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Human-specific *Alu* insertion/deletion polymorphisms in Gujjar population of Jammu region of (J&K) State

RAKESH KUMAR PANJALIYA, PARVINDER KUMAR, VIKAS DOGRA and SUBASH GUPTA

Institute of Human Genetics, University of Jammu, Jammu Tawi - 180 006 (India).

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ABSTRACT

Human populations are polymorphic with respect to insertion/deletion to *Alu* elements, the short interspersed elements (SINEs) found in about 500 000 copies in multiple chromosomal locations of human genome. During present study four *Alu* insertion/deletion polymorphisms (*Alu* ACE, *Alu* APO, *Alu* PV-92, *Alu* PLAT) were studied in Gujjar population of Jammu region of J&K state. Blood samples of 50 unrelated healthy donors constituted the material for the present study. DNA was isolated and amplified by PCR using target specific Oligonucleotide primers and finally subjected to Agarose gel electrophoresis. All the four markers showed high insertion frequencies and high Heterozygosities values. The agreement with Hardy-Weinberg equilibrium was evaluated by chi-square test for goodness of fit for the differences between the observed frequencies and the expected frequencies. Two markers (*Alu* APO and *Alu* PV92) showed significant differences between the observed frequencies and the expected frequencies which can be attributed to small sample size. In near future this study may assist in the study of genomic diversity of other populations of the region.

Key words: SINEs, *Alu*, polymorphism, retroposition, genome, heterozygosity, insertion, deletion.

INTRODUCTION

Human genome contains a significant portion of repetitive DNA sequences. *Alu* insertion elements are the most abundant class of short interspersed elements (SINEs) in the human genome, numbering more than million per haploid genome (Watkins *et al.* 2003). The *Alu* family of repetitive elements was originally defined as a fraction of renatured repetitive DNA that was distinctively cleaved with the restriction enzyme *Alu*I (Houck *et al.*1979). *Alu* elements are derived from the 7SL RNA gene and they share about 90% sequence homology with 150 bp in the middle that is not found in the *Alu* family (Ullu *et al*. 1984). These elements mobilize by retroposition through an RNA polymerase III intermediate (Rogers, 1983). *Alu* elements first appeared in primate genome about 65 million years ago (Deininger and Daniels, 1986) and have since undergone amplification from a few master genes (Deininger *et al*. 1992) to their repetitive status today at a rate of approximately 8 X 10^{-3} *Alu* elements per year. Some *Alu* elements have retroposed so recently that they are not yet fixed that is their insertion in specific location of genome is polymorphic which make them useful markers for the population structure analysis. The advantage of *Alu* insertions as markers is that the ancestral state of polymorphism is always known – the absence of element since there is no mechanism for their removal after the insertion. The other advantage is uniqueness of insertion at specific location since there is no sequence specificity for insertion sites meaning that all loci carrying a particular *Alu* insertion derived from unique event and hence they are identical by descent. These properties are reason that polymorphic *Alu* insertions were used as markers

in numerous population structure analyses even at micro-geographical scale. During the present study, genomic DNA samples of 50 healthy individuals, randomly selected from Gujjar population from different geographic locations of Jammu region, were analyzed for the four *Alu* (*Alu* ACE, *Alu* APO, *Alu* PLAT and *Alu* PV 92) insertion / deletion polymorphism.

MATERIAL AND METHODS

Materials

Present study is an account of the genomic diversity with respect to *Alu* insertion/ deletion polymorphism amongst the Gujjar population of Jammu region. Before collecting the blood samples of the 50 randomly selected healthy individuals, the Gujjar community was apprised about the nature of work and outcomes of the study.

Methods

5 ml of whole blood was collected randomly from 50 healthy individuals of the Gujjar community of J&K State. These samples were stored at –20^{0}C until DNA was isolated. DNA was isolated by using phenol:chloroform method (Sambrook and Russel, 2001) and salting out method (Miller *et al*., 1988). Target specific Oligonucleotide primers of four *Alu* (*Alu* ACE, *Alu* APO, *Alu* PLAT and *Alu* PV 92) markers were used to amplify the target loci.

PCR reactions were carried out in a 25ìl volume containing 100 ng DNA, 200 µM dNTPs, 1.5 mMMgCl_2, 25 ng each primer, 1.25 U Taq polymerase,50 mM KCl 10 mM Tris – HCl (pH 8.4). 30 cycles of 94°C for 4 min, 58°C for 1 min, 72°C for 1 min were used for ACE in a thermocycler, 30 cycles of 94°C for 4 min, 54°C for 1 min, 72°C for 1 min were used for PV92, 30 cycles of 94°C for 4 min, 50°C for 1 min, 72°C for 1 min were used for APO and 30 cycles of 94°C for 4 min, 60°C for 1 min, 72°C for 1 min were used for PLAT. PCR products of each marker was visualized in UV– light after separation in a 2% Agarose gel and ethidium bromide staining.

Allele frequencies of all the four *Alu* polymorphic markers were calculated by gene counting method. The estimated allele frequencies were then used to apply the chi-square test to calculate whether the difference between the observed frequencies and the expected frequencies was significant or not. Heterozygosity of each individual *Alu* marker was calculated using Nei's 1973 method. Average heterozygosity was also calculated.

RESULTS

Distribution of four *Alu* (*Alu* ACE, *Alu* APO, *Alu* PV-92, *Alu* PLAT) insertion/deletion genotypes and allele frequencies in Gujjar population of Jammu region of J&K state are given in Table 1.The heterozygosity values in all the four markers and average heterozygosity value are given in Table 2.The observed frequencies and expected frequencies in all four *Alu* (*Alu* ACE, *Alu* APO, *Alu* PV92, *Alu* PLAT) markers with corresponding ÷^{2} values at one degree of freedom studied are given in Table 3.

Table 1: Distribution of four *Alu (Alu ACE, Alu APO, Alu PV92, Alu PLAT)* insertion genotypes and allele frequencies in Gujjar population of Jammu region of J&K state

Alu markers		II	ID	DD	Total	I	D	Total
Alu ACE	N	8	22	20	50	38	62	100
	Frequency	0.16	0.44	0.40	100	0.38	0.62	100
Alu APO	N	NIL	44	6	50	44	56	100
	Frequency	ZERO	0.88	0.12	100	0.44	0.56	100
Alu PV92	N	11	14	25	50	36	64	100
	Frequency	0.22	0.28	0.50	100	0.36	0.64	100
Alu PLAT	N	16	25	9	50	57	43	100
	Frequency	0.32	0.50	0.18	100	0.57	0.43	100

Table 2: Showing heterozygosity and average heterozygosity of four *Alu* *(Alu ACE, Alu APO, Alu PV92 and Alu PLAT)* polymorphic loci in 50 individuals of Gujjar population of Jammu region of J&K

Alu marker	Heterozygosity
Alu ACE	0.4712
Alu APO	0.4928
Alu PV92	0.4608
Alu PLAT	0.4920
Average heterozygosity	0.4805

DISCUSSION

In the present study *Alu* ACE frequency was calculated to be 0.38 and on making a comparison of the present findings with the available data on other different Indian populations it was found to be lower than the *Alu* ACE frequency as reported by Majumder *et al*. (1999) in 14 ethnic populations of India. *Alu* ACE frequency observed in the present study was also found lower when compared with the four ethnic populations groups from Punjab (Kaur *et al*., 2002), two tribal populations of South India (Veerraju *et al*., 2001),

Table 3: Showing the observed frequencies and expected frequencies in all four *Alu* (*Alu* ACE, *Alu* APO, *Alu* PV92, *Alu* PLAT) markers with corresponding ÷^{2} values studied in Gujjar population of Jammu region of J&K

Alu marker	Phenotypes	Observed frequencies	Expected frequencies
Alu ACE	ID	8	7.22
	DD II	22	22.5619.22
		20	$x^2 = 0.22$
			(0.70 > p > 0.05)
Alu APO	II	0	9.68
	ID	44	24.64
	DD	6	15.68
			$x^2 = 30.87 (p < 0.001)$
Alu PV92	II	11	6.48
	ID	14	23.04
	DD	25	20.48
			$x^2 = 7.70$
			(0.01 > P >0.001)
Alu PLAT	II	16	16.24
	ID	25	24.51
	DD	9	9.25
			$x^2 = 0.02$
			(0.90 > P > 0.80)

two caste populations of Tamil Nadu except Gavara Naidu which had the *Alu* ACE insertion frequency of 0.379 (Vijaya *et al.,* 2007). It was also lower than the Yadava population of Andhra Pradesh (Ravindrnath *et al*., 2005). *Alu* ACE heterozygosity was calculated to be 0.4712 which was close to the heterozygosity values reported by Kaur *et al*. (2002) in four ethnic populations of Punjab India. *Alu* ACE heterozygosity value was higher than the value reported from two tribal populations of South India by Veerraju *et al*. (2001) and it was close to the values reported by Majumder *et al*. (1999) in 14 ethnic populations of India. *Alu* APO marker showed 0.44 insertion frequency which was higher than only one population (Munda) group and lower than 11 populations studied by Majumder *et al.* (1999). *Alu* APO insertion frequency was also lower when compared with other Indian populations (Kaur *et*

al., 2002; Ravindranath *et al*., 2005; Vijaya *et al.,* 2007; Veerraju *et al*., 2001).

Alu APO marker showed highest value of heterozygosity (0.4928) which was close to the heterozygosity values in other Indian populations (Majumder *et al*., 1999; Kaur *et al*., 2002; Ravindranath *et al*., 2005; Vijaya *et al*., 2007). This marker showed highest level of heterozygosity among the four markers used in the study of the genomic diversity of Gujjar population during the present study.

Alu PV92 insertion frequency was found to be 0.36 in Gujjar population. It was higher than Brahmins but lower than other three populations of Punjab (Kaur *et al.,* 2002). *Alu* PV92 insertion frequency was also higher than Reddiyar and Tamil Yadaver population groups of Tamil Nadu (Vijaya *et al.,* 2007) and 4 (Brahmins (UP), Gaud, Muslims and Rajputs) of total populations studied by Majumder *et al.* (1999). *Alu* PV92 insertion frequency was also lower than the two tribal populations studied by Veerraju *et al.,* (2001).

In the present study insertion frequency was highest in *Alu* PLAT marker which was found to be 0.57. This value is close to the insertion values of *Alu* PLAT in other Indian populations (Majumder *et al*., 1999; Kaur *et al.,* .2002; Ravindranath *et al*., 2005; Vijaya *et al*., 2007).

Alu PLAT marker was found to be the second most diverse marker among the four markers used for the study of genomic diversity in Gujjar population and showed heterozygosity value 0.4920 which was almost similar to the heterozygosity values reported by Kaur *et al.* (2002) in four ethnic populations of Punjab.

After studying the allele frequencies and heterozygosity values for four *Alu* (*Alu* ACE, *Alu* APO, *Alu* PV92, and *Alu* PLAT) polymorphic loci, all the markers showed insertion frequencies lower than or equal to those found in other populations of India studied by previous workers (Majumder *et al.,* 1999; Kaur *et al.,* 2002; Ravindranath *et al*., 2005; Vijaya *et al*., 2007) but in comparison to other world populations, the insertion frequencies in the present population are quite high. The average heterozygosity in the present population was recorder as 0.4792. It may be pertinent to point out here that Majumder *et al.* (1999) reported consistently high levels of average heterozygosity in 14 ethnic populations from India ranging from 0.351 to 0.499. The present study population also exhibits high levels of heterozygosity thus showing that Gujjar population is highly diverse with respect to these markers because high values of heterozygosity accounts for high diversity. But Gujjar population is a strictly endogamous population and therefore should have more of homozygotes as compared to heterozygotes i.e. should have low levels of heterozygosity as against the present finding. The reason behind the high heterozygosity levels of studied markers and thus high genomic diversity in Gujjar population may be the derivation of this strictly endogamous population from the non-endogamous Hindus who adopted Islam as their religion during the reign of Aurengjeb and became Muslims. This suggests the effect of recent historic events on the distribution of *Alu* specific markers on the present day Gujjar population of Jammu region of J&K state. Chi- square test revealed that in two markers *Alu* ACE and *Alu* PLAT the chi square value was below the tabulated value at one degree of freedom which showed that the differences between the observed frequencies and the expected frequencies was not significant and the population was in Hardy-Weinberg equilibrium with respect to *Alu* ACE and *Alu* PLAT markers (Table 3). But in case of *Alu* APO and *Alu* PV92 the chi-square value calculated was higher than the tabulated value at one degree of freedom which revealed that there was significant difference between observed frequencies and the expected frequencies and the population was not in Hardy–Weinberg equilibrium with respect to *Alu* APO and *Alu* PV92 marker. The unexpected results obtained for *Alu* APO and *Alu* PV92 markers may be due to sampling error as the population size used in the present study was quite small.

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