© IJHG 2021 Int J Hum Genet, 21(2): 70-78 (2021)
PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330 DOI: 10.31901/24566330.2021/21.02.779

Distribution of Selected Single Nucleotide Polymorphisms in the Brahmin, Rajput and Bania Populations of Jammu District of Jammu and Kashmir, North India

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KEYWORDS Caste Populations. DNA Markers. Genetic Structure. Genetic Affinities

ABSTRACT A total of 461 randomly selected unrelated subjects belonging to three selected caste populations of Jammu district of Jammu and Kashmir (J&K) viz., the Brahmin, Rajput, and Bania were typed using standard PCR-RFLP technique for a battery of five SNPs (Single Nucleotide Polymorphisms) namely NAT2, ADH2, PSCR, T2, and ALAD. The objective of the present study was to characterize these populations genetically and assess the degree of genetic differentiation and genetic affinities among them. The results revealed that the present caste populations were moderately differentiated (G_{ST} =0.0105).The genetic distance analysis demonstrated that the Rajput and Bania were in close genetic affinities while the Brahmin population was somewhat distant. In conclusion, the present investigation documented the underlying genomic uniformity in the people of the Jammu district.

INTRODUCTION

Generally, the human DNA polymorphisms are categorized into two major groups, one based on nucleotide substitution, i.e., single nucleotide polymorphisms (SNPs), and the other is an insertion/deletion (InDel) polymorphism in which a specific nucleotide sequence is either present (insertion) or absent (deletion). While not as common as SNPs, InDels are widely spread across the genome (Weber et al. 2002). Due to their abundance in the genome and ease of their conventional and high-throughput genotyping, both SNPs and InDels are the most commonly used DNA markers for the analyses of population genetic variation. They are highly informative in ascertaining relationships between individuals and populations (Deininger and Batzer 1999) and offer great opportunities for detailed analysis of genetic diversity present within and between different human populations and human evolution in general.

Nowhere in the world are people distributed in such a large number of ethnic, caste, religious and linguistic groups as in India (Bhasin 2006). It is believed that the Indian populations are genetically unique and harbour the second highest genetic diversity stock after the Africans (Tamang and Thangraj 2012). Therefore, India offers an excellent opportunity to study human socio-cultural and genetic variability. Several genome diversity studies based on bi-allelic InDel and SNP polymorphisms have revealed that there was a high level of genomic diversity among human populations of various ethnic groups inhabiting different regions of the country (Majumder et al.1999; Mukherjee et al. 2000; Bamshad et al. 2001, Veerraju et al. 2001, 2008; Chakrabarti et al. 2002, Kaur et al. 2002, Basu et al. 2003, Vishwanathan et al. 2004; Ravindranath et al. 2005; Mastana 2007; Vijaya et al. 2007; Gauniyal et al. 2008, 2011; Kanthimathi et al. 2008; Saraswathy et al. 2008, 2009; Tripathi et al. 2008; Meitei et al. 2010; Dada et al. 2011; Kshatriya et al. 2011, 2019; Mondal et al. 2011; Yadav and Arora 2011; Panjaliya et al. 2010, 2012, 2013; Saini et al. 2012; Sharma et al. 2012; Krishnaveni and Prabhakaran 2015; Chinniah et al. 2016; Deva et al. 2016; Laybourn et al. 2016; Panmei et al. 2016; Singh et al. 2016, 2017; Bala et al. 2019; Singh and Bhanwer 2019; Singh et al. 2020). These studies also demonstrated that most of the genetic diversity in people of India was found within populations rather than between populations. The North Indian state of Jammu and Kashmir (J&K) is linguistically and ethnically diverse, but only a few studies have been reported in the literature on the genomic diversity among its people (Panjaliya et al. 2010, 2012, 2013; Singh et al. 2020).

Objectives

The objectives of the present human population genetics study were, first to establish the

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genomic profiles of three major endogamous caste populations of Jammu district of North Indian state of Jammu and Kashmir viz., the Brahmin, Rajput, and Bania using a battery of 5 SNP markers viz., NAT2 (rs1799929), ADH2 (rs2066701), PSCR (rs34672281), T2 (rs6313) and ALAD (rs1139488) and second to compare the allele and genotype frequency distribution of each of the studied markers in the present caste populations with those of the people inhabiting different states of North India. Finally, the genetic structure was studied to evaluate heterozygosity, gene differentiation, and genomic similarities and differences among the present populations.

MATERIAL AND METHODS

Sample Collection

The ethical clearance for this study was obtained from the Institutional Ethical Committee of the Punjabi University, Patiala (Punjab). After the informed consent, intravenous blood samples (3-5 ml each) were collected in EDTA.Na, vials from a total of 461 subjects (154 Brahmin, 155 Rajput, 152 Bania) from the Jammu district of Jammu and Kashmir state in different field works spread over April 2018- December 2019. In the laboratory in the Department of Human Genetics, Punjabi University, Patiala. DNA was extracted from the blood samples following the salting out method of Miller et al. (1988). The quality and quantity checks of the extracted DNA were carried out using agarose gel electrophoresis and spectrophotometer.

Amplification and Genotyping

The DNA was amplified using the polymerase chain reaction (PCR) technique in 0.5 ml Eppendorf vials containing 7.5µl of Master Mix (G-Biosciences), 0.75µl each of forward and reverse primers, 5.0µl of nuclease-free water (New England Bio Labs), and 1.0µl of template DNA. Amplification was carried in a Thermal Cycler (Applied Biosystems 2720), and amplicons were digested using a respective restriction enzyme, followed by 1.5-2.5% agarose gel electrophoresis to separate DNA fragments. Genotypes were read under UVP GelDoc-It Imaging System and recorded. The primer sequences, annealing temperature, and restriction enzymes used for typing the present markers are listed in Table 1.

Table 1: Primer sequences, annealing temperatures, and restriction enzymes used for genotyping various SNPs

SNP(Accession ID)	*Primer sequence*	*Annealing temperature*	*Restriction enzyme*	*Reference*
NAT2 (rs1799929)	Forward Primer: 5' - GAC ATT GAA GCA TAT TTT GAA A – 3' Reverse Primer: 5' - GAT GAA AGT ATT TGA TGT TTA G – 3'	63^{0}C	KpnI	Cascorbi et al. (1996)
ADH2 (rs2066701)	Forward Primer: 5' - ATA TTT ATT TTA CCC TAA ACT TAT – 3' Reverse Primer: 5' - GAG CTA AAA CAT ACT TTG GAT AG – 3'	53^{0}C	RsaI	Mulligan et al. (2003)
PSCR(rs34672281)	Forward Primer: 5' - GGG TTC TAA AGG GAA GAA A – 3' Reverse Primer : 5' - CCT AAC AGA GGT CAC AAG G – 3'	65^{0}C	TaqI	Stinissen and Broeckhovn (1991)
T2 (rs6313)	Forward Primer: 5'- CTG CAG CTT TTT CTC TAG GG – 3' Reverse Primer : 5'- CGT CTG CTA CAA GTT CTG GCT T – 3'	57^{0}C	MspI	Mukherjee et al. (2000)
ALAD (rs1139488)	Forward Primer: 5' - AGA CAG ACA TTA GCT CAG TA – 3' Reverse Primer: 5' - GGC AAA GAC CAC GTC CAT TC – 3'	58.9^{0}C	RsaI	Astrin et al. (1991)

Statistical Analysis

The allele frequencies were calculated from the genotype count data by the gene counting method following Mourant et al. (1976). For each marker, the Hardy-Weinberg Chi-square (χ^2_{HW}) test was applied to check significant deviations, if any, from the genetic equilibrium in the studied caste populations of the Jammu district. The contingency Chi-square (χ^2) test was used to study inter-population differences. The extent of gene differentiation was determined by the method of Nei's measures of gene diversity (Nei 1973), and genetic similarities and differences were studied by Nei's genetic distance measure *D* (Nei 1972). The dendrograms were constructed using program PHYLIP, version 3.695 (Felsenstein 2005).

RESULTS

The distribution of genotypes and allele frequencies of the five studied SNPs viz., NAT2, ADH2, PSCR, T2, and ALAD in the Brahmin, Rajput, and Bania populations of Jammu district of Jammu and Kashmir (J&K) is presented in Table 2. All markers were found to be highly polymorphic in each of the present caste populations. The goodness of fit Hardy-Weinberg (χ^2_{HW}) test revealed statistically non-significant differences between the observed and expected genotype distributions for all the studied SNPs suggesting that each studied population was in genetic equilibrium.

All markers showed three possible genotypes in the present castes (++, +-, - -) (Table 2). The table shows that the frequency of the + allele of NAT2 polymorphism was observed 0.6623 in the Brahmin, which was comparatively higher than that recorded in the Rajput (0.5677) and Bania (0.5197) populations. The + allele frequency of the ADH2 marker was found to be highest in the Rajput (0.4677), followed by the Bania (0.4342), and the Brahmin showed a relatively low frequency of the allele (0.3182).The frequency of the + allele of the PSCR marker was recorded high in the Bania (0.4770), followed by the Brahmin (0.4156) and Rajput (0.3710). Like NAT2, for the T2 marker also the + allele frequency was found to be highest in the Brahmin (0.5584), followed by the Rajput (0.5290) and Bania (0.4803). The + allele frequency of ALAD was observed most elevated in the Brahmin (0.2597) and lowest in the Bania (0.1743), while the Rajput showed a value (0.1839) in between them.

Contingency Chi-square (χ^2)

The contingency Chi-square (χ^2) test values of the 5 studied SNPs viz., NAT2, ADH2, PSCR, T2, and ALAD in the present caste populations of the Jammu district of J&K are presented in Table 3. The table shows statistically significant differences in the genotype distribution of 3 SNPs (NAT2, ADH2, ALAD) between the present Brahmin and Rajput populations and between the Brahmin and Bania populations. On the other hand, such differences between the Rajput and Bania populations were observed only for a single SNP (PSCR). Therefore, this statistical test demonstrated comparatively more genotypic homogeneity between the latter caste populations of the Jammu district.

Heterozygosity

The heterozygosity (*h*) estimates and average heterozygosity (*H*) for the studied 5 SNP loci in the Brahmin, Rajput, and Bania populations of the Jammu district of J&K are presented in Table 4. The table shows that barring *ALAD* locus, all the remaining 4 SNP loci showed consistently high values of heterozygosity (*h*>40%) in the studied populations, ranging from 0.4473 in the Brahmin to 0.4992 in the Bania at *NAT2* locus, ranging from 0.4339 in the Brahmin to 0.4979 in the Rajput at *ADH2* locus, ranging from 0.4667 in the Rajput to 0.4989 in the Bania at *PSCR* locus, ranging from 0.4932 in the Brahmin to 0.4992 in the Bania at *T2* locus and ranging from 0.2878 in the Bania to 0.3845 in the Brahmin at *ALAD* locus. The average heterozygosity (*H*) was 0.4489 in the Brahmin, 0.4508 in the Rajput, and 0.4553 in the Bania, suggesting that the latter caste was genetically more heterogeneous than the former two castes (Table 4).

Gene Diversity

The results of gene diversity analysis of the five studied SNPs in the Brahmin, Rajput, and Bania populations of the Jammu district are given in Table 5.The intra-populational gene diversity (H_S) varied widely from 0.3242 at the *ALAD* locus

Table 2: Distribution of genotypes and allele frequencies and goodness of fit Chi-square (χ^2_{HW}) test values of different SNPs in the caste populations of Jammu district of Jammu and Kashmir

SNP	*Population*	*n*		*Genotype count*			*Allele frequency*		(χ^2_{HW}) *(d.f. 1)*	*p*
				+ +	+ -	- -	+	-		
NAT2	Brahmin	154	Observed	72 (46.75)	60 (38.96)	22 (14.29)	0.6623	0.3377	2.5611	0.1095
			Expected	67.55	68.88	17.56				
	Rajput	155	Observed	47 (30.32)	82 (52.90)	26 (16.78)				
			Expected	49.96	76.07	28.96	0.5677	0.4323	0.9394	0.3324
	Bania	152	Observed	46 (30.26)	66 (43.42)	40 (26.32)				
			Expected	41.05	75.88	35.06	0.5197	0.4803	2.5776	0.1083
ADH2	Brahmin	154	Observed	18 (11.69)	62 (40.26)	74 (48.05)				
			Expected	15.59	66.81	71.59	0.3182	0.6818	0.8008	0.3708
	Rajput	155	Observed	32 (20.65)	81 (52.26)	42 (27.09)				
			Expected	33.91	77.17	43.91	0.4677	0.5323	0.3802	0.5374
	Bania	152	Observed	25 (16.45)	82 (53.95)	45 (29.60)				
			Expected	28.65	74.68	48.66	0.4342	0.5658	1.4585	0.2271
PSCR	Brahmin	154	Observed	28 (18.18)	72 (46.75)	54 (35.07)				
			Expected	26.60	74.80	52.59	0.4156	0.5844	0.2166	0.6416
	Rajput	155	Observed	22 (14.19)	71 (45.81)	62 (40.00)				
			Expected	21.33	72.33	61.33	0.3710	0.6290	0.0531	0.8177
	Bania	152	Observed	31(20.39)	83 (54.61)	38 (25.00)				
			Expected	34.58	75.83	41.58	0.4770	0.5230	1.3553	0.2443
T2	Brahmin	154	Observed	53 (34.42)	66 (42.86)	35 (22.72)				
			Expected	48.02	75.94	30.03	0.5584	0.4416	2.6422	0.1040
	Rajput	155	Observed	48 (30.97)	68 (43.87)	39 (25.16)				
			Expected	43.38	77.23	34.38	0.5290	0.4710	2.2176	0.1364
	Bania	152	Observed	39 (25.66)	68 (44.74)	45 (29.60)				
			Expected	35.06	75.88	41.05	0.4803	0.5197	1.6398	0.2003
ALAD	Brahmin	154	Observed	9 (5.84)	62 (40.26)	83 (53.90)				
			Expected	10.39	59.22	84.38	0.2597	0.7403	0.3392	0.5602
	Rajput	155	Observed	7 (4.52)	43 (27.74)	105 (67.74)				
			Expected	5.24	46.51	103.24	0.1839	0.8161	0.8871	0.3462
	Bania	152	Observed	7 (4.61)	39 (25.66)	106 (69.73)				
			Expected	4.62	43.75	103.62	0.1743	0.8257	1.7984	0.1799

n = Sample size, + + = Homozygous for the presence of the restriction site, + - = Heterozygous for the presence and absence of restriction site, - - =Homozygous for the absence of restriction site, + = allele for the presence of restriction site, - = allele for the absence of restriction site.
Numbers in parentheses are percentages.

Table 3: Contingency Chi-square (χ^2) test (d.f. 2) values of different SNPs in the caste populations of Jammu district of Jammu and Kashmir

SNP	*Brahmin vs. Rajput*		*Brahmin vs. Bania*		*Rajput vs. Bania*	
	χ^2	*p*	χ^2	*p*	χ^2	*p*
NAT2	8.9907*	0.0111	11.2277*	0.0036	4.6813	0.0962
ADH2	15.2690*	0.0004	10.9719*	0.0041	0.9400	0.6250
PSCR	1.2755	0.5284	3.7029	0.1570	8.1948*	0.0166
T 2	0.4904	0.7825	3.3974	0.1829	1.3304	0.5141
ALAD	6.2594*	0.0437	8.2738*	0.0159	0.1706	0.9182

*Statistically significant ($p \leq 0.05$)

Table 4: Distribution of heterozygosity (*h*) and average heterozygosity (*H*) of different SNPs in the caste populations of the Jammu district of Jammu and Kashmir

SNP locus	*Heterozygosity (h)*		
	Brahmin	*Rajput*	*Bania*
NAT2	0.4473	0.4908	0.4992
ADH2	0.4339	0.4979	0.4913
PSCR	0.4858	0.4667	0.4989
T2	0.4932	0.4983	0.4992
ALAD	0.3845	0.3002	0.2878
Average heterozygosity (*H*)	0.4489	0.4508	0.4553

to 0.4969 at the *T2* locus with an average of 0.4517. The value of inter-subpopulational gene diversity (D_{ST}) ranged from a low of 0.0021 at *T2* to a high of 0.0082 at *ADH2* with an average of 0.0048. The gene diversity in the total population (H_T) fluctuated from 0.3271 at *ALAD* locus to 0.4990 at *T2* locus with an average of 0.4565. Thus in the present caste material of the Jammu district, the gene diversity in the total population (0.4565) was found to be primarily attributable to intra-subpopulation gene diversity (0.4517), and only a tiny fraction was due to inter-subpopulational gene diversity (0.0048). The coefficient of gene differentiation (G_{ST}), a measure of gene differentiation, ranged from a low of 0.0042 at the *T2* locus to as high as 0.0170 at the *ADH2* locus, showing that the former locus was the least differentiated and the latter was the most differentiated in the present Jammu populations. The average G_{ST} value over all five loci in the present material was recorded 0.0105, which demonstrated moderate genetic differentiation in the present caste populations of Jammu district of J&K.

Genetic Distance

A UPGMA (unweighted pair group method with arithmetic mean) dendrogram was constructed (Fig. 1) to comprehend the overall genomic similarities and differences in the present caste material of the Jammu district, based on *D* (genetic distance) values. The figure showed that the Rajput and Bania were in close genetic affinities while the Brahmin population was distant from both of them.

DISCUSSION

The allele frequency results obtained from the present study on caste populations of Jammu dis-

Table 5: Measures of gene diversity (H_T, H_S, D_{ST}) and coefficient of gene differentiation (G_{ST}) estimates of different SNPs in the caste populations of Jammu district of Jammu and Kashmir.

SNP locus	*Total gene diversity of the population (H_T)*	*Intra-subpopulational gene diversity (H_S)*	*Inter-subpopulational gene diversity (D_{ST})(H_T-H_S)*	*Coefficient of gene differentiation (G_{ST})(D_{ST} / H_T)*
NAT2	0.4861	0.4791	0.0070	0.0114
ADH2	0.4826	0.4744	0.0082	0.0170
PSCR	0.4876	0.4838	0.0038	0.0077
T2	0.4990	0.4969	0.0021	0.0042
ALAD	0.3271	0.3242	0.0029	0.0089
Average	0.4565	0.4517	0.0048	0.0105

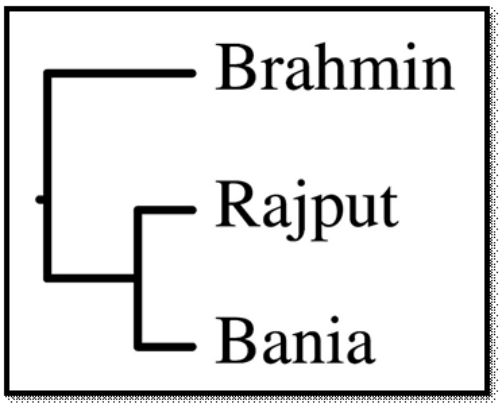

Fig.1. UPGMA dendrogram constructed using data from 5 studied SNPs showing genetic relationships among the present Brahmin, Rajput, and Bania populations of the Jammu district of Jammu and Kashmir

trict of North Indian state of Jammu and Kashmir were compared with such data available in the literature on other populations of North India inhabiting the states of Punjab, Rajasthan, and Uttar Pradesh (Table 6). The frequency of the + allele for NAT2 polymorphism ranged from 0.519 to 0.662 in the present Jammu populations, which fitted into the range reported in various North Indian populations (0.423-0.730). For the ADH2 marker, the frequency of the + allele in the present populations ranged from 0.318 to 0.467, which fitted in the range observed in different North Indian populations (0.429-0.519). The frequency of the + allele of the PSCR marker in the present Jammu populations (range 0.371 to 0.477) fitted in the range found in populations of North India (0.176-0.475). For the T2 marker, the frequency of the + allele ranged from 0.480 to 0.558 in the present populations, which fitted in the range observed in the populations of North India (0.407-0.650). The ALAD marker + allele frequency range in the present Jammu populations (0.174-0.259) did not fit into the range found in the North Indian populations (0.571-0.602). Thus, in terms of the allele frequencies, barring ALAD, the + allele frequencies observed in present caste populations of the Jammu district of Jammu and Kashmir were similar to those of various North Indian populations.

Table 7 shows values of average heterozygosity and genic differentiation in populations of North India. The range of average heterozygosity (*H*) in the present populations of Jammu district of J&K (0.4489-0.4553) was higher than that reported in five populations of Punjab (0.3816-0.4163, Singh et al. 2016) but lower than that of two Tharu tribal groups of Uttar Pradesh (0.462-0.467, Chakrabarti et al. 2002). Therefore, the present populations showed higher genetic vari-

Table 6: Distribution of various SNPs in populations inhabiting different geographical regions of North India

Region *State* *District*	*Population*	*Sample size*	*Frequency of the + allele*					*Reference*
			NAT2	*ADH2*	*PSCR*	*T2*	*ALAD*	
NORTH INDIA								
Jammu and Kashmir								
Jammu district	Brahmin	154	0.662	0.318	0.415	0.558	0.259	Present study
Jammu district	Rajput	155	0.567	0.467	0.371	0.529	0.183	
Jammu district	Bania	152	0.519	0.434	0.477	0.480	0.174	
Punjab	Bania	184	-	-	-	0.497	-	Singh et al. (2016)
	Brahmin	194	-	-	-	0.546	-	
	Jat Sikh	208	-	-	-	0.461	-	
	Khatri	199	-	-	-	0.450	-	
	Scheduled Castes	236	-	-	-	0.466	-	
Uttar Pradesh	Katharia Tharu	37	0.730	0.429	0.203	0.514	0.571	Chakrabarti et al. (2002)
	Rana Tharu	54	0.722	0.519	0.176	0.407	0.602	
Rajasthan	Bhil	47	0.659	-	0.475	0.619	-	Dada et al. (2011)
	Mina	31	0.466	-	0.285	0.466	-	
	Saharia	30	0.703	-	0.469	0.560	-	
	Garasia	53	0.423	-	0.437	0.423	-	
	Damaria	26	0.532	-	0.450	0.514	-	
	Rajput	34	0.613	-	0.424	0.650	-	

Table 7: Average heterozygosity and genic differentiation in populations of North India

State	*Population*	*Number of markers*	*Average heterozygosity (H)*	*Genic differentiation (G_{ST} / F_{ST})*	*Reference*
Jammu and Kashmir	Brahmin	5	0.4489	0.0105	Present study
	Rajput	5	0.4508		
	Bania	5	0.4553		
Punjab	Brahmin	7	0.4023	0.0166	Singh et al. (2016)
	Bania	7	0.3816		
	Jat Sikh	7	0.3888		
	Khatri	7	0.4163		
	Scheduled Castes	7	0.4013		
Uttar Pradesh	Katharia Tharu	25	0.467	-	Chakrabarti et al. (2002)
	Rana Tharu	25	0.462		
Rajasthan	Bhil	12	-	0.049	Dada et al. (2011)
	Mina	12	-		
	Saharia	12	-		
	Garasia	12	-		
	Damaria	12	-		
	Rajput	12	-		

ation than the populations of Punjab, but the extent of variation was lower than the populations of Uttar Pradesh. As for the measure of genic differentiation, the present populations showed a value (G_{ST}=0.0105) similar to populations of Punjab (F_{ST}=0.0166**,** Singh et al. 2016) but much lower than that reported in populations of Rajasthan **(**G_{ST}=0.049, Dada et al. 2011**).** This suggests that the degree of genic differentiation with respect to the studied loci was comparable to populations of Punjab but was much lower than that of populations of Rajasthan.

A UPGMA dendrogram was constructed using genetic distance (*D*) values based on a database available on three common SNPs viz., NAT2, PSCR, and T2 (Fig. 2) for the present populations and select North Indian populations, in this case, populations of Rajasthan and Uttar Pradesh, to understand the overall genomic similarities and differences among them. The figure showed that the Brahmin population of the Jammu district of Jammu and Kashmir was placed together in a sub-cluster with the Saharia population of Rajasthan. In contrast, the present Rajput and Bania populations were positioned in a sub-cluster along with the Damaria population of Rajasthan.

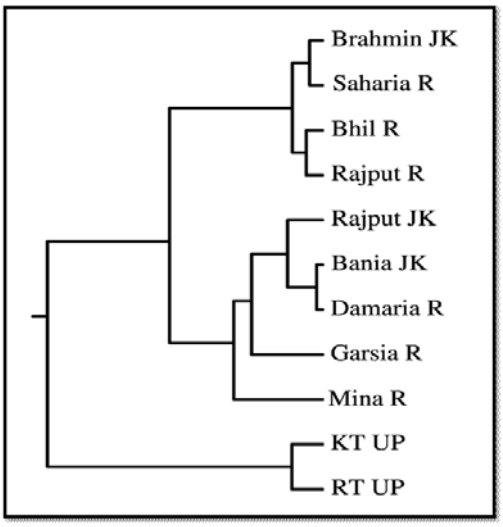

Fig. 2. UPGMA dendrogram constructed using data from 3 common SNPs (NAT2, PSCR, and T2) among the present caste populations of Jammu and Kashmir and various other populations reported from North Indian states of Jammu and Kashmir (JK), Rajasthan (R), and Uttar Pradesh (UP)
KT= Katharia Tharu, RT= Rana Tharu

CONCLUSION

The present genomic study on the Brahmin, Rajput, and Bania caste populations of the Jammu district of the North Indian state of Jammu and Kashmir revealed a high level of genetic variability, and they were moderately differentiated. The genetic distance analysis demonstrated that the Rajput population was in close genetic affin-

ities with the Bania population than the Brahmin population. In general, the allele frequencies of different SNPs in the present caste populations of Jammu and Kashmir fitted into the range reported in various other populations of North Indian states of Punjab, Rajasthan, and Uttar Pradesh, and genetic distance analysis using limited common markers and populations demonstrated close genetic affinities with populations of Rajasthan.

RECOMMENDATIONS

Further studies on populations inhabiting other districts of Jammu and Kashmir using the present and other molecular markers are required to fully appreciate the genomic diversity present in the people of this North Indian state.

ACKNOWLEDGMENT

Anu Raina is thankful to the University Grants Commission, Govt. of India, New Delhi, for the financial assistance for this work.

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Paper received for publication in February, 2021
Paper accepted for publication in March, 2021