

## FOR THE RECORD

Birajalaxmi Das,<sup>1</sup> M.Phil., Ph.D.; V. S. Arun,<sup>1</sup> M.Sc.; and M. Seshadri,<sup>1</sup> Ph.D.

# Molecular Genetic Data at Two Tetranucleotide Repeat Loci (D12S66 and D12S67) in Two Indian Tribal Populations

**POPULATION:** Molecular genetic polymorphism study was undertaken in two tribal population groups of India at two tetranucleotide repeat loci on chromosome 12 (D12S66 and D12S67). The two tribal groups studied were Bison Horn Maria and Muria, belonging to Bastar district of Madhya Pradesh in Central India. For this study, 75 random, unrelated individuals were analyzed for D12S66 locus, whereas 76 individuals were analyzed for D12S67 locus.

**KEYWORDS:** forensic science, D12S66, D12S67, Indian tribal populations

Genomic DNA was extracted using a rapid non-enzymatic method (1). Duplex PCR amplification was performed for the two microsatellites (D12S66 and D12S67) using locus specific primers flanking the repeat region (2). Amplification was carried out in eppendorf thermocycler<sup>TM</sup> using Taq polymerase (Roche Molecular Diagnostics, GmbH, Germany). Amplimers were electrophoresced on 6% denaturing urea gel (7M) and analyzed in ALF DNA Sequencer (Amersham Biosciences Ltd., Uppasala, Sweden) using the software Fragment manager. Allelic ladders for both the loci were developed in our laboratory and used for the correct assignment of the allele sizes. Nomenclature of alleles for both the loci

<sup>1</sup> Low Level Radiation Studies Section, Bio-Science Group, Bhabha Atomic Research Center, Trombay, Mumbai – 400 085, India.

TABLE 1—Allele frequencies at D12S66 locus.

Allele (repeats)	Bison Horn Maria (n = 60)		Muria (n = 90)	
	No. observed	Frequency	No. observed	Frequency
8	2	0.033 ± 0.023	2	0.022 ± 0.016
9	-	-	4	0.044 ± 0.022
10	10	0.167 ± 0.049	11	0.122 ± 0.036
11	39	0.650 ± 0.062	54	0.600 ± 0.052
12	6	0.100 ± 0.039	9	0.100 ± 0.030
13	1	0.017 ± 0.017	5	0.056 ± 0.024
14	2	0.033 ± 0.023	5	0.056 ± 0.024
H	0.53		0.50	
h	0.55 ± 0.07		0.61 ± 0.05	
PD	0.80		0.80	
PIC	0.57		0.63	
Exact Test (P value)	0.475 ± 0.001		0.005 ± 0.0002	

H = Observed heterozygosity, h = Expected heterozygosity, PD = Power of discrimination, PIC = Polymorphic information content, n = Number of chromosomes.

TABLE 2—Allele frequencies at D12S67 locus.

Allele	Bison Hon Maria (n = 64)		Muria (n = 88)	
	No. observed	Frequency	No. observed	Frequency
36	-	-	1	0.011 ± 0.011
37	2	0.031 ± 0.022	5	0.057 ± 0.025
38	2	0.031 ± 0.022	5	0.057 ± 0.025
39	9	0.141 ± 0.044	13	0.148 ± 0.038
40	15	0.234 ± 0.053	17	0.193 ± 0.042
41	18	0.281 ± 0.057	22	0.250 ± 0.046
42	3	0.047 ± 0.027	14	0.159 ± 0.039
43	13	0.203 ± 0.051	8	0.091 ± 0.031
44	2	0.031 ± 0.022	3	0.034 ± 0.020
H		0.88		0.77
h		0.81 ± 0.02		0.85 ± 0.02
PD		0.90		0.93
PIC		0.83		0.86
Exact Test (P value)		0.010 ± 0.0002		0.015 ± 0.0003

No. = Number, H = Observed heterozygosity, h = Expected heterozygosity, PD = Power of discrimination, PIC = Polymorphic information content, n = Number of chromosomes.

(D12S66 and D12S67) was based on the number of repeat units (3,4).

Allele and genotype frequencies, gene diversities and Exact tests were performed using the program ARLEQUIN Ver. 1.1 (5). The polymorphic information content (PIC) was determined according to Botstein et al. (6) and the Power of Discrimination (PD) was calculated as described by Fisher (7).

A total number of 7 alleles (8–17 repeats) and 15 genotypes were observed at D12S66 locus, whereas 9 alleles (36–44 repeats) and 26 genotypes were observed at D12S67 locus among these two population groups (Tables 1–2). At D12S66, allele 11 was predominant among these two population groups, whereas allele 41 was predominant for the locus D12S67. Both the population groups did not

conform Hardy-Weinberg Equilibrium expectations at D12S67 locus. At D12S66 locus, Bison Horn Maria population group conform to Hardy-Weinberg equilibrium, whereas Muria group, it deviated. The allele frequency data are comparable with the results published earlier in other Indian population groups (8). Because of high PIC and PD values, these two loci would be very useful and informative for forensic investigations in India.

Access to the complete dataset is available via electronic mail from communicating author.birajalaxmi@yahoo.co.in, msesh@apsara.barc.ernet.in.

#### *Acknowledgments*

We express our sincere thanks to Dr. V. R. Rao, Dr. B. N. Sarkar, Dr. D. Chowdhury and Dr. P. Padmanabhan of Anthropological Survey of India, for the collection of samples. We are also thankful to Arun V. S. for experimental assistance.

#### **References**

1. Lahiri D, Nurnberger J. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991;19:5444. [\[PubMed\]](#)
2. Roewer L, Arnemann J, Spurr N, Grzeschik K, Epplen J. Simple repeat sequences on the human Y chromosome are equally polymorphic as their autosomal counterparts. *Human Genetics* 1992;89:389–94. [\[PubMed\]](#)

3. Minaguchi A, Haga T. Polymorphism of the D12S66 system in the Japanese population and its detection using degraded DNA. *The Bulletin of Tokyo Dental College* 2000;41:15–20. [\[PubMed\]](#)
4. Minaguchi K. D12S67, a bipartite locus: Differential amplification of parts of the nucleotide sequence reveals considerable polymorphism. *Electrophoresis* 1997;18:1923–7. [\[PubMed\]](#)
5. Schneider S, Kueffer JM, Roessli D, Excoffier L. ARLEQUIN version 1.1: a software for population genetic data analysis. Switzerland: Genetics and Biometry Laboratory, University of Geneva, 1997.
6. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet* 1980;32:314–31. [\[PubMed\]](#)
7. Fisher R. Standard calculations for evaluating a blood group system. *Heredity* 1951;5:95–102. [\[PubMed\]](#)
8. Das Birajalaxmi, Chauhan PS, Seshadri M. Genetic variation observed at two tetrameric short tandem repeat loci on chromosome 12 (D12S66 and D12S67) among five distinct ethnic groups of India: detection of two new alleles. *Ann Hum Biol* 2002;29(5):513–25. [\[PubMed\]](#)

Additional information and reprint requests:

M. Seshadri, Ph.D.  
Low Level Radiation Studies Section  
Bio-Science Group  
Bhabha Atomic Research Center  
Trombay, Mumbai – 400 085  
India