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Human- specific *Alu* Insertion/Deletion Polymorphisms in Various Population Groups of Jammu Region

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ABSTRACT In present study, six *Alu* insertion/deletion polymorphisms (*Alu* ACE, *Alu* APO, *Alu* PV-92, *Alu* PLAT, *Alu* FXIIIB and *Alu* D1) were studied in five different populations (Brahmins, Rajputs, Scheduled Castes, Gujjars and Jat Sikhs) of Jammu region. Blood samples were collected randomly from 400 unrelated healthy individuals. DNA was extracted and amplified by PCR using six target specific Oligonucleotide primers of *Alu* family and finally subjected to Agarose gel electrophoresis. Allele frequencies were used to calculate average heterozygosity. All the six markers were found to be highly polymorphic with high heterozygosity values. The genetic distance analysis revealed a close genomic affinity between Schedule Castes and *Jat* Sikhs suggesting recent diversification of these two population groups whereas *Gujjars* and Brahmins stood apart genetically.

INTRODUCTION

Alu insertion elements are the most abundant class of short interspersed elements (SINEs) in primate genomes, being present in approximately 1.2 million copies in the human genome and having been found in every primate sequenced thus far (Lander et al. 2001; Watkins 2003; Chimpanzee Sequencing and Analysis Consortium 2005; Gibbs et al. 2007; Locke et al. 2011).These elements mobilize through an RNA polymerase III intermediate by the process called retropostion into a new position in the genome (Rogers, 1983; Luan et al. 1993; Luan and Eickbush 1995; Cost et al. 2002). Some *Alu* elements have retroposed so recently that they are not yet fixed and their insertion at specific location of genome is polymorphic. This polymorphic nature of *Alu* elements make them ideal for studying different aspects of evolutionary biology, genetic ancestry and relatedness. The insertion of an *Alu* element can be regarded as a unique event as once inserted, these elements are rarely removed. *Alu* elements are homoplasy free markers as their ancestral state at any locus is known to be the absence of *Alu* element (Batzer and Deininger 1991; Murata et al. 1993; Batzer et al. 1994; Shedlock and Okada 2000; Okada et al. 2004; Ray et al. 2006; Ray 2007; Xing et al. 2007). These properties of polymorphic *Alu* elements makes them a better option in the recent years to trace human ancestry and phylogenetic relationships (Batzer et al. 1996; Stoneking et al. 1997; Majumder et al. 1999; Watkins et al.2001).

Jammu and Kashmir is well known for its natural, cultural and genetic heritage and has remained unexplored with respect to study of genomic diversity as well as phylogenetic study.

In this context, the researchers have analyzed six *Alu* (*Alu* ACE, *Alu* APO, *Alu* PV-92, *Alu* PLAT, *Alu* FXIIIB and *Alu* D1) insertion / deletion polymorphism in five different population groups of Jammu region namely Brahmins, Rajputs, Scheduled Castes, Gujjars and Jat Sikhs. All these population groups have a significant socio-cultural hierarchy in Indian culture.

The present study aims to generate the allele frequencies of the said *Alu* markers among five different selected populations of Jammu region.

MATERIAL AND METHODS

Study Population

In the present investigation 400 unrelated healthy individuals were selected from five different populations namely Brahmins (100), Rajputs (100), Scheduled Castes (100), Gujjars (50) and Jat Sikhs (50) of Jammu region. The brief description of the different populations is as follows:

Brahmins: The Brahmins of Jammu province have mainly been farmers by occupation with the exception of Mohyals, who were concentrated in Mirpur and were in Government jobs. Brahmin community is highly stratified in hierarchical order, at the top of which stands that clan of the Brahmins who claims to be the descendant of King Porus (Dewan 2007).

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Rajputs: Rajputs are the members of one of the major group of the 'Hindu Kshatriya Varna' in the Indian sub-continent particularly North-India. Rajput populations are found in Himachal Pradesh, Jammu, Punjab, Uttrakhand, Madhya Pradesh, Bihar, Gujarat, Maharashtra and Uttar Pradesh (Dewan, 2007).

Scheduled Caste (SC): They are the Indian population groupings that are explicitly recognized by the Constitution of India, previously called the "depressed classes" by the British. In the present study the Barwala, Chamar and Megh populations were included which are recognized as Scheduled Castes by Constitution (Jammu and Kashmir) Scheduled Castes Order, 1956.

Gujjars: The Gujjars are well known semi-nomadic pastoral community who were basically Hindus; however during the reign of Aurangzeb, they changed their religion and became Muslims (Pandita 2003).

Jat Sikhs: *Jat* Sikh refers to a sub group of the Sikh ethno-religious group from the Indian sub-continent. They comprise of at least half of the Sikh population in Punjab (Crenshaw 1995).

Blood Sampling

5 ml of blood was collected with prior informed consent of the individuals by venipuncture into EDTA coated vacutainers and were stored at –20°C until further analysis.

DNA Extraction

DNA extraction was carried out by phenol: chloroform method (Sambrook and Russell 2001) with slight modifications. Quantitative and qualitative analysis of DNA was carried out by both agarose gel electrophoresis and spectrophotometry. Target specific Oligonucleotide primers of six *Alu* (*Alu* ACE, *Alu* APO*, Alu* PLAT, *Alu* PV 92, *Alu* FXIIIB and *Alu* D1) markers were used to amplify the target loci as studied by Majumder et al. (1999). The primer sequence and their annealing temperature are given in Table 1.

Polymerase Chain Reaction

PCR reactions were carried out in a 25µl volume containing 100 ng DNA, 200 µM dNTPs, 1.5 mM $MgCl_2$, 25 ng each primer, 1.25 U Taq polymerase, 50 mM KCl 10 mM Tris – HCl (pH 8.4). 30 cycles of 94º C for 4 min, 58º C for 1 min, 72º C for 1 min were used for ACE; 30 cycles of 94º C for 4 min, 54º C for 1 min, 72º C for 1 min for PV92; 30 cycles of 94º C for 4 min, 50º C for 1 min, 72º C for 1 min for APO; 30 cycles of 94º C for 4 min, 60º C for 1 min, 72º C for 1 min for PLAT; 30 cycles of 94º C for 4 min, 56º C for 1 min, 72º C for 1 min for FXIIIB and 35 cycles of 94º C for 4 min, 60º C for 1 min, 72º C for 1 min were used for D1. PCR products were visualized under UV– light after separation in a 2% Agarose gel and ethidium bromide staining.

Statistical Analysis

Allele frequencies were calculated by gene counting method. Chi-square test was applied to calculate the significant difference between the observed and expected genotype frequencies. Heterozygosity values at individual loci and the average heterozygosity based on all loci were calculated using the estimated allele frequencies for each population according to Nei (1973). Extent of gene differentiation among the popu-

Table 1: Oligonucleotide primers and annealing temperature of loci studied

Locus	*Primer sequence*	*Annealing temp(°C)*
Alu ACE	5'-CTG GAG ACC ACT CCC ATC CTT TCT-3'5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'	58
Alu APO	5'-AAG TGC TGT AGG CCA TTT AGA TTA G-3'5'-AGT CTT CGA TGA CAG CGT ATA CAG A-3'	50
Alu PV92	5'-AAC TGG GAA AAT TTG AAG AGA AAG T-3'5'-TGA GTT CTC AAC TCC TGT GTG TTA G-3'	54
Alu PLAT	5'-GTG AAA AGC AAG GTC TAC CAG-3'5'-GAC ACC GAG TTC ATC TTG AC-3'	60
Alu FXIIIB	5'-TCA ACT CCA TGA GAT TTT CAG AAG T-3'5'-CTG GAA AAA ATG TAT TCA GGT GAG T-3'	56
Alu D1	5'-TGC TGA TGC CCA GGG TTA GTA AA-3'5'-TTT CTG CTA TGC TCT TCC GGT GAG T-3'	60

Table 2: Distribution of insertion allele frequencies of six *Alu* markers in five different human populations of Jammu region

Populations	*Alu ACE*	*Alu APO*	*Alu PV92*	*Alu PLAT*	*Alu FXIIIB*	*Alu D1*
Brahmins	0.72	0.615	0.55	0.55	0.485	0.44
Rajputs	0.575	0.74	0.475	0.60	0.65	0.49
Scheduled Castes	0.61	0.845	0.41	0.51	0.69	0.495
Gujjars	0.38	0.44	0.36	0.57	0.57	0.57
Jat Sikhs	0.62	0.81	0.30	0.56	0.67	0.5

Table 3: Showing Nei's expected heterozygosities at individual locus and average heterozygosity based on six loci in five population groups of Jammu region

Human populations	*Alu ACE*	*Alu APO*	*Alu PV92*	*Alu PLAT*	*Alu FXIIIB*	*Alu D1*	*All loci (SE)*
Brahmins	0.4032	0.4740	0.4950	0.4950	0.4995	0.4900	0.4761(0.0150)
Rajputs	0.4800	0.3800	0.4988	0.4800	0.4550	0.4900	0.4639(0.0178)
Scheduled Castes	0.4758	0.2660	0.4830	0.4900	0.4278	0.5000	0.4405(0.0363)
Gujjars	0.4712	0.4928	0.4608	0.4920	0.4902	0.4920	0.4834(0.0056)
Jatt Sikhs	0.4800	0.3000	0.4200	0.4900	0.4928	0.4900	0.4454(0.0312)

lation groups was assessed by performing gene diversity analysis separately for each locus and also for all loci jointly. Genomic relationships among the populations were assessed by constructing the dendrograms by neighbour-joining (NJ) using PHYLIP 3.5c software.

RESULTS

Distribution of insertion allele frequencies of six *Alu* (*Alu* ACE, *Alu* APO, *Alu* PV- 92, *Alu* PLAT, *Alu* FXIIIB and *Alu* D1) in five different populations of Jammu region of J&K are given in Table 2.

Genomic Diversity within Populations

The heterozygosity values of each locus and average heterozygosity based on all loci is given in Table 3. All loci are found to be highly polymorphic in most of the populations. Minimum heterozygosity value was found in *Alu* APO marker (0.2660 in Scheduled Caste). The maximum attainable heterozygosity value that is 0.50 for biallelic marker is observed in one marker (*Alu* D1 in Scheduled Caste). It is observed that the average heterozygosity value based on all loci in different populations is also high (> 0.40). The average heterozygosity values range from 0.4405 (Scheduled Caste) to 0.4834 (Gujjar).

Genomic Diversity between Populations

The result of gene diversity analysis for individual locus and all loci is given in Table 4. Total genomic diversity (H_T) based on all loci is 0.4757. Most of the genomic diversity is attributed to genomic diversity between individuals within populations (H_S). Inter- subpopulation gene diversity (D_{ST}) based on all loci is 0.0145. The extent of gene diversity between populations with respect to the total gene diversity, coefficient of gene differentiation (G_{ST}), varies from 0.0009 (*Alu* PLAT) to 0.0973 (*Alu* APO). G_{ST} based on all loci is 0.0306. Thus highest contribution to inter-population variability is made by *Alu* APO (9.7 %) and least by *Alu* PLAT (0.09 %). Based on all loci only 3 % of the total genomic diversity is attributable to between populations.

Table 4: Showing results of gene diversity analysis for the individual locus and for all loci considered jointly in five different populations of Jammu region

Locus	*Gene diversity in total population (H_T)*	*Gene diversity of sub-populations(H_S)*	*Inter sub-population gene diversity (D_{ST})*	*Coefficient of gene differentiation (G_{ST})*
Alu ACE	0.4800	0.4586	0.0214	0.0440
Alu APO	0.4200	0.3791	0.0409	0.0973
Alu PV92	0.4800	0.4795	0.0005	0.0014
Alu PLAT	0.4937	0.4890	0.0047	0.0009
Alu FXIIIB	0.4810	0.4683	0.0127	0.0260
Alu D1	0.4998	0.4927	0.0071	0.0142
All loci	0.4757	0.4612	0.0145	0.0306

F- Statistics

The measure of deviations of the genotypic frequencies from normal frequencies in terms of

Table 5: Showing the F-Statistics values for six different *Alu* markers in five different populations of Jammu region

Locus	H_I	H_S	H_T	F_{IS}	F_{ST}	F_{IT}
Alu ACE	0.4975	0.4586	0.4800	-0.0848	0.0445	-0.0364
Alu APO	0.3825	0.3791	0.4200	-0.0089	0.0973	0.0892
Alu PV92	0.4175	0.4795	0.4800	0.1293	0.0010	0.1302
Alu PLAT	0.4775	0.4890	0.4937	0.0235	0.0095	0.0328
Alu FXIIIB	0.4575	0.4683	0.4810	0.0230	0.0264	0.0488
Alu D1	0.3425	0.4927	0.4998	0.3040	0.0142	0.1573

heterozygosity deficiency or excess that is the inbreeding coefficient (F_{IS}) values range from -0.0089 (*Alu* APO) to 0.3040 (*Alu* D1). *Alu* ACE and *Alu* APO show excess of heterozygotes whereas other four markers show heterozygote deficiency Table 5. Co-ancestry coefficient or fixation index (F_{ST}) ranges from 0.0010 (*Alu* PV 92) to 0.0973 (*Alu* APO). The overall inbreeding coefficient (F_{IT}) values range from -0.0364 (*Alu* ACE) to 0.1573 (*Alu* D1)

Genomic Affinities between Populations

The genomic affinities among five human populations are depicted in unrooted neighbour-joining tree (Fig. 1) which reveals close genomic affinities between Scheduled castes and *Jat* Sikhs whereas Brahmins and *Gujjars* show genetic diversion from other populations. Genomic affinities of these populations along with the 14 ethnic Indian populations based on six polymorphic loci are depicted in Figure 2.

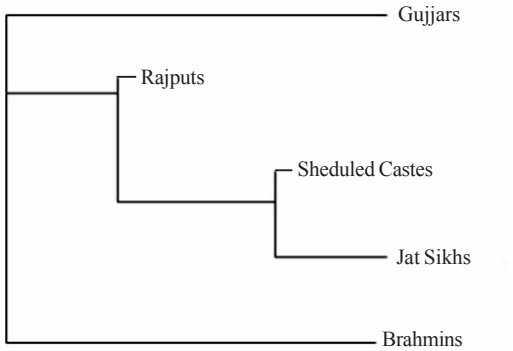

Fig. 1. Unrooted neighbour-joining tree depicting genomic affinities among five populations of Jammu region based on six human specific *Alu* insertion/ deletion polymorphisms

DISCUSSION

The allele frequencies and heterozygosity values observed in the present study are comparable with the previously studied populations of north India. High *Alu* insertion frequencies are observed in the present population groups except *Alu* PV92 when compared to other markers and *Alu* APO showed highest insertion frequencies in all populations which is in concordance with the allele frequencies observed by Saini et al. (2012) in same populations of north-west India. The *Alu* insertion frequencies are also found close to other Indian populations studied by different workers (Majumder et al. 1999; Kaur et al. 2002; Ravindranath et al. 2005; Vijaya et al. 2007; Yadav and Arora 2010). Most of the populations in present study exhibit high levels of gene diversity (> 0.40) with respect to the six *Alu* markers and minimum heterozygosity value is observed in *Alu* APO marker (0.2660 in Scheduled Caste). The maximum attainable heterozygosity value that is 0.50 for biallelic marker has been observed in one marker (*Alu* D1) in Scheduled Caste population. It is observed that the average heterozygosity value based on all loci in different populations is also high (> 0.40). The heterozygosity analysis reveals that gene diversity in the five different population groups (Rajputs, Brahmins, Scheduled Castes, Gujjars and Jat Sikhs) is quite high and consistent with the studies carried out in other Indian populations by different workers (Majumder et al. 1999; Yadav and Arora 2010; Kshatriya et al. 2011; Saini et al. 2012).

The total genomic diversity (H_T) for individual locus as well as for all loci when taken together is also quite high (>0.40). Total genomic diversity (H_I) based on all loci is 0.4757 and genomic diversity between individuals within populations (H_S) is found to be 0.4612 which are close to H_S values reported in other Indian populations (Majumder et al. 1999; Kanthimathi et al. 2007; Saini et al. 2012) but higher than the Manipur populations (Meitei et al. 2010). Intersubpopulation gene diversity (D_{ST}) based on all loci is 0.0145, the value close to Haryana populations (Yadav and Arora 2010). Coefficient of

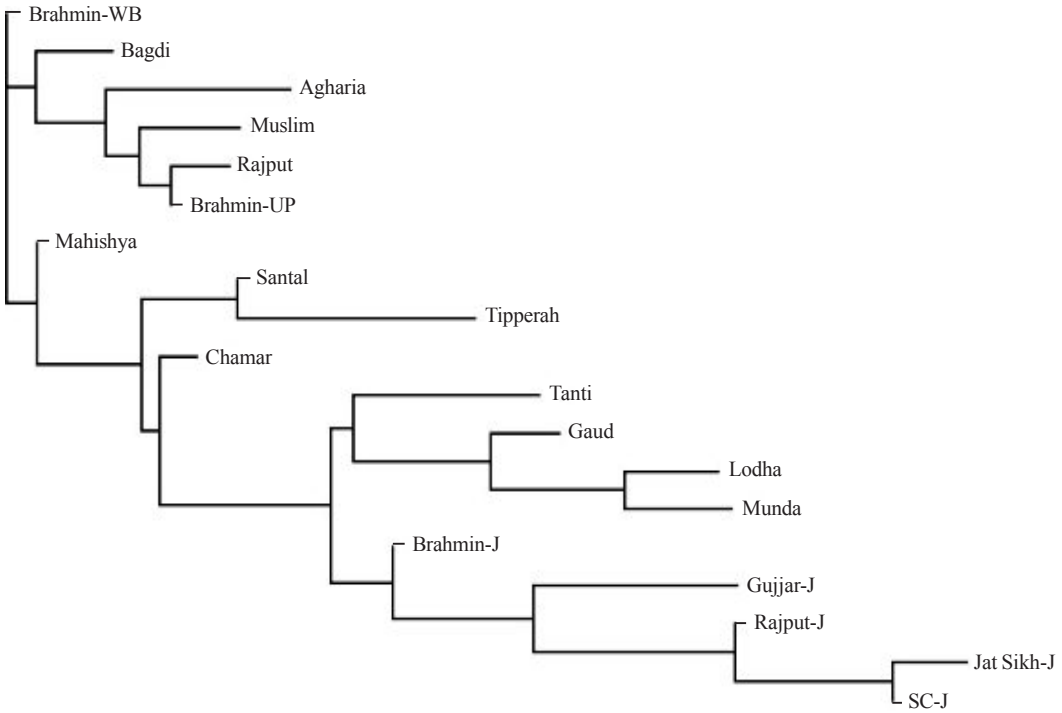

Fig. 2. Unrooted neighbour-joining tree depicting genomic affinities among nineteen populations (five populations from Jammu region and fourteen populations studied by Majumder et al. 1999) based on six human specific *Alu* insertion/deletion polymorphisms

gene differentiation (G_{ST}) based on all loci is 0.0306, which is close to the value based on eight polymorphic loci in eight populations groups of Haryana (Yadav and Arora 2010) and Dravidian caste populations of Tamil Nadu (Kanthimathi et al. 2007) but lower than population groups studied by Majumder et al. (1999) and Meitei et al. (2010). Thus only 3 % of the total genomic diversity is attributable to between-populations which is less than in other Indian populations (6.8 %) (Majumder et al. 1999).

The genomic affinities among the caste populations do not co-relate well with their socio-economic affiliation (Fig. 1). Jat Sikhs show close affinity with Scheduled Castes and their diversion seems to be a recent event in evolutionary history. Gujjars do not show close affinities with other four populations. Rajputs show close affinity with Scheduled Castes and *Jat* Sikhs. On reconstructing the dendrogram with the 14 other ethnic Indian populations (Majumder et al. 1999) based on six *Alu* (*Alu* ACE, *Alu* APO, *Alu* PV92, *Alu* PLAT, *Alu* FXIIIB and *Alu* D1) polymorphic loci the populations of Jammu region do not show close affinities with these populations (Fig. 2). The populations from Jammu region tend to form a cluster within the centre of the dendrogram. There is no clear cut separation into genetically distinct groups like the Brahmins from Jammu, UP and WB which do not show close affinities. The results obtained from the present study are in accordance with the other Indian populations studied so far using *Alu* insertion/deletion polymorphic markers. These Indian populations show not only higher degree of genomic differentiation among but also greater levels of heterozygosity than among most other global populations except Africa. Still there is need to generate more genetic data on North Indian populations and as well as other important neighbouring regions like Afghanistan, Pakistan, Iran and Iraq.

CONCLUSION

The Indian subcontinent is a rich mixture of endogamous groups, which are maintaining their genetic identity due to high degree of endogamy.

In order to fully understand the nature and extent of genetic heterogeneity of the Indian sub-continent, an extensive database on different polymorphic DNA markers is needed. Although, extensive data on various blood group markers, protein and enzyme polymorphic markers and HLA etc. is already available, only scanty data on DNA markers is available for North-West India. The present study is an effort to generate a basic gene frequency distribution data at six polymorphic *Alu* (*Alu* ACE, *Alu* APO, *Alu* PV92, *Alu* PLAT, *Alu* FXIIIB and *Alu* D1) in five different population groups of Jammu region namely Rajputs, Brahmins, Scheduled Castes, Gujjars and Jat Sikhs.

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