

## Original Article

# Y-STR Haplotype Diversity among the Population of District Haridwar, Uttarakhand, India

Kamal Chauhan<sup>1</sup>, B.K. Mohapatra<sup>2</sup>, U.S. Thakur<sup>3</sup>, C. Behera<sup>4\*</sup>

<sup>1</sup>Laboratory Assistant, <sup>2</sup>Senior Scientific Officer Grade I, <sup>3</sup>Senior Scientific Assistant Central Forensic Science Laboratory (CBI), CGO Complex, Lodhi Road, New Delhi, India

<sup>4</sup>Associate Professor, Department of Forensic Medicine, AIIMS, Ansari Nagar, New Delhi, India

\*Corresponding author email id: drchitta75@rediffmail.com

## ABSTRACT

Seventeen Y-chromosomal short tandem repeat (Y-STR) loci in a population sample of Dist. Haridwar, Uttarakhand, India was analysed to find out the haplotype diversity (HD). Blood samples were collected from 85 unrelated male individuals and genomic DNA isolation was carried out by the standard organic extraction method, followed by multiplex PCR amplification using AmpF/STR Y-filer polymerase chain reaction (PCR) amplification kit and subsequent genotyping. A total of 75 haplotypes were observed among the studied samples, out of which 69 were unique. Allele frequency and gene diversity were calculated. HD and discrimination capacity were found to be 0.991 and 0.8823, respectively. Haplotypes of Dist. Haridwar, Uttarakhand population was compared with that of other Indian populations using analysis of molecular variance tool to measure the genetic relatedness between various populations of India.

**Keywords:** Discrimination capacity, Genotyping, Haplotype diversity, PCR, Polymorphism, Uttarakhand, Y-STR

## INTRODUCTION

Short tandem repeats (STRs) or microsatellites are highly polymorphic markers dispersed throughout the genome, which make them suitable for studying genetic polymorphism. The Y-chromosome is inherited patrilineally. Nowadays, Y-chromosome-specific STRs (Y-STRs) are extensively being used in forensic genetics due to its significant role in solving sexual assault cases, paternity testing, individual identification and studying migration patterns<sup>[1-8]</sup>.

Indian subcontinent is inhabited by a very diverse population consisting of more than five thousand anthropologically defined population groups<sup>[9-16]</sup>. Population genetic studies based on DNA polymorphism have revealed the presence of a large extent of genetic variation among several Indian population groups<sup>[17-21]</sup>. We analysed DNA samples of the Dist. Haridwar,

Uttarakhand population to find out the haplotype diversity (HD) using seventeen Y-STR loci. The Y-STR loci chosen for this study include: DYS 19, DYS 389I, DYS 389II, DYS 390, DYS 391, DYS 392, DYS 393, DYS 385a/b, DYS 437, DYS 438, DYS 439, DYS 448, DYS 456, DYS 458, DYS 635 and Y\_GATA\_H4. Haplotypes of the Dist. Haridwar, Uttarakhand population was compared with the haplotypes of 10 other Indian populations using analysis of molecular variance (AMOVA) tool aiming to find out the genetic relatedness between various populations of India<sup>[22]</sup>.

The Uttarakhand's name is derived from the Sanskrit words *uttered* (उत्तर) meaning 'north' and *Khanda* (खण्ड) meaning 'land', altogether simply meaning 'Northern Land'. Uttarakhand has a total area of 53,483 km<sup>2</sup> of which 86% is mountainous and 65% is covered by forest. The state is bordering Himachal Pradesh in the north-

west and Uttar Pradesh in the south and has international borders with Nepal and China. The state is very rich in natural resources. The famous peaks of Nanda Devi, Kedarnath, Trishul, Bandarpunch and Mt Kamet, the major Glaciers including Gangotri, Pindari, Milam and Khatling are located in Uttarakhand. The Ganga, the Yamuna, Ramganga and Sharda are principal rivers of this region. The state of Uttarakhand is looking after twelve major ecological areas of the country. These include Nanda Devi National Park, the Valley of Flowers, Gangotri, Govind and the Rajaji National Parks, Kedarnath, Mussoorie, Binsar, Sanadi, Govind and the Ascod Sanctuaries. All these areas support many rare plants and animal communities.

Uttarakhand (Figure 1) has a multiethnic population spread across two geocultural regions: the Gahrwal and the Kumaon. A large portion of the population is Rajput (various clans of erstwhile landowning rulers and their descendants), including members of the native Garhwali, Kumaoni and Gujjar communities, as well as a number of immigrants. According to a 2007 study by the centre for the study of developing societies, Uttarakhand has the highest percentage of Brahmins of any state in India, with approximately 20% of the population being a Brahmin. Approximately one-fifth of the population belongs to the



**Figure 1: Map of Uttarakhand**

scheduled castes (an official term for the lower castes in the traditional Hindu caste system). Scheduled tribes (an official term for natives outside the Indian social system), such as the Raji, who live near the border with Nepal, constitute less than 3% of the population. More than four-fifths of Uttarakhand’s residents are Hindus. Muslims, Sikhs, Christians, Buddhists and Jains make up the remaining population with the Muslims being the largest minority.

## MATERIALS AND METHODS

### Samples

Liquid blood samples collected in 2 ml EDTA vacutainer tubes taken from 85 unrelated healthy male individuals of Dist. Haridwar, Uttarakhand. Blood samples were stored at 15°C till further analysis.

### DNA Analysis

Genomic DNA isolation from the blood samples was carried out via standard organic extraction method (Phenol–Chloroform extraction method)<sup>[23]</sup>. Amplification of the extracted Genomic DNA samples was done via multiplex PCR using AmpF<sub>l</sub> STR Y-filer PCR Amplification Kit™ (Life Technologies, Singapore) for 17 Y-STR loci using GeneAmp 9700 thermal cycler. PCR amplification was carried out as per the manufacturer’s instructions. The amplified products were analysed by capillary electrophoresis on an ABI Prism 3130 *xl* Automated Genetic Analyzer<sup>[24]</sup> using LIZ 500 as Internal Lane DNA standard. Allelic designations for different loci were obtained by GeneMapper ID software (v. 3.2).

### Statistical Analysis

Allele frequencies were calculated by direct counting. Gene diversity (GD) was calculated using the formula:

$$GD = \frac{n}{n-1} [1 - \sum P_i^2]$$

*i* - 1

where,  $P_i$  is the frequency of *i* the allele and *n* is the number of samples analysed<sup>[25]</sup>.

All candidate loci were evaluated individually by determining the contribution each locus would make to increase the HD afforded by the SWGDAM core loci. The HapYDive programme was used to determine HDs (<http://www.ipatimup.pt/app/>)<sup>[26]</sup>.

The discrimination capacity (DC) was also determined as  $DC = h/n$  where  $h$  is the number of different haplotypes observed in the population<sup>[25]</sup>.

To find out the genetic relatedness between the population of Dist. Haridwar, Uttarakhand and other Indian population groups, we used online AMOVA tool provided by the Y-chromosome haplotype reference database (YHRD, www.yhrd.org). A total of 11 population samples with 724 haplotypes were included in this study. The studied samples belong to following populations: Punjab, India (Balmiki)<sup>[27]</sup> population sample with 62 haplotypes; Tamil Nadu, India (Iyengar)<sup>[27]</sup> population samples with 66 haplotypes; Madhya Pradesh, India (Kanyakubja Brahmin)<sup>[27]</sup> population samples with 78 haplotypes; Maharashtra, India (Konkanastha Brahmin)<sup>[27]</sup> population samples with 71 haplotypes; West Bengal, India (Paliya)<sup>[28]</sup> population samples with 53 haplotypes; Jharkhand, India (Sakaldwipi Brahmin)<sup>[27]</sup> population samples with 65 haplotypes; Himachal Pradesh, India (Saraswat Brahmin)<sup>[29]</sup> population samples with 61 haplotypes; Kashmir, India (Saraswat Brahmin)<sup>[29]</sup> population samples with 58 haplotypes; Rajasthan, India (Saraswat Brahmin)<sup>[29]</sup> population samples with 60 haplotypes; Tripura, India (Tripuri)<sup>[27]</sup> population samples with 65 haplotypes and Dist. Haridwar, Uttarakhand, India population sample with 85 haplotypes. Population pairwise distances between the Uttarakhand population and other Indian populations (Rst values) were calculated. Online AMOVA tool of YHRD was used for molecular variance analysis with 10,000 permutations for calculating  $P$  value<sup>[30]</sup>.

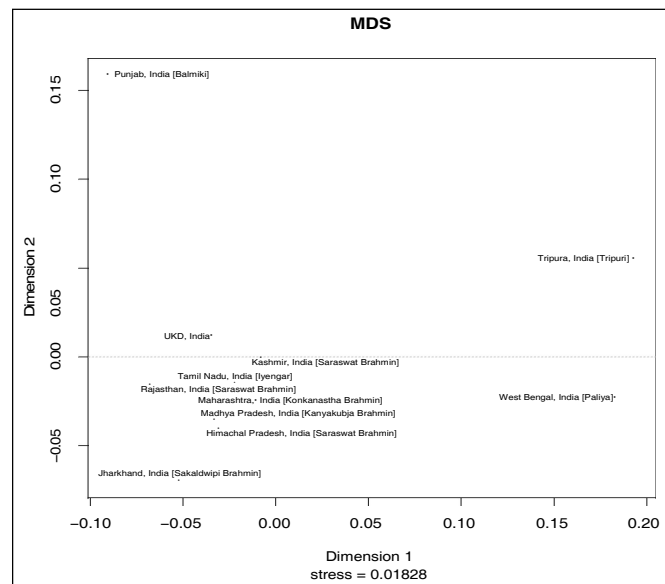
## RESULTS

Blood samples collected from 85 healthy unrelated male individuals of Dist. Haridwar, Uttarakhand, India deposited in the laboratory from time to time were analysed for 17 Y-STR loci to find out the HD. A total of 75 different haplotypes were observed by combined analysis of 17 Y-STR loci in the studied samples. The observed haplotype details are discussed in Table 1. Out of 75 haplotypes, 67 haplotypes were unique (89.333%) and observed only once, 2 haplotypes were observed thrice (2.667%) and 6 haplotypes were observed twice (8%). HD value for the population of Dist. Haridwar, Uttarakhand was found to

be 0.991. DC value for the studied samples was calculated to be 0.8823.

Observed alleles for the 17 Y-STR loci along with their allelic frequencies are mentioned in Table 2. GD per locus ranged from 0.364 to 0.9255 with an average GD value of 0.6892. The lowest GD (0.364) has been found at locus DYS391, wherein the most frequent allele has been allele 10 with a frequency of 76.5%. The highest GD (0.9255) has been found in case of the bi-allelic marker DYS385a/b. The highest frequency shown by different alleles for each of the 17 Y-STR loci has been highlighted in Table 2.

For extensive analysis of the genetic relatedness, haplotypes of the population of Dist. Haridwar, Uttarakhand, India were compared via AMOVA with haplotypes of other populations of India. The details of Indian populations used for comparative analysis are discussed in Table 3. AMOVA pairwise distances based on Rst values between the population of Dist. Haridwar, Uttarakhand and other Indian populations are described in Table 4 as well as shown vide MDS plot in Figure 2. Results revealed that population of Dist. Haridwar, Uttarakhand, India are not closely related to other Indian populations.



**Figure 2: MDS plot showing Analysis of molecular variance (AMOVA) pairwise distances based on Rst values between Dist. Haridwar, Uttarakhand population of India & other Indian populations**

**Table 1: Y-chromosome haplotype distribution in a sample of 85 males from Dist. Haridwar, Uttarakhand, India**

Haplo-type	N	DYS 456	DYSI 389	DYS 390	DYSII 389	DYS 458	DYS 19	DYS 385	DYS 393	DYS 391	DYS 439	DYS 635	DYS 392	YGAT AH4	DYS 437	DYS 438	DYS 448
1	3	15	14	24	31	16	15	11,14	13	10	10	23	11	12	14	9	20
2		15	13	22	30	16	15	15,18	12	10	11	20	11	13	14	9	19
3		15	13	24	31	15	13	14,16	15	11	12	21	11	12	14	10	20
4		15	14	23	30	18	14	13,20	13	10	11	23	10	11	16	11	19
5	2	16	13	22	29	17	14	15,16	12	10	11	20	11	11	16	9	18
6		15	13	22	29	16	16	15,17	12	10	11	21	11	11	14	9	19
7		15	13	23	31	15	14	14,14	13	10	12	21	11	12	16	9	20
8		15	13	22	29	17	15	9,16	12	10	10	22	14	11	16	10	20
9		14	14	24	30	16	15	15,15	13	10	12	21	11	11	14	10	18
10		15	14	23	30	17	14	14,15	12	10	11	24	11	11	16	9	19
11		15	12	22	28	19	16	9,18	12	10	8	21	14	12	16	10	18
12		16	13	22	28	17	15	15,17	13	10	12	20	11	11	14	9	19
13		15	13	25	30	16	16	11,14	13	10	12	23	11	13	14	11	21
14		13	12	24	29	17	15	13,19	13	11	13	21	11	11	15	9	19
15		15	14	24	31	16	14	12,12	13	10	12	21	13	12	15	11	20
16		16	13	23	28	19	15	16,16	13	10	12	21	11	12	14	9	19
17		16	14	22	30	18	15	14,15	13	10	10	23	11	12	14	11	18
18		16	13	22	28	17	15	15,17	13	10	12	20	11	11	14	9	19
19	3	16	13	25	30	18	16	11,14	13	11	10	23	11	12	14	11	20
20	2	15	14	22	30	16	15	14,15	12	10	10	22	11	10	14	10	19
21		14	14	23	31	17	14	13,19	13	10	11	24	12	11	15	11	19
22		15	13	22	30	16	15	15,18	12	10	11	20	11	12	14	9	19
23		15	13	22	30	16	16	15,16	12	10	11	20	11	12	14	9	19
24		15	13	25	31	15	16	11,14	13	11	11	23	11	13	14	11	20
25		14	14	23	31	17	14	13,19	13	10	11	24	12	11	15	11	19
26		16	13	23	28	19	15	16,16	13	10	12	30	11	12	14	9	19
27		16	14	21	32	17	15	13,17	12	11	11	20	11	11	14	11	19
28		14	13	24	29	16	15	15,16	13	10	13	20	11	11	14	10	18
29		16	13	22	30	16	15	16,17	12	10	11	21	11	12	14	9	19
30		15	14	23	30	17	14	13,19	14	10	10	25	10	12	15	11	19
31		13	12	24	28	16	15	13,17	12	10	11	20	11	11	15	9	18
32	2	15	13	24	31	16	16	12,14	13	10	10	23	11	13	14	11	20
33		15	13	22	29	18	15	14,19	15	10	11	25	10	12	14	11	19
34		17	13	22	33	16	15	14,15	14	10	12	20	11	12	16	10	19
35		15	14	23	30	17	14	13,19	14	10	10	25	10	12	15	11	19
36		15	13	23	29	18	14	13,20	13	10	11	24	10	12	16	11	19
37		15	13	26	31	16	15	12,15	13	10	10	24	11	12	14	12	20
38		16	14	21	32	17	15	13,17	12	11	11	20	11	11	14	11	19

Y-STR Haplotype Diversity among the Population of District Haridwar, Uttarakhand, India

Table 1 cont.....

Haplo-type	N	DYS 456	DYSI 389	DYS 390	DYSII 389	DYS 458	DYS 19	DYS 385	DYS 393	DYS 391	DYS 439	DYS 635	DYS 392	YGAT AH4	DYS 437	DYS 438	DYS 448
39		15	14	25	32	17	15	11,14	13	10	10	23	11	13	14	11	19
40		15	14	25	33	16	15	11,14	13	10	10	23	11	13	14	11	20
41		15	13	23	29	18	14	13,20	13	10	11	24	10	12	16	11	19
42		15	13	22	29	17	15	9,16	12	10	12	22	14	11	16	10	20
43		15	13	22	29	16	16	15,15	12	10	11	24	11	10	14	10	19
44		15	14	25	33	15	15	11,14	13	10	10	24	11	13	14	11	20
45		15	13	22	29	17	15	15,15	12	11	11	20	11	11	14	9	19
46	2	17	14	22	30	17	15	15,16	12	10	11	21	11	12	14	9	19
47		17	13	22	29	18	15	16,17	12	10	11	20	11	12	14	9	19
48		15	14	25	33	15	15	11,14	13	10	10	24	11	13	14	11	20
49		15	13	22	29	16	15	15,18	12	11	11	20	11	11	14	9	19
50	2	15	13	25	31	17	16	11,14	13	11	10	23	11	12	14	12	20
51		15	12	22	29	20	14	14,16	12	10	13	21	12	10	16	10	21
52		15	13	25	30	17	15	11,15	13	11	10	24	11	13	14	11	20
53		16	14	21	34	18	15	15,16	12	10	11	21	11	11	14	9	19
54		15	13	24	30	18	15	13,16	13	11	13	22	11	11	16	10	19
55		14	13	23	29	17	14	15,21	12	10	13	21	11	11	14	9	21
56	2	16	13	22	30	16	15	16,17	12	10	11	21	11	12	14	9	19
57		15	14	24	32	15	16	11,14	13	11	10	23	11	12	14	11	20
58		16	13	21	29	16	14	15,17	12	10	12	20	11	13	14	9	20
59		16	14	24	30	15	14	12,23	14	10	12	24	12	11	14	11	19
60		15	14	22	32	16	15	15,17	12	10	11	21	11	12	14	9	19
61		15	13	25	31	15	16	11,14	13	10	10	23	11	13	14	11	20
62		14	13	23	29	17	14	14,17	12	10	11	20	11	11	14	11	20
63		17	14	22	31	16	14	15,17	12	11	11	20	11	12	14	9	19
64		14	13	24	29	16	16	15,16	13	10	13	20	11	11	14	10	18
65		15	14	23	30	17	14	13,16	13	10	11	23	11	11	15	9	21
66		15	14	23	31	16	15	13,17	14	11	10	24	10	12	16	11	19
67		13	14	22	31	18	15	15,18	12	10	11	20	12	11	14	9	20
68		16	13	22	28	17	15	15,17	13	10	12	20	11	11	14	9	19
69		16	13	23	30	17	14	14,16	15	11	11	20	11	11	14	10	19
70		14	13	23	29	17	14	13,19	12	10	11	23	11	11	14	9	21
71		15	13	25	31	16	15	11,14	13	10	10	23	11	13	14	11	20
72		16	13	25	32	16	16	11,14	13	11	10	23	11	12	14	11	20
73		15	14	24	30	16	15	11,13	13	11	11	24	13	12	14	11	19
74		17	13	26	29	16	15	15,16	14	10	11	19	13	11	14	11	18
75		15	14	25	30	20	15	13,18	13	10	11	26	10	11	16	11	19

**Table 2: Allele frequency and gene diversity values of 17 Y-STR loci in the Dist. Haridwar, Uttarakhand population of India**

Loci→ Allele↓	DYS 456	DYS 389I	DYS 390	DYS 389II	DYS 458	DYS 19	DYS 393	DYS 391	DYS 439	DYS 635	DYS 392	GATA H4	DYS 437	DYS 438	DYS 448	Geno type	*DYS 385a/b
8									0.012							9,16	0.0235
9									0					<b>0.4</b>		9,18	0.0118
10								<b>0.765</b>	0.318		0.094	0.047		0.165		11,13	0.0118
11								0.235	<b>0.435</b>		<b>0.776</b>	0.376		<b>0.4</b>		11,14	<b>0.2118</b>
12		0.047					0.388		0.165		0.059	<b>0.424</b>		0.035		11,15	0.0118
13	0.035	<b>0.576</b>				0.012	<b>0.506</b>		0.071		0.035	0.153				12,12	0.0118
14	0.082	0.376				0.247	0.071				0.035		<b>0.729</b>			12,14	0.0235
15	<b>0.553</b>				0.094	<b>0.541</b>	0.035						0.094			12,15	0.0118
16	0.247				<b>0.388</b>	0.2							0.176			12,23	0.0118
17	0.082				0.318											13,16	0.0235
18					0.141										0.106	13,17	0.0471
19					0.035					0.012					<b>0.506</b>	13,18	0.0118
20					0.024					<b>0.259</b>					0.329	13,19	0.0706
21			0.035							0.2					0.059	13,20	0.0353
22			<b>0.353</b>							0.059						14,14	0.0118
23			0.2							<b>0.259</b>						14,15	0.0588
24			0.188							0.153						14,16	0.0353
25			0.188							0.035						14,17	0.0118
26			0.035							0.012						14,19	0.0118
27																15,15	0.0353
28				0.082												15,16	0.1059
29				0.247												15,17	0.0824
30				<b>0.318</b>						0.012						15,18	0.0471
31				0.224												15,21	0.0118
32				0.071												16,16	0.0235
33				0.047												16,17	0.0471
34				0.012													
GD	0.626	0.53	0.771	0.783	0.726	0.613	0.594	<b>0.364</b>	0.685	0.807	0.387	0.661	0.433	0.659	0.628		<b>0.9255</b>
GD=Gene diversity value																	
Haplotype diversity or HD = <b>0.991</b>																	

\*Table 2 shows allele frequencies for each Y-STR locus except DYS385a/b for which genotype frequencies are reported. The genotype frequencies for DYS385a/b were calculated for the combination of two alleles. Bold values indicate highest allele frequency for corresponding Y-STR locus & highest genotype frequency for DYS385a/b in the studied population.

**Table 3: Details of studied Indian populations**

S.No.	Population Name	Location	No. of Haplotypes
1.	Dist. Haridwar, Uttarakhand	Uttarakhand, India	85
2.	Balmiki	Punjab, India	62
3.	Iyengar	Tamil Nadu, India	66
4.	Kanyakubja Brahmin	Madhya Pradesh, India	78
5.	Konkanastha Brahmin	Maharashtra, India	71
6.	Paliya	West Bengal, India	53
7.	Sakaldwipi Brahmin	Jharkhand, India	65
8.	Saraswat Brahmin	Himachal Pradesh, India	61
9.	Saraswat Brahmin	Kashmir, India	58
10.	Saraswat Brahmin	Rajasthan, India	60
11.	Tripuri	Tripura, India	65

**Table 4: Analysis of molecular variance (AMOVA) pairwise distances based on Rst values between Dist. Haridwar, Uttarakhand population of India and other Indian populations**

Population*	UKD	PnBl	TNI	MPBr	MBr	WBPI	JhBr	HPSBr	KSBBr	RSBr	TTi
UKD	–	0.0000	0.0194	0.0024	0.0045	0.0000	0.0001	0.0000	0.0083	0.0035	0.0000
PnBl	0.1063	–	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TNI	0.0228	0.1576	–	0.0478	0.0544	0.0000	0.0019	0.0324	0.0345	0.0002	0.0000
MPBr	0.0402	0.2334	0.0189	–	0.1505	0.0000	0.1000	0.2699	0.0040	0.0061	0.0000
MBr	0.0358	0.1833	0.0185	0.0076	–	0.0000	0.0031	0.0363	0.0068	0.0068	0.0000
WBPI	0.1999	0.3429	0.1900	0.2387	0.2296	–	0.0000	0.0000	0.0000	0.0000	0.0006
JhBr	0.0762	0.2871	0.0524	0.0339	0.0511	0.2418	–	0.0086	0.0000	0.0004	0.0000
HPSBr	0.0714	0.2833	0.0242	0.0032	0.0221	0.2211	0.0355	–	0.0045	0.0003	0.0000
KSBBr	0.0285	0.1620	0.0209	0.0374	0.0357	0.1693	0.1026	0.0394	–	0.0000	0.0000
RSBr	0.0341	0.2009	0.0578	0.0328	0.0352	0.2353	0.0555	0.0534	0.0615	–	0.0000
TTi	0.2125	0.3339	0.1983	0.2661	0.2309	0.0813	0.3038	0.2678	0.1889	0.2819	–

\*Populations: UKD – Dist. Haridwar, Uttarakhand, India; PnBl – Punjab, India (Balmiki); TNI – Tamil Nadu, India (Iyengar); MPBr – Madhya Pradesh, India (Kanyakubja Brahmin); MBr – Maharashtra, India (Konkanastha Brahmin); WBPI – West Bengal, India (Paliya); JhBr – Jharkhand, India (Sakaldwipi Brahmin); HPSBr – Himachal Pradesh, India (Saraswat Brahmin); KSBBr – Kashmir, India (Saraswat Brahmin); RSBr – Rajasthan, India (Saraswat Brahmin); TTi – Tripura, India (Tripuri).

## DISCUSSION

Population genetic studies have revealed a large extent of genetic diversity among various Indian populations<sup>[20]</sup>. The present study was carried out with an aim to study the HD of the population of Dist. Haridwar, Uttarakhand at 17 Y-STR loci. Although data on various Indian populations have been reported, there are no published data available about the genetic structure of the population

of Dist. Haridwar, Uttarakhand elucidating the HD based on 17 Y-STR loci<sup>[15–20]</sup>. Among the 17 Y-STR loci analysed, the highest GD (0.9255) was observed for locus DYS 385a/b and the lowest GD (0.364) was observed in the case of locus DYS. HD and DC for the studied population were found to be 0.991 and 0.8823, respectively, which imply that the 17 Y-STR loci studied in the population of Dist. Haridwar, Uttarakhand are highly

polymorphic. Comparative study of haplotypes of the Dist. Haridwar, Uttarakhand population with other Indian populations suggests that Uttarakhand population is not closely related to other Indian populations.

## CONCLUSION

A higher degree of HD and DC indicates that 17 Y-STR loci used in the current study are highly polymorphic among the population of Dist. Haridwar, Uttarakhand. Thus, this set of Y-STRs can be used for forensic purposes like paternity testing, individual identification, genetic mapping and others and this will add to the databank of various studies conducted on the Indian population as no previous Y-STR data is available in the literature for this population.

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