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Autosomal DNA Polymorphisms of Four South India Tribal Populations

A. Deva, R. Nandakumar*, **P. Raghu, K. Prabhakaran**

PG and Research Department of Zoology, Periyar E.V.R. College (Autonomous),
(Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India

*E-mail address: drnkzoo@gmail.com

ABSTRACT

Background: The entry and dispersal of modern humans is India of remains unclear and extending with many interesting evidences South India, assumed to be a major corridor for their with many ancient genetic deposits such as Dravidian tribal with Negrito features. As the relationships between the genetic polymorphs and diseases in human being revealed globally, it is worthy to investigate the genomic architecture of population in south India. **Objective:** To examine what evolutionary forces have most significantly impacted south Indian tribal genetic variation, and to test whether the phenotypic similarities of some south Indian tribal groups to Africans represent a signature of close relationship to Africans or are due to convergence. **Methods:** Blood samples from 193 unrelated individuals of both sexes are drawn from the Dravidian tribal settlements of Tamil Nadu and Kerala. South India are genotyped for four Aluindel (Alu FXIIIB, Alu ACE, AluTA25 and Alu PLAT) allele profile by PCR genotyping method. **Results:** All loci are highly polymorphic and average heterozygositiesare substantial (range: 0.37-0.44). Genetic differentiation is high (Gst = 3.7%) in all the study populations.

***Keywords*:** Tribal; Dravidian; Genome Diversity; Heterozygosity; Autosomal Markers

1. INTRODUCTION

India has served as a major passageway for the dispersal of modern humans, and Indian demographics have been influenced by multiple waves of human migrations [1]. Because of

its long history of human settlement and its enormous social, linguistic, cultural diversity and the population history of India has intrigued anthropologists and human geneticists for a long time [1]. The people of south India encompassing states of Tamil Nadu, Karnataka, Kerala, and Andhrapradesh speak the Dravidian languages. The origins of these languages are thought to be most likely Mediterranean [2], Negroid [3], or Mongoloid [4]. Further, it is inferred from historic documents that there have been many population and political invasions in to India and hence has been a melting pot of races [5], there are about 3000 castes and 461 tribes and more than 25,000 sub-castes existing in India today: predominantly a mixture of populations from Middle East, Central Asia and Mongolia [6]. The caste system is very rigid in Tamil Nadu and Kerala is characterized by endogamy, social restriction on inter-caste marriages and occupation based social classes [2].

It is well documented that the Dravidians of south India practiced a culture and unique social institution with a very ancient linguistic family further subdivided into many gene pools, differing in their origin, migration and population settlement [7-9]. Transposable Elements (TEs) are powerful drivers of evolution. Constitutes the majority of genomic DNA in many eukaryotes and they dramatically shape genetic content by causing mutations, rearrangements, and sequence duplications. Of increasing significance is the link between these transposon-mediated mutations and disease [10,11]. Alu (member of a SINE family) insertion/deletion polymorphisms offer several advantages over other nuclear DNA polymorphisms for human evolutionary studies. They are rapid, simple and stable with newly inserted elements and rarely undergo deletion. It is recently proved that the ACE deletion/deletion polymorphism could affect the athletic ability in Turkish population [12].

The present study aimed to investigate the genomic diversity, genetic differentiation and genomic affinities of the four South Indian tribal populations based on four human-specific Alu insertion/ deletion polymorphisms in the nuclear genome.

2. MATERIALS AND METHODS

Study Populations

One hundred ninety there healthy unrelated individuals belonging to four different population groups from South India are included in this study. Blood samples (5-10 ml) are drawn from healthy, adult volunteers with prior informed consent. The tribal groups are confined to the villages of hilly tracts and valleys of four different districts of Tamil Nadu and Kerala India. They include Malaivedan from Madurai, Dindukkal and Theni Districts (n = 70), Malaipandaram from Kollam of Kerala and Thirunalveli (n = 49), Kanikaran from Kanyakumari and Thirunalveli (n = 44) and Mannan from Idukki of Kerala (n = 30). The genomic DNA is extracted from whole blood using by the salting out procedure and is suspended in 10 mM Tris and 0.1mM EDTA for genotyping. The four polymorphic loci are genotyped by a standard 30-cycle PCR Table1. Appropriate annealing temperatures and additives are optimized for each system. The PCR protocols followed for the present study have been reported previously [13,14]. After PCR amplification, ampliconsare separated by electrophoresis. Later the sample containing EtBr stained gel, is visualized under UV and documented.

3. STATISTICAL ANALYSIS

Table 1. PCR Primer and Conditions.

Locus	Primer sequence	Annealing Temperature ºC	Amplified Product size		Agarose gel %
			+	-	
Alu ACE	5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' 5'-GAT GTG GCC ATC ACA TTC GTC AGA-3'	58.2	490 bp	190 bp	2
Alu TPA25	5'-GTA AGAGTTCCGTA ACAGGACAGCT-3' 5'-CCCCACCCTAGGAGA ACTTCTCTTT-3'	56.9	457 bp	134 bp	2
Alu FXIIIB	5'-TCAACTCCATGAGA TTTTCAGAAGT-3' 5'-CTGGAAAAAATGTATT CAGGTGAGT-3'	54	700 bp	500 bp	2
Alu PLAT	5'-GTGAAAAGCAAGG TCTACCAG-3' 5'-GACACCGAGTTCAT CTTGAC-3'	53	570	260	2

Table 2. Ethnographic Notes.

Population	Collection districts	Living place	Occupation	Languages spoken	Food habits	Marriage Practice/Cultural activity
Malaivedan	Madurai, Theni, Dindukkal	Madakulam, Thuvariman, Vedarpuliyankula, Oldvethalakundu, Ayakkulam, Uthimoodu,	Hunting and gathering were their traditional occupations.A number of the Malai Vedan have become agricultural and plantation labourers.	Dravidian language groups -Tamil	Non – vegetarians , Rice , Pork, Tuber roots and tubers.	The Malaivedan do not have social diviions. Monogamy is the norm and marriage between cross – cousins is preferred

Malai Pandaram (Hill Pandaram)	Kanikkaran	Mannan
Kollam dt of Kerala and Thirunelveli	Thirunelveli, Kanniyakumari	Iduki dt of Kerala
Achchankovil, Sokkampatti	Periyamailar, Inchikkuli, Puravilai, Penu	Kovilmalai
Hunting and gathering were their traditional occupations. At present, they on longer hunt, but the collection of minor forest produce is still one of their major occupations. Settled cultivation, wage labour and animal husbandry are their other occupations.	Traditionally the Kanikkar were hunters, gatherers and shifting cultivation. The present- day occupation of the community is settled cultivation, Besides,they work as wage labourers in the forest deparment	Their traditional occupations are hunting and gathering , trapping of birds and animals and shifting cultivation. At present their occupations are agricultural labour, settled cultivation, animal husbandry and mat- weaving
Dravidian language groups -Malayalam, Tamil	Dravidian language groups - Malayalam, Tamil	Dravidian language groups-Tamil, Malayalam
They are non- vegetarians and eat wild game.	Non – vegetarians but do not eat beef, pork wild tubers rice	Non – vegetarians ragi, rice root and tubers
The Mala pandaram does not have social divisions in a strict sense. Community endogamy and family lineage exogamy of are their marriage rules. Cross–cousin marriages are popular among them	Cross – cousins. Divorce is permisable pupubery girls after attening 2 – 3 Years 20 – 25.	tribal communities are somewhat unique in that they observe the 'Matriarchal' system whereby kings are always chosen from their women's side.,Divorce is usually not permitted remarriage of widows is rare

Allele frequencies are estimated by direct counting with the help of the complete programme and POPGENE, [15]. (Tables 3, 4 and 5). MEGA6: Molecular Evolutionary Genetics Analysis [16]. Software was used to construct the dendrograms by the neighbor joining method using the data for the Indian populations and the population study in the present for these constructions alleles are grouped based on Alu specificities. Origin Pro 8 software. NJ trees are constructed by MEGA: 6 [16]. Software with the inputs from available Indian and global alu data.

4. RESULTS AND DISCUSSION

The present study populations are compared with other tribal populations of south India. [11]. Allele frequencies for the '+' (Insertion alleles) for the four Alu DNA markers are given in Table 3 and Fig. 1. The average heterozygosities of all loci are presented in the Table 4. And Fig. 2. Interestingly all the study population harbour an average heterozygosity value of 0.425. The present study population also exhibits high levels of heterozygosity which is similar to other Indian population studied [2]. The amount of genetic differentiation among populations, Gst values (a measure of the inter populations variability) for all polymorphic loci is observed as 3.72%. The total genomic diversity (H_T) is found to be 0.578. However, most of the genomic diversity is attributable to diversity between individuals within the populations (H_S 0.549).

The genomic affinities among four study populations are represented in Figure 3, using four Alu insertion allele frequency data of four loci by a standard NJ tree. This tree is divided into two clusters: Malaivedan with Kanikaran and Malai Pandaram with Mannan. It is seen that the affinities among the study populations do correlate well with their sociocultural affiliation. Instead, populations that occupy closer geographical habitats show, by and large, closer genomic affinity. It is observed that Malai Pandaram is genetically more distant from other study populations.

To determine the genetic relationships of the present study populations with other Indian tribal populations, the data of four Alu indel loci (Alu FXIIIB, Alu ACE, AluTA25 and Alu PLAT) presented by [11,17,18], that are common with the present study are used. The NJ tree consisting of the 18 tribal Indian populations including the four study populations is presented in Figure 4.

The present investigation is conducted with the goal of analysing the extent of genetic variation at a number of polymorphic autosomal loci from samples of diverse tribal populations from southern India, with a particular focus on the origins of particular groups that show phenotypic similarities to Africans (i.e. "Negrito"characteristics). Populations with "Negrito" features have been reported in southern Asia, southeast Asia and island southeast Asia, leading to the suggestion that they might represent the signature of an ancient migration from Africa [1] .

Therefore, observed variations in the allele frequencies among populations are highly informative in assessing the genomic diversity of a population. The allele frequency distribution pattern of these populations is comparable with those observed in other Indian populations [11,14,17-24].

The average heterozygosity values reflect the genetic heterogeneity in the study populations. There is a significantly greater inter-individual variation within each study population than between the populations; hence the extent of population differentiation is very high and the incident of average Gst value for all markers in the four tribal groups 0.0372 (3.72%). Earlier studies have reported Gst values ranging from as low as 3.12% by [11] to as high as 8.3 % [17], the centroid analysis suggests that there is considerable amount of gene flow in the set of population under consideration. Further it can be explained that higher heterozygosity values and gene flow may be due to their small population sizes and closer proximity.

The present study reveals that the four Dravidian tribal populations from South India are highly polymorphic, highly heterozygous in nature, with higher genomic differentiation and

genetically distant from other Indian tribal and world populations. The probable explanation for above results could be their small population sizes, strict endogamy practices and their geographical isolation over a long period of time. In conclusion, the present study suggests that the tribal groups of southern India share a common ancestry, regardless of phenotypic characteristics, and are more closely related to other Indian groups than to African groups. Based on four autosomal loci, they appear to show high levels of genetic diversity and genetic differentiation. Genetic drift has been the major evolutionary force to shape genetic variation in these populations. This represents an important feature of tribal populations of south India, which has to be taken into account in any attempt to reconstruct the history of these populations.

Table 3. Allele frequencies at four Alu indel polymorphic loci in four south Indian tribal populations.

Locus	Malaivedan	Malai Pandaram	Kanikaran	Mannan
Alu FXIIIB	0.365	0.465	0.297	0.75
Alu ACE	0.461	0.020	0.545	0.396
Alu PLAT	0.519	0.489	0.757	0.714
Alu TAP25	0.694	0.561	0.738	0.62

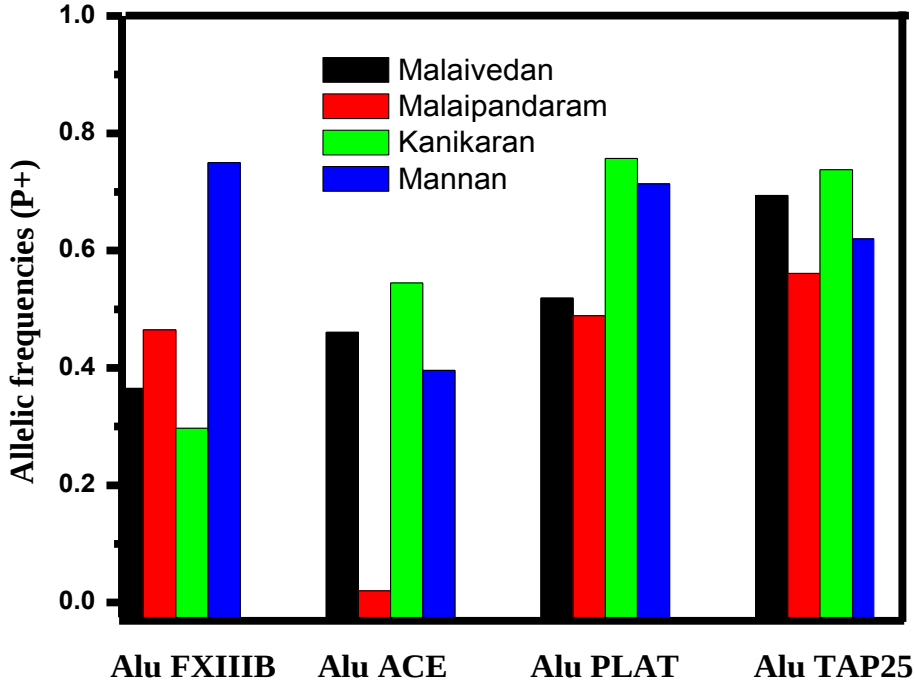

Fig. 1. Allele frequencies at four Alu indel polymorphic loci in four south Indian tribal populations.

Table 4. Heterozygosities at individual locus and average hetrozygosity based on four polymorphi loci in four south Indian tribal populations.

	Hetrozygosity			
Locus	**Malaivedn**	**Malaipandarm**	**Kanikaran**	**Mannan**
Alu FXIIIB	0.438	0.438	0.438	0.438
Alu ACE	0.377	0.377	0.377	0.377
Alu PLAT	0.443	0.443	0.443	0.443
Alu TAP25	0.443	0.443	0.436	0.443
Average Heterozygosities	0.425	0.425	0.425	0.425

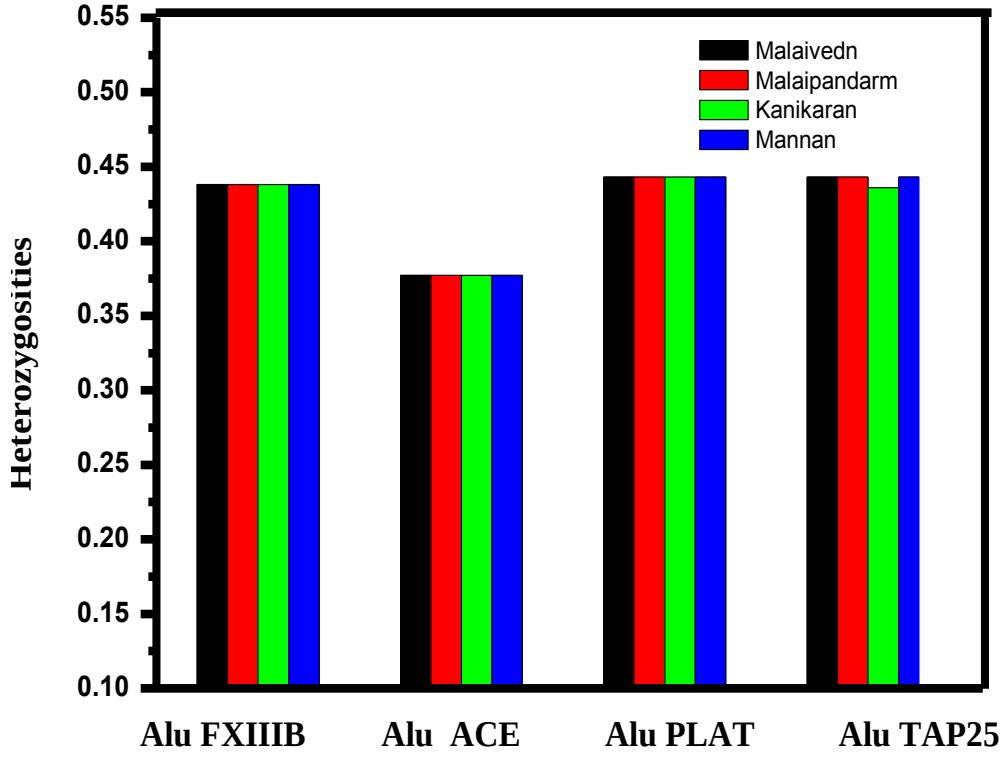

Fig. 2. Heterozygosities at individual locus and average hetrozygosity based on four polymorphic loci in four south Indian tribal populations.

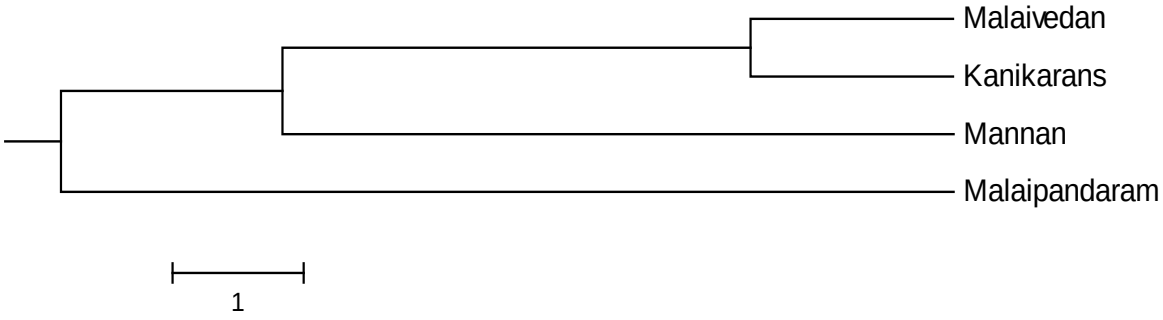

Fig. 3. Neighbour – joining tree depicting genomic affinity among the Caste of four Tribal Populations of south Indi.

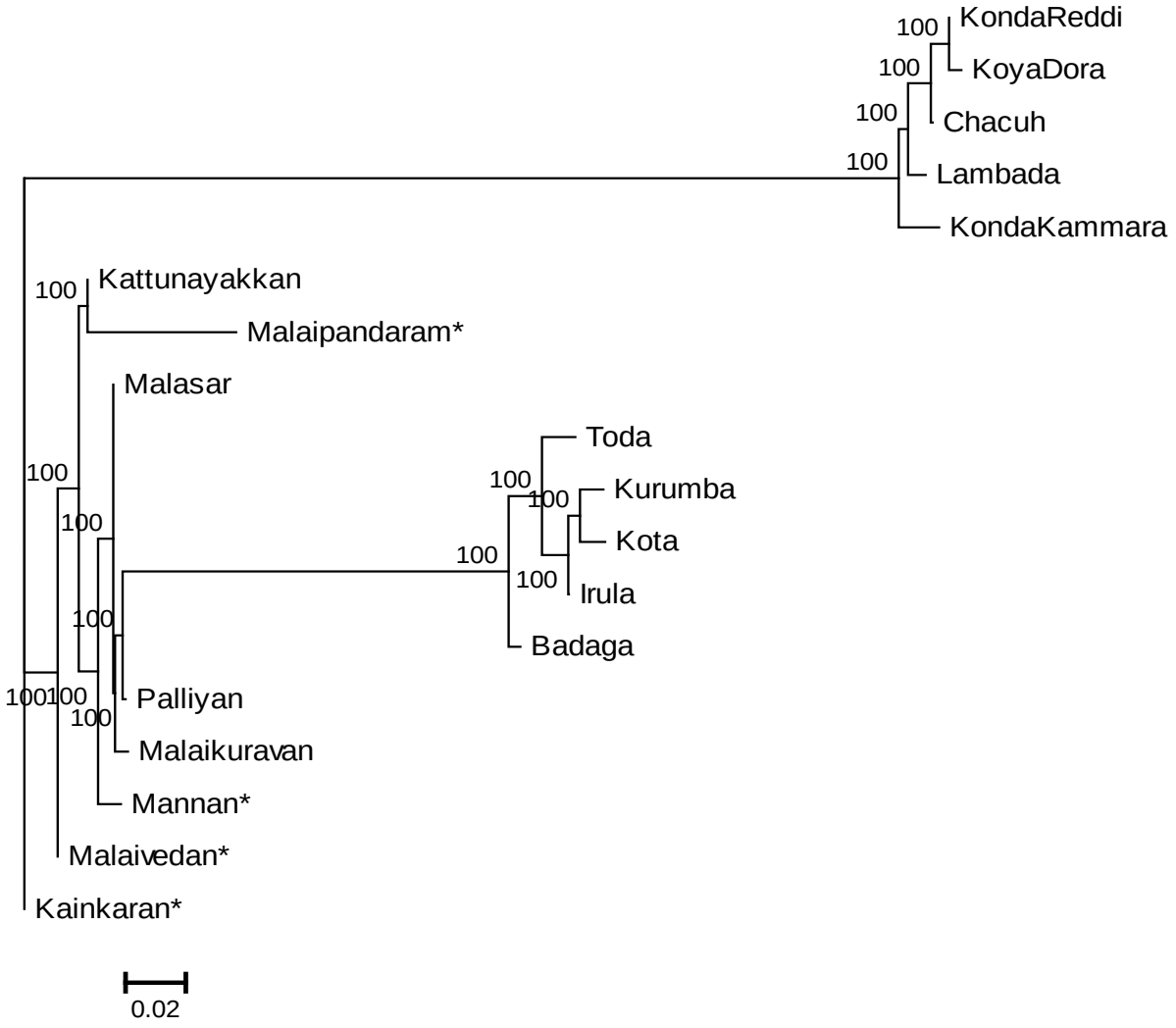

Fig. 4. Neighbour – Joining tree depicting genomic affinity among the of Eighteen Tribal Populations of south India.

Table 3. Results of gene diversity analysis for individual loci and for all loci jointly considered in the study populations.

Locus	H_S	H_T	G_{ST}
Alu FXIIIB	0.831	0.851	0.119
Alu ACE	0.736	0.782	0.175
Alu PLAT	0.455	0.486	0.058
Alu TAP25	0.174	0.191	0.020

5. CONCLUSION

Genetic drift therefore probably played a significant role in shaping the patterns of genetic variation observed in southern Indian tribal populations. Analyses of population relationships showed that Indian populations are closely related to one another, regardless of phenotypic characteristics, and do not show any particular affinities to Africans.

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