

FOR THE RECORD

Kumarasamy Thangaraj,¹ Ph.D.; Gyaneshwer Chaubey,¹ M.Sc.; Vijay Kumar Singh,¹ M.Sc.; Alla G. Reddy,¹ M.Sc.; P. P. Pavate,² Ph.D.; and Lalji Singh,¹ Ph.D.

Genetic Profile of Nine Autosomal STR Loci Among Halakki and Kunabhi Populations of Karnataka, India

POPULATION: Blood samples were collected from a total of 84 healthy and unrelated Halakki (44) and Kunabhi (40) populations, with their informed written consent. The geographic location of the sampled area is shown in Fig. 1. Both the populations are endogamous, and they belong to Dravidian linguistic family. Halakki is a tribal group having a population size of approximately 3383. They claim that they originally belong to Gujarat and Rajasthan, and migrated through Andhra Pradesh to Karnataka. Kunabhi is also a tribal population, who are approximately 35,214 in number. The male Kunabhi can be identified by their tattoo marks. A necklace is the symbol of married women. They were hunters and gatherers, but at present they practice agriculture.

KEYWORDS: forensic science, autosomal STR haplotypes, Dravidian tribe, Kunabhi, Halakki, Karnataka, DNA typing, population genetics, D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820

The analysis of genetic variation, in the short tandem repeats (STRs) of autosomal DNA, provides unique information on the genetic diversity of the populations. The nine typed autosomal STRs are highly polymorphic and help in human identification. We have analyzed nine autosomal STR loci (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820) in 84 samples of Kunabhi and Halakki populations of Uttara Kannada District of Karnataka, India.

DNA was isolated from the above samples following protocols published elsewhere (1). DNA samples were quantified by spectrophotometer and size fractionated in 0.8% agarose gel. One nanogram of genomic DNA of the above samples was used to amplify nine STR loci (AmpFISTR[®] profiler plus kit, Applied Biosystems) in a multiplex reaction, following the manufacturer's instruction (2). Two positive and two negative controls were used along with every set of PCR reactions. PCR amplicons were analyzed in an ABI Prism 3700 Genetic Analyzer (Applied Biosystems) using GeneScan and Genotyper software (Perkin Elmer) to obtain the allelic description. Laboratory experiments were carried out at the Center for Cellular and Molecular Biology, Hyderabad, following all the quality control measures. The allele frequencies were computed by the gene counting method. Arlequin software version 2.00 was used to obtain observed and expected heterozygosity, and AMOVA and the exact test for Hardy-Weinberg equilibrium probabilities (3). Polymorphism information content (PIC), power of discrimination (PD), power of exclusion (PE), and typical paternity index (TPI) were calculated for

each locus using Powerstats v12 software (<http://www.premega.com/geneticidtools/powerstats/>). Bonferroni corrections were obtained by using SISA (<http://home.clara.net/sisa/>).

The allele frequency data along with observed and expected heterozygosity, and the exact test for the two populations are presented in Tables 1 and 2. An allele frequency distribution shows that all the loci were highly polymorphic with a uniform pattern sharing in both the populations. For the Halakki, loci D3S1358 and D7S820 had the least number of alleles, while locus FGA depicted a dispersed allelic distribution. For the Kunabhi, loci vWA and D5S818 had the least number of alleles, while locus D21S11 depicted a dispersed allelic distribution. The more the variance in a locus the less the information obtained, as probabilities of recurrent mutations may camouflage the record of population history, which presumably gets erased at repeat loci with higher mutation rates. Both Kunabhi and Halakki populations showed a very high degree of heterozygosity. Both the populations showed no deviation from Hardy-Weinberg expectations at the nine loci. A statistical evaluation of these two studied populations with several Indian populations [Brahmin, Kshatriya, Vysya, Akuthota, Kamma, Kapu, Pokanati, Panta, Vanna, Balija, Ediga, Ekila, Gandla, Jangam, Kurava, Thogata, Yadava, Chakali, Mangali, Vaddi, Madiga, Mala, Erakula, Sugali, Yanadi, Dudekula, Sheik and Golla (4,5); Iyengar, Gowda, Lingayat, Muslim (Karnataka) (6), Agharia, Satnami, Gond, Teli (7), Garo, Naga, Kuki and Hmar (8)] suggests that the level of genetic differentiation among regions was low at all loci.

Our study supports the existing view that there is no significant variation among various caste and tribal populations of India (9). AMOVA analysis revealed substructuring in Indian populations. These microsatellite loci studied were found to be highly polymorphic and informative, and although these two populations'

¹ Center for Cellular and Molecular Biology, Uppal Road, Hyderabad, India.

² Department of Anthropology, Karnatak University, Dharwad 580 003, India.

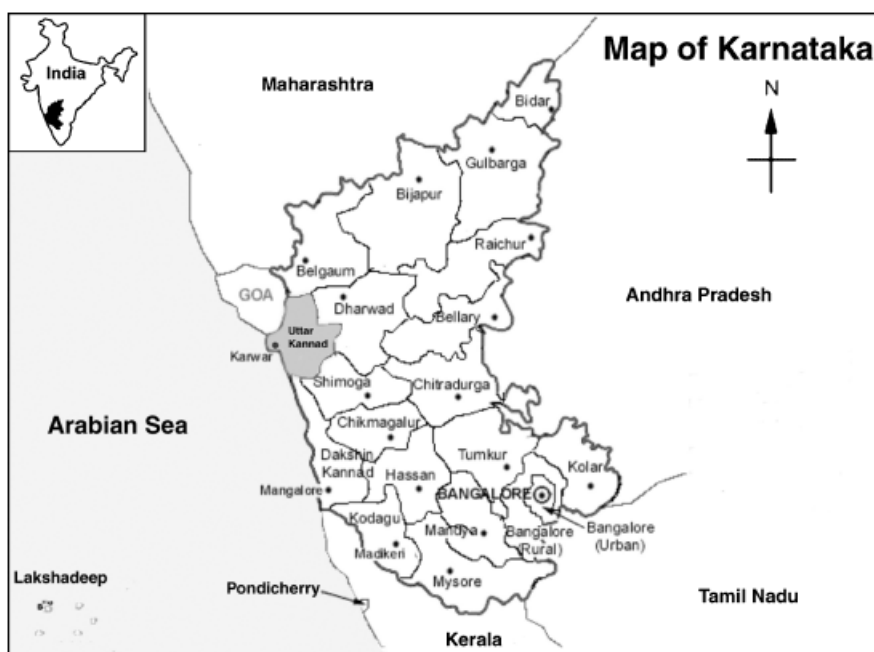


FIG. 1.

TABLE 1—Allele frequencies of nine autosomal STR loci in Kunabhi populations of Karnataka, India.

Allele	Loci								
	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
7	—	—	—	—	—	—	0.013	—	—
8	—	—	—	0.013	—	—	—	—	—
9	—	—	—	0.263	—	—	0.038	0.050	0.163
10	—	—	—	0.125	—	—	0.088	0.138	0.038
11	—	—	—	0.050	—	—	0.463	0.100	0.280
12	—	—	—	0.113	—	0.113	0.275	0.288	0.188
13	—	0.163	—	—	—	0.013	0.075	0.213	0.213
13.2	0.075	0.050	—	0.075	—	—	—	0.088	0.125
14	0.225	0.075	—	—	—	0.250	0.013	0.075	—
14.2	0.350	0.263	0.013	0.213	—	—	—	0.025	—
15	0.187	0.275	0.013	0.088	—	0.138	0.013	—	—
16	0.113	0.100	0.125	0.025	—	0.225	—	—	—
17	0.025	0.038	0.100	—	—	0.075	—	—	—
18	—	—	0.200	—	—	0.063	—	—	—
19	—	—	0.075	—	—	0.038	—	—	—
20	—	—	0.138	—	—	—	—	—	—
21	—	—	0.050	—	—	0.013	—	—	—
22	—	—	0.050	—	—	0.013	—	—	—
23	—	—	0.138	—	—	0.013	—	—	—
24	—	—	0.025	—	0.013	—	—	—	—
24.2	—	—	0.013	—	0.175	—	—	—	—
25	—	—	0.025	—	0.138	—	—	—	—
27	—	—	—	—	0.088	—	—	—	—
28	—	—	—	—	0.125	—	—	—	—
29	—	—	—	—	0.100	—	—	—	—
30	—	—	—	—	0.150	—	—	—	—
30.1	—	—	—	—	0.050	—	—	—	—
31	—	—	—	—	0.050	—	—	—	—
31.2	—	—	—	—	0.038	—	—	—	—
HO	0.650	0.725	0.725	0.775	0.825	0.400	0.384	0.615	0.775
HE	0.696	0.822	0.896	0.785	0.894	0.862	0.839	0.823	0.845
p^*	0.445	0.476	0.450	0.631	0.387	0.575	0.499	0.653	0.497
PD	0.836	0.930	0.956	0.888	0.951	0.909	0.910	0.914	0.931
PIC	0.630	0.790	0.870	0.740	0.870	0.830	0.800	0.770	0.810
PE	0.355	0.468	0.510	0.553	0.646	0.114	0.105	0.310	0.553
TPI	1.430	1.820	2.000	2.220	2.860	0.830	0.810	1.300	2.200

Mean number of pairwise difference = 6.890.

Average gene diversity = 0.811.

Mismatch observed mean = 9.456.

Mismatch observed variance = 1.346.

STR, short tandem repeat; HO, observed heterozygosity; HE, expected heterozygosity; PD, power of discrimination; PIC, polymorphism information content; PE, probability exclusion; TPI, typical paternity index; p^* , p value after Bonferroni correction.

TABLE 2—Allele frequencies of 9 autosomal STR loci in Halakki populations of Karnataka, India.

Allele	Loci								
	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
7	—	—	—	—	—	—	—	0.013	0.013
8	—	—	—	—	—	—	—	0.250	0.237
9	—	—	—	—	—	—	—	0.175	0.062
10	—	—	—	0.013	—	—	—	0.062	0.175
11	—	—	—	0.175	—	0.025	0.038	0.200	0.225
12	—	—	—	0.200	—	0.025	0.275	0.250	0.287
13	—	0.025	—	0.150	0.0250	0.175	0.363	0.050	—
14	0.025	0.125	—	0.025	0.112	0.300	0.162	—	—
15	0.150	0.363	—	0.038	0.087	0.162	0.162	—	—
16	0.062	0.300	—	0.062	0.013	0.162	—	—	—
17	0.237	0.188	—	0.162	0.125	0.050	—	—	—
18	0.287	—	0.025	0.125	0.162	0.025	—	—	—
19	0.237	—	0.050	0.050	0.338	0.025	—	—	—
20	—	—	0.112	—	0.100	0.038	—	—	—
21	—	—	0.125	—	0.025	0.013	—	—	—
22	—	—	0.213	—	0.013	—	—	—	—
23	—	—	0.150	—	0.187	—	—	—	—
24	—	—	0.188	—	—	—	—	—	—
25	—	—	0.087	—	—	—	—	—	—
26	—	—	0.050	—	—	—	—	—	—
HO	0.352	0.867	0.654	0.858	0.876	0.640	0.700	0.450	0.825
HE	0.748	0.795	0.866	0.822	0.870	0.835	0.752	0.807	0.803
p^*	0.543	0.587	0.812	0.981	0.469	0.481	0.712	0.574	0.712
PD	0.870	0.879	0.952	0.940	0.940	0.929	0.860	0.911	0.907
PIC	0.710	0.760	0.840	0.800	0.850	0.810	0.690	0.770	0.760
PE	0.654	0.702	0.702	0.702	0.800	0.367	0.608	0.695	0.654
TPI	2.93	3.42	3.42	3.42	5.16	1.46	2.56	3.33	2.93

Mean number of pairwise difference = 6.950.

Average gene diversity = 0.870.

Mismatch observed mean = 6.280.

Mismatch observed variance = 0.981.

STR, short tandem repeat; HO, observed heterozygosity; HE, expected heterozygosity; PD, power of discrimination; PIC, polymorphism information content; PE, probability exclusion; TPI, typical paternity index; p^* , p value after Bonferroni correction.

size was very small, they were useful for human identification purposes.

The complete dataset is available through electronic mail from the corresponding author at lalji@ccmb.res.in

Acknowledgments

We acknowledge all the anonymous donors who have donated their blood samples for this study.

References

1. Thangaraj K, Joshi MB, Reddy AG, Gupta NJ, Chakravarty BN, Singh L. CAG repeat expansion in the androgen receptor gene is not associated with male infertility in Indian populations. *J Androl* 2002;23:815–8.
2. Applied Biosystems. PCR protocol for AmpFISTR Profiler Plus. USA: PE Applied Biosystems Human Identification Group, Applied Biosystems; 2001.
3. Schneider S, Rosslie D, Excoffier L. Arlequin Ver 2.000, A software for population genetics data analysis. University of Geneva, Geneva: Genetics and Biometry Laboratory; 1997. <http://anthropologie.unige.ch/arlequin>.
4. Reddy BM, Sun G, Dutta R, Deka R. STR data for the AmpF/STR Profiler Plus loci among Golla population of southern Andhra Pradesh, India. *J Forensic Sci* 2001;46:734–5.

5. Reddy BM, Naidu VM, Madhavi VK, Thangaraj K, Langstieh BT, Venkataramana P, et al. STR data for the AmpF/STR Profiler Plus loci among 27 population of different social hierarchy from southern part of Andhra Pradesh, India. *Forensic Sci Int* 2005;149:81–97.
6. Rajkumar R, Kashyap VK. Distribution of alleles of 15 STR loci of the PowerPlex™ Multiplex system in four predominant population groups of south India. *Forensic Sci Int* 2002;126:173–7.
7. Trivedi R, Chattopadhyay P, Maity B, Kashyap VK. Genetic polymorphism at nine microsatellite loci in four high altitude Himalayan desert human populations. *Forensic Sci Int* 2002;127:150–5.
8. Dutta R, Chattopadhyay P, Kashyap VK. STR data for the AMPF/ STR profiler plus loci among four predominant populations of eastern India. *J Forensic Sci* 2000;45:1353–7.
9. Kivisild T, Rootsi S, Metspalu M, Mastana M, Kaldma K, Parik J, et al. The genetic heritage of the earliest settlers persists both in Indian tribal and caste populations. *Am J Hum Genet* 2003;72:313–32.

Additional information and reprint requests:

Lalji Singh, Ph.D.

Center for Cellular and Molecular Biology

Uppal Road

Hyderabad 500 007

India

Tel: (91)-40-27160789

Fax: (91)-40-27160591

E-mail: palji@ccmb.res.in