis, personal identification and in anthropological studies in Santal population.

A Neighbor-Joining (N-J) tree was constructed based on pairwise Nei's genetic distance by comparing the allele frequencies of the 15 studied loci (Table 3) with 28 other published geographically close population namely, Bengali (Dhaka-Bangladesh), Chakma, Tripura and Marma (Chittagong Hill Tracts-Bangladesh), Karmali, Kora, Maheli, Lodha, Mahishya, Bauri and Namasudra, Rajbanshi Paliya and Dhimal (West-Bengal India), Munda (Jharkhand-India), Bhil (Gujarat-India), Gond and Brahmin (Madhya Pradesh-India) Adi Pasi (Aarunachal Pradesh-India), Balmiki (Punjab-India), Mahadev Koli (Maharashtra-India), Iyengar, Kurumans, and Tamil (Tamilnadu-India), Nepalese (Nepal), Sindhi (Sindh-Pakistan), Han (Cntral China) and Karimojong (Uganda).

Table 3. Nei's genetic distances between Santal population and 28 other published populations.

POPULATION	Santal	Bengali	Chakma	Tripura	Marma	Bhil	Gond	Brahmin	Adi Pasi	Munda	Balmiki	Mahadev Koli	Iyengar	Kurumans	Karmali	Kora	Maheli	Lodha	Mahishya	Bauri	Namasudra	Rajbanshi	Paliya	Dhimal	Tamil	Nepalese	Sindhi	Han	Karimojong
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	**0.046**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0.022	0.012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0.024	0.011	-0.002	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0.022	0.011	-0.004	-0.004	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0.021	0.020	0.03	0.028	0.031	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0.020	0.018	0.023	0.026	0.021	0.031	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0.025	0.016	0.023	0.019	0.024	0.019	0.026	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0.027	0.024	0.032	0.036	0.03	0.034	0.034	0.039	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	**0.018**	0.010	0.018	0.018	0.016	0.029	0.025	0.028	0.031	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	**0.012**	0.004	0.012	0.010	0.013	0.016	0.018	0.017	0.027	0.012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	**0.019**	0.004	0.011	0.011	0.010	0.021	0.018	0.019	0.026	0.010	0.005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0.021	0.003	0.010	0.010	0.009	0.027	0.021	0.018	0.028	0.011	0.012	0.009	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0.027	0.013	0.011	0.015	0.013	0.031	0.027	0.022	0.031	0.019	0.019	0.013	0.013	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0.032	0.021	0.016	0.017	0.013	0.034	0.035	0.034	0.033	0.029	0.025	0.024	0.022	0.018	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	**0.003**	0.005	0.011	0.013	0.012	0.016	0.008	0.010	0.022	0.010	0.005	0.011	0.008	0.014	0.022	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	**0.014**	0.003	0.014	0.011	0.013	0.017	0.017	0.014	0.021	0.009	0.003	0.005	0.009	0.016	0.021	0.003	0	0	0	0	0	0	0	0	0	0	0	0	0
18	**0.012**	0.007	0.015	0.012	0.014	0.015	0.015	0.017	0.026	0.009	0.005	0.010	0.016	0.020	0.025	0.001	0.001	0	0	0	0	0	0	0	0	0	0	0	0
19	0.025	0.005	0.017	0.013	0.013	0.026	0.024	0.017	0.027	0.012	0.011	0.007	0.010	0.014	0.024	0.012	0.007	0.014	0	0	0	0	0	0	0	0	0	0	0
20	0.041	0.032	0.02	0.028	0.022	0.042	0.046	0.044	0.043	0.038	0.03	0.029	0.030	0.027	0.024	0.032	0.034	0.036	0.038	0	0	0	0	0	0	0	0	0	0
21	0.025	0.004	0.013	0.010	0.014	0.023	0.017	0.017	0.028	0.012	0.006	0.005	0.008	0.017	0.027	0.008	0.003	0.011	0.002	0.040	0	0	0	0	0	0	0	0	0
22	**0.018**	0.008	0.021	0.022	0.019	0.023	0.019	0.018	0.031	0.016	0.008	0.008	0.013	0.019	0.029	0.007	0.007	0.010	0.011	0.040	0.009	0	0	0	0	0	0	0	0
23	**0.017**	0.005	0.017	0.013	0.014	0.023	0.017	0.018	0.027	0.011	0.004	0.005	0.012	0.019	0.023	0.005	0.002	0.004	0.008	0.037	0.003	0.008	0	0	0	0	0	0	0
24	**0.014**	-0.001	0.009	0.008	0.007	0.017	0.015	0.014	0.023	0.005	-0.002	0.000	0.005	0.014	0.016	0.003	-0.003	0.005	0.001	0.027	-0.002	0.005	0.000	0	0	0	0	0	0
25	0.022	0.006	0.013	0.010	0.017	0.024	0.025	0.023	0.032	0.014	0.003	0.006	0.018	0.021	0.029	0.010	0.002	0.005	0.011	0.035	0.006	0.017	0.005	0.000	0	0	0	0	0
26	**0.018**	0.007	0.011	0.008	0.008	0.023	0.024	0.019	0.029	0.014	0.009	0.006	0.01	0.015	0.021	0.011	0.007	0.012	0.009	0.028	0.010	0.012	0.009	0.003	0.01	0	0	0	0
27	0.033	0.008	0.018	0.016	0.02	0.035	0.034	0.024	0.040	0.021	0.015	0.016	0.004	0.024	0.030	0.019	0.014	0.019	0.015	0.039	0.010	0.022	0.017	0.009	0.013	0.017	0	0	0
28	0.024	0.015	0.008	0.015	0.011	0.031	0.030	0.029	0.033	0.022	0.017	0.015	0.015	0.015	0.018	0.017	0.018	0.022	0.020	0.018	0.021	0.024	0.023	0.009	0.020	0.012	0.025	0	0
29	**0.059**	0.037	0.04	0.035	0.034	0.061	0.053	0.049	0.060	0.042	0.041	0.036	0.038	0.042	0.045	0.040	0.037	0.041	0.030	0.06	0.029	0.038	0.037	0.031	0.043	0.036	0.038	0.052	0

Note: Boldface font indicates the smallest or largest genetic distances (below diagonal) from Santal population.

The analysis showed that the Santals lie genetically close to the Munda population belonging to a cluster which includes Kora, Lodha, Karmali, Maheli, Gond, Bhil, Tamil, and Mahadev Koli from different states of India (Fig 1).

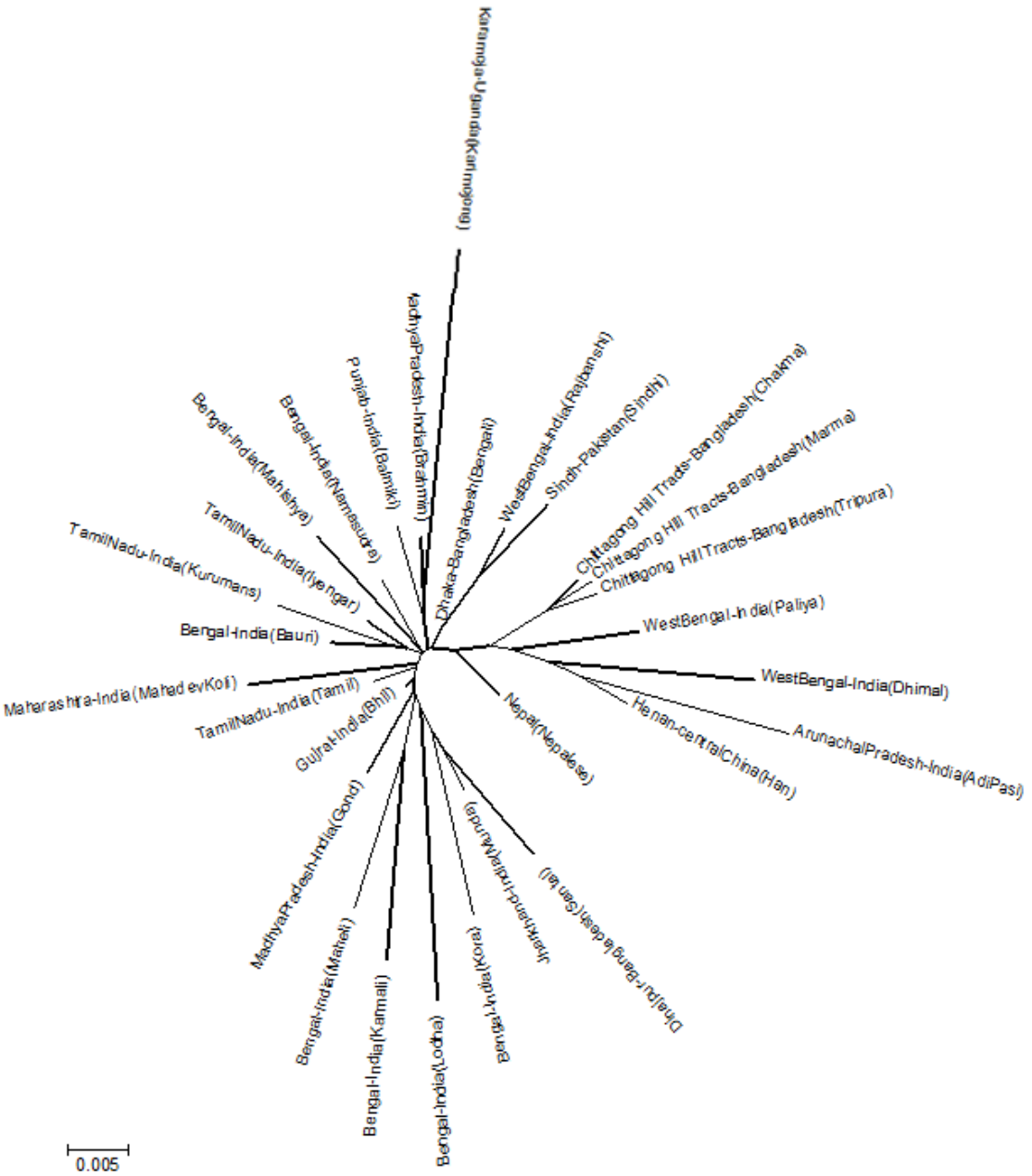

Figure 1. Neighbor-joining tree bases on Nei's DA distance among Santals and 28 other geographically close populations.

A previous study conducted from this laboratory based on Y-chromosomal STRs also revealed a close genetic relationship between Santal population from Dinajpur, Bangladesh and Munda population from Jharkhand, India[25]. The genetic affinity of Santal population with these populations may be due to a possible sharing of paternal lineage and genes during migration.

In conclusion, this study demonstrated that the 15 autosomal STR loci reported here offer a highly discriminating system for use in parentage testing and forensic identification of individuals in this population. From population genetic point of view, the Santals are closely related to Munda population from Jharkhand, India. On the other hand, they are relatively isolated and showed significant genetic variation from the Bengali mainstream population and other tribal populations belonging to the mongoloid stock such as Chakma, Tripura and Murma from Bangladesh as well as Han Chinese from central China; Brahmin from Madhya Pradesh, India; Nepalese from Nepal; and Karimojong tribe from Uganda.

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