J Hum Ecol, 68(1-3): 11-15 (2019)
PRINT: ISSN 0970-9274 ONLINE: ISSN 2456-6608
DOI: 10.31901/24566608.2019/68.1-3.3163

Distribution of Human-Specific *Alu* Insertion/Deletion Polymorphisms in the Gaddi Tribe of Kangra District, Himachal Pradesh (North India)

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KEYWORDS Gaddi Brahmin. Gaddi Rajput. Genetic Differentiation. Genetic Affinities

ABSTRACT The objective of the study was to characterize genetically two major Gaddi tribe populations of Himachal Pradesh viz., the Gaddi Brahmin and Gaddi Rajput inhabiting the Kangra district. For this, a total of 325 subjects were studied for the distribution of 6 *Alu* markers - ACE, APO, PV92, CD4, PLAT and TPA25 using standard techniques. The results demonstrated genetic homogeneity between the present two Gaddi populations and the degree of genetic differentiation was observed low (G_{ST} = 0.0019). The genetic distance analysis revealed that the present Gaddi populations have close genetic affinities.

INTRODUCTION

The people of the Indian subcontinent present a great diversity of cultural, morphological, linguistic and genetic traits (Majumder 1998). Immigrations over several millennia in the pre-historic and historical times into the region facilitated gene flow that introduced new genes that altered the genetic composition of the indigenous people resulting in considerable genetic heterogeneity in the contemporary populations of India.

The early genetic studies on the people of India were based on distribution of various blood groups, serum proteins and red-cell enzyme polymorphisms (Mourant et al. 1976; Tills et al. 1983; Bhasin et al. 1992). More recently, DNA polymorphisms including *Alu* Insertion/deletion (Ins/Del) have provided new tools to investigate genetic variation in man. The *Alu* Ins/Del markers have been reported to be highly polymorphic and have been widely used in human variation studies worldwide (Batzer and Deininger 1991; Stoneking et al. 1997).

Himachal Pradesh is home to eight tribes, viz., the Bhot/Bodh, Gaddi, Gujjar, Jad/Lamba/Khampa, Kanaura/Kinnara, Lahaula, Pangwala and Swangla (Census of India 1981).

The Gaddi is the most dominant tribe inhabiting the Chamba and Kangra districts of the state.

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Objectives

The main objectives of the present study were first, to establish the genomic profile of the Gaddi Brahmin and Gaddi Rajput populations inhabiting Kangra district of Himachal Pradesh using a battery of 6 *Alu* insertion/deletion markers viz., ACE, APO, PV92, CD4, PLAT and TPA25. Second, to study the extent of genetic variation, degree of gene differentiation and genetic relationships among the studied populations.

MATERIAL AND METHODS

After informed consent, intravenous blood samples (3-5 ml each) were collected in EDTA. Na_2 vials from a total of 325 subjects (150 Gaddi Brahmin and 175 Gaddi Rajput) from the Kangra district of Himachal Pradesh. Ethical clearance for this study was obtained from the Institutional Ethical Committee of Punjabi University, Patiala (Punjab).

Laboratory Analysis

In the laboratory at Punjabi University, Patiala DNA was extracted from the blood samples following the salting out method of Miller et al. (1988). After the quality and quantity checks the extracted DNA samples were amplified by the PCR technique using primer sequences given by Majumder et al. (1999) and Stoneking et al. (1997). The amplified PCR products were genotyped for *Alu* insertion/deletion markers ACE,

APO, PV92, CD4, PLAT and TPA25 on 2% agarose gel using standard techniques and the results were visualized in a UVP GelDoc-It Imaging System and recorded.

Statistical Analysis

The allele frequencies were calculated by the gene counting method. The genotype distribution was checked for the Hardy-Weinberg equilibrium by the Goodness of Fit Chi-square (χ^2) test. The inter-population differences were studied by the Contingency χ^2 test. The gene diversity was estimated following Nei (1973). The genetic relationships among the present and other populations reported from North India were assessed using the genetic distance measure *D* (Nei 1972) and a dendrogram was constructed using program PHYLIP, version 3.695 (Felsenstein 2005). To estimate the relative amount of gene flow from another population (external source) experienced by the Gaddi Brahmin and Gaddi Rajput, a regression analysis of the mean observed heterozygosity on distance from centroid was done considering the 12 populations of North India following Harpending and Ward (1982).

RESULTS

The distribution of genotypes and allele frequencies of the six *Alu* markers viz., ACE, APO, PV92, CD4, PLAT and TPA25 in the Gaddi Brahmin and Gaddi Rajput populations of Kangra district of Himachal Pradesh are listed in Table 1. Barring *Alu* PV92 (χ^2 HW =4.2743, d.f. =1, $p<0.05$) and *Alu* TPA25 (χ^2 HW =4.3118, d.f. =1, $p<0.05$) in the Gaddi Rajput population, the goodness of fit χ^2 test revealed statistically non-significant differences between the observed and expected genotype distributions, demonstrating that the present populations were in genetic equilibrium for the studied markers. The pair-wise comparisons were done by performing the Contingency Chi-square (χ^2) test using genotype numbers of six studied *Alu* Ins/Dels and no heterogeneity was found between the studied populations ($p>0.05$).

Heterozygosity

The heterozygosity (*h*) values for the studied 6 *Alu* Ins/Del markers along with the average heterozygosity (*H*) over markers are presented in Table 2. All markers were found to be highly polymorphic in Gaddi Brahmin and Gaddi Rajput populations. It was observed that barring *Alu* CD4 and *Alu* APO, the remaining four genetic markers showed consistently high levels of *h* (>40%) in both the populations. *h* was found high at most of the markers ranging from 0.4851 at *Alu* PV92 to 0.4999 at *Alu* TPA25 in the Gaddi Brahmin and ranging from 0.4788 at *Alu* ACE to 0.4927 at *Alu* PLAT in the Gaddi Rajput. The average heterozygosity (*H*) over all the six markers was found to be almost same in the Gaddi Brahmin (0.3856) and Gaddi Rajput (0.3878) (Table 2).

Gene Diversity

The results of gene diversity analysis are given in Table 3. The intra-subpopulational gene

Table 1: Distribution of allele frequencies and goodness of fit χ^2 HW test values of 6 studied *Alu* Ins/Del markers studied in the Gaddi Brahmin and Gaddi Rajput populations of Kangra district of Himachal Pradesh

Marker	*Gaddi Brahmin*					*Gaddi Rajput*				
	Genotypes			*Allele frequencies*		*Genotypes*			*Allele frequencies*	
	++ (*II*)	+- (*ID*)	-- (*DD*)	+ (*I*)	- (*D*)	++ (*II*)	+- (*ID*)	-- (*DD*)	+ (*I*)	- (*D*)
Alu ACE	43	70	28	0.5532	0.4468	63	85	27	0.6029	0.3971
Alu APO	123	24	0	0.9184	0.0816	139	31	5	0.8829	0.1171
Alu PV92	20	80	45	0.4138	0.5862	23	98	54	0.4114	0.5886
Alu CD4	112	29	1	0.8908	0.1092	142	32	1	0.9029	0.0971
Alu PLAT	42	83	21	0.5719	0.4280	50	95	29	0.5603	0.4397
Alu TPA25	31	82	32	0.4966	0.5034	25	99	51	0.4257	0.5743

+ (*I*) =Insertion allele, - (*D*) =Deletion allele

Table 2: Distribution of heterozygosity (*h*) and average heterozygosity (*H*) of the 6 studied *Alu* Ins/Del markers in the Gaddi Brahmin and Gaddi Rajput populations of Kangra district of Himachal Pradesh

Marker	*Heterozygosity (h)*	
	Gaddi Brahmin	*Gaddi Rajput*
Alu ACE	0.4943	0.4788
Alu APO	0.1499	0.2068
Alu PV92	0.4851	0.4843
Alu CD4	0.1946	0.1753
Alu PLAT	0.4897	0.4927
Alu TPA25	0.4999	0.4889
Average heterozygosity (*H*)	0.3856	0.3878

diversity (H_S) varied widely from 0.1783 at *Alu* APO to 0.4945 at *Alu* TPA25. The value of D_{ST} (inter-subpopulational gene diversity) varied from a low of 0.0001 at *Alu* PLAT to a high of 0.0024 at *Alu* TPA25 and it was found nil in case of *Alu* PV92 and *Alu* CD4. The total genomic diversity (H_T) based on all 6 markers was found to be 0.3874. Most of this diversity was attributable to intra-subpopulational gene diversity (H_S = 0.3867) and only a small fraction was due to inter-subpopulational gene diversity (D_{ST} = 0.0007).

The coefficient of gene differentiation (G_{ST}) was found to be nil at *Alu* PV92. G_{ST} value ranged from a low of 0.0001 at *Alu* PLAT to as high as 0.0050 at *Alu* TPA25, indicating that the former marker was the least differentiated and the latter marker was the most differentiated in the present studied Gaddi tribe populations of Himachal Pradesh. The average G_{ST} value over all SNP loci was recorded 0.0019 which demonstrated little genetic differentiation in the Gaddi Brahmin and Gaddi Rajput.

DISCUSSION

The results obtained from the present work were compared with such data available on other populations of North India. Due to the paucity of the studies from Himachal Pradesh on all the 6 *Alu* Ins/Del markers included in the present study, a database available on common 5 *Alu* Ins/Del markers viz., ACE, APO, PV92, CD4 and PLAT on 10 populations reported from North India viz., the Brahmin, Rajput, Gujjar and Jat Sikh of Jammu and Kashmir (Panjaliya et al. 2012), the Brahmin, Khatri, Scheduled Castes and Jat Sikh of Punjab (Kaur et al. 2002) and the Katharia Tharu and Rana Tharu of Uttar Pradesh (Chakrabarti et al. 2002) was considered.

Table 4 presents the distribution of insertion + (*I*) allele frequencies in various populations of North Indian states. The frequency of the allele for marker *Alu* ACE ranged from a low of 0.3800 in the Gujjar to a high of 0.7200 in the Brahmin of Jammu and Kashmir. For marker *Alu* APO, it varied from 0.4400 in the Gujjar of Jammu and Kashmir to 0.9270 in the Jat Sikh of Punjab. *Alu* PV92 exhibited low frequency of + (*I*) allele in most of them, varying from 0.3000 in the Jat Sikh population of Jammu and Kashmir to 0.8210 in the Rana Tharu of Uttar Pradesh. Barring Katharia Tharu (0.0140) and Rana Tharu (0.0190) of Uttar Pradesh, the frequency of the allele for the *Alu* CD4 marker was observed high in each of the 10 populations of North India, ranging from 0.8400 in the Jat Sikh of Jammu and Kashmir to 0.9479 in the Scheduled Castes of Punjab. In *Alu* PLAT

Table 3: Measures of gene diversity (H_T, H_S, D_{ST}) and coefficient of gene differentiation (G_{ST}) estimates of the 6 studied *Alu* Ins/Del markers investigated in the Gaddi Brahmin and Gaddi Rajput populations of Kangra district of Himachal Pradesh

Marker	*Total gene diversity of the population (H_T)*	*Intra-subpopulational gene diversity (H_S)*	*Inter-subpopulational gene diversity (D_{ST})*	*Coefficient of gene differentiation (G_{ST})*
Alu ACE	0.4878	0.4866	0.0012	0.0025
Alu APO	0.1789	0.1783	0.0006	0.0035
Alu PV92	0.4847	0.4847	0.0000	0.0000
Alu CD4	0.1850	0.1849	0.0000	0.0004
Alu PLAT	0.4913	0.4912	0.0001	0.0001
Alu TPA25	0.4969	0.4945	0.0024	0.0050
Average	0.3874	0.3867	0.0007	0.0019

Table 4: Distribution of Insertion + (*I*) allele frequency of 5 *Alu* Ins/Del markers common among the present Gaddi populations of Himachal Pradesh and various other populations reported from North Indian states

State	*Population*	*Frequency of Insertion + (I) Allele*					*Reference*
		ACE	*APO*	*PV92*	*CD4*	*PLAT*	
Jammu and Kashmir	Brahmin	0.7200	0.6150	0.5500	0.8900	0.5500	Panjaliya et al. (2012)
	Rajput	0.5750	0.7400	0.4750	0.8600	0.6000	
	Gujjar	0.3800	0.4400	0.3600	0.8700	0.5700	
	Jat Sikh	0.6200	0.8100	0.3000	0.8400	0.5600	
Himachal Pradesh	Gaddi Brahmin	0.5532	0.9184	0.4138	0.8908	0.5719	Present study
	Gaddi Rajput	0.6029	0.8829	0.4114	0.9029	0.5603	
Punjab	Brahmin	0.4895	0.9062	0.3541	0.9166	0.4468	Kaur et al. (2002)
	Khatri	0.6250	0.8750	0.4062	0.9375	0.5119	
	S.C.	0.4791	0.9062	0.5000	0.9479	0.4896	
	Jat Sikh	0.6979	0.9270	0.3958	0.8437	0.4559	
Uttar Pradesh	Katharia Tharu	0.5690	0.8470	0.6940	0.0140	0.8190	Chakrabarti et al. (2002)
	Rana Tharu	0.6230	0.7260	0.8210	0.0190	0.7920	

S.C.=Scheduled Castes

the + (*I*) frequency varied from 0.4468 in the Brahmin of Punjab to 0.8190 in the Katharia tharu of Uttar Pradesh. The allele frequencies observed in the present Gaddi Brahmin and Gaddi Rajput populations inhabiting Kangra district of Himachal Pradesh fitted well in these ranges.

To comprehend the overall genetic similarities and differences among 12 North Indian populations, an UPGMA (unweighted pair group method with arithmetic mean) dendrogram (Fig. 1) was constructed using the Nei's genetic distance (*D*) matrix. The figure shows that the Gujjar population of Jammu and Kashmir separated in a single line cluster from the remaining 11 populations of North India at an early stage of evolution. The Katharia Tharu and Rana Tharu of Uttar Pradesh were placed together in one sub-cluster. The Khatri, Jat Sikh, Brahmin and Scheduled Caste populations of Punjab were positioned together with the Jat Sikh, Brahmin and Rajput of Jammu and Kashmir and the present Gaddi populations in another sub-cluster. It is interesting to note that the Gaddi Brahmin and Gaddi Rajput populations inhabiting Kangra district of Himachal Pradesh were placed together in a sub-cluster demonstrating their close genetic affinities.

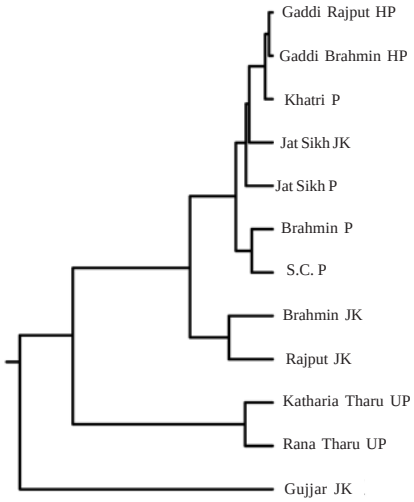

Fig. 1. UPGMA dendrogram based on 5 *Alu* Ins/Del markers common among the present 2 Gaddi populations of Himachal Pradesh and 10 other populations reported from North India: JK=Jammu and Kashmir, HP=Himachal Pradesh, P=Punjab, UP= Uttar Pradesh, S.C.=Scheduled Castes

The centroid analysis was performed by plotting average heterozygosity *(H)* of populations against the genetic distance from centroid (*rii*) (Fig. 2). The figure shows that the present Gaddi Brahmin and Gaddi Rajput populations exhibited lower heterozygosity values than predicted by the model and appeared below the theoretical regression line suggesting lesser gene flow from external source (another population) experienced by them.

CONCLUSION

With little genetic differentiation observed between the Gaddi Brahmin and Gaddi Rajput

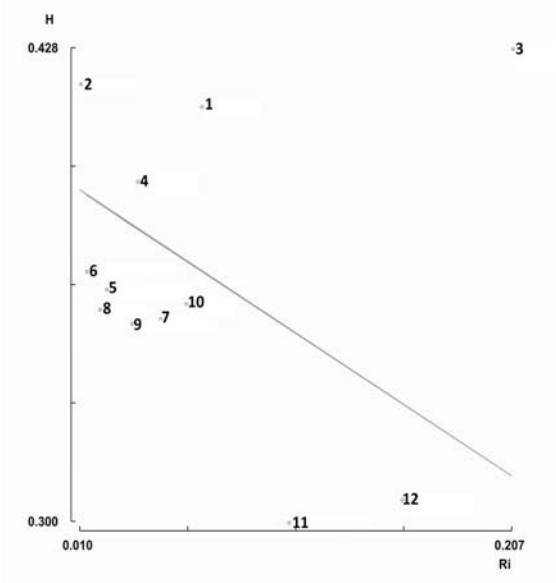

Fig. 2. Average heterozygosity versus distance from centroid (*rii*) of 5 *Alu* Ins/Del markers common among the present 2 Gaddi populations of Himachal Pradesh and 10 other populations reported from North India.
1=Brahmin, 2=Rajput, 3=Gujjar, 4=Jat Sikh (Jammu and Kashmir);
5=Gaddi Brahmin, 6=Gaddi Rajput (Himachal Pradesh) 7=Brahmin, 8=Khatri, 9=Scheduled Castes, 10=Jat Sikh (Punjab);
11=Katharia Tharu, 12=Rana Tharu (Uttar Pradesh)

populations inhabiting Kangra district of Himachal Pradesh, the present study demonstrated their underlying genomic uniformity. The genetic distance analysis also revealed close genetic affinities between the present two Gaddi populations.

RECOMMENDATION

To fully appreciate the genomic diversity in the people of Himachal Pradesh further studies using molecular markers, including *Alu* insertion/deletion markers, are required.

ACKNOWLEDGMENT

Manju Bala is thankful to DST, Govt. of India, New Delhi for the financial assistance for this work.

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Paper received for publication in August, 2019
Paper accepted for publication in August, 2019