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ACE Gene I/D Polymorphism-A Global Perspective in relation to Indian Population

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ABSTRACT

Alu elements are a family of interspersed repeats in the human genome. Alu ACE insertion (I)/deletion (D) polymorphism is a marker useful to study the genomic diversity of worldwide population. The genomic diversity among the populations may help to understand the evolutionary and migrational history of population. The present investigation is to examine the allele and genotype frequency of three population of south India- Paliyar tribe, Nadar and Pallar. Accurately, 147 samples were collected and genotype was done using PCR-SSP. According to Hardy-Weinberg equilibrium equation, there have no significant difference in Pallar and Nadar population whereas, significant difference was observed in Paliyar tribe population (p-0.0001). The results of phylogenetic tree analysis and PCA showed that south Indian Paliyar tribal population separated from other population in India. It might be due to under selection pressure.

Keywords: ACE gene, Caste and tribe, South India, Phylogenetic analysis

INTRODUCTION

India has multi ethnic, endogamy population which provides the interesting insights of genomic diversity and migration (Majumder *et al,* 1999). Indian populations are highly well defined endogamous groups by morphological, social and genetic features of evolutionary history (Vishwanathan *et al.*2004). Indian tribes and non-tribal populations are possibly the original inhabitants of India. However, their evolutionary history and genetic structure have been disputed (Basu *et al.* 2003). The genomic diversity of population history and genetic structure are studied through the polymorphic variation. Alu is a well known polymorphic DNA marker with seven polymorphisms. In the human genome, Alu genes are the largest family of mobile elements found repeatedly. The term 'repetitive element' describes that multiple copies of various DNA sequences present in the human genome. Interspersed elements can be subdivided on the basis of size, with Short Interspersed Elements (SINEs) being less than 500 bp long

(Deininger *et al*. 1998; Okada *et al.*1991). Almost 30 years ago, Alu SINEs were identified originally as a component in human DNA. The origin and amplification of Alu elements are evolutionarily coincided with the radiation of primates in the past 65 million years (Shedlock *et al.* 2000). Alu sequences are considered to be ancestrally derived from the 7SL RNA gene and activated through RNA polymerase III-derived transcript in a process termed as 'retro position' (Sengupta *et al.* 2016; Majumder *et al.*1999; Veeraraju *et al.*2001,; Barbalic *et al.* 2004). Cook *et al.* 2013 reported the interaction between the mobile elements results insertion, deletion, duplication and alteration in the complex genomic structure.

ACE Gene Polymorphism

Angiotensin Converting Enzyme (ACE) is a zinc-containing peptidylpeptide hydrolase plays an important role in the rennin angiotensin system (RAS) and it is involved in the conversion of decapeptide angiotensin I to active octapeptide angiotensin II (Dickson and Sigmund *et al.,*2017; Durango *et al*., 2006). ACE is expressed in several tissues such as lung, vascular endothelium, kidney epithelium and Leydig cells in the testis. ACE not only degrades vasoactive peptides but also contributes in the metabolism of neurotransmitters (Cann RL, 2001). ACE isozyme production is regulated by variety of hormones including glucocorticoids in endothelium and androgens in the testis (Bagheri *et al*. 2010). Angiotensin I-converting enzyme (ACE) is a key enzyme, which catalyzes the conversion of angiotensin I to angiotensin II, an effective vasopressor. Therefore, any alteration in ACE activity can cause several pathological conditions including vasoconstriction, heart failure, coronary thrombosis and ventricular remodeling (Choudhury *et al.* 2012 and Gard, 2010). The human ACE gene is located on chromosome 17q23.3 with 26 exons and 25 introns. The ACE gene polymorphism was primarily reported by Rigat *et al.* 1992. This polymorphism is defined by the presence (insertion, I) or absence (deletion, D) of 287 bp fragments present in intron 16 which leads to three form of genotypes such as I/I, I/D, and D/D. The occurrence of D allele or D/D genotype is associated with disorders include elevated plasma, tissue specific ACE activity and Hypertension (Pereira *et al.,* 2008). In the present investigation, ACE insertion (I)/deletion (D) polymorphism was evaluated among the three South Indian populations such as Nadar, Pallar and Paliyar to relate the level of endogamy caste and tribal population.

MATERIALS AND METHODS

Subjects:

The present population study is based on cross sectional study. The populations selected for the present study are Pallar, Nadar and Paliyar tribe. A total number of 200 samples were collected from Dindigul and Kodaikanal region of Tamil Nadu. From this, 147 samples were chosen for genotyping. The research was completed in accordance with the ethical principles outlined by the Indian Council of Medical Research (ICMR) guidelines for medical research with human subjects. Ethical clearance was obtained from the Institutional Ethics Committee of Mother Teresa Women's University, Kodaikanal. All subjects were informed about the contents and aims of the study and they had given their written consent. A detailed questionnaire was used while collecting the samples. All the volunteers above 18 years of age were selected and equal number of male and females were elected for sampling. A relative of the subject until second degree was eliminated in the sampling. Since, the concerned gene was an autosomal gene.

DNA Analysis:

The DNA samples were collected from mouth wash which is a non-invasive technique and extracted by modified salting out method (Ausubel *et al*. 1999). For genotyping purpose, one sample per household was collected randomly from the populations. Genotyping was done using sequence specific primers to amplify the fragment from the isolated DNA using F-5'CTGGAGACCACTCCCATCCTTTCT3' and R-5'GATGTGGCCATCACATTCGTCAGA 3' primers. The PCR cycling conditions as follows: initial denaturation was set at 94^0C for 10 minutes. For 30 cycles, denaturation at 94^0C for 20 seconds followed by primer annealing at 58^0C for 30 seconds which followed by extension at 72^0c for 30 seconds. Final extension of 72^0c for 7 minutes was given. The final volume of the reaction mixture was 25 µl which contain 1X PCR buffer, 2 mM $MgCl_2$, 0.5 mM dNTPs, 0.5 µM of forward primer, 0.5 µM of reverse primer, 1 U/µl of Taq DNA polymerase and 100 ng DNA.

The PCR product was analyzed by electrophoresis on 1.5% of agarose gel. This polymorphism is defined by the presence (insertion, I) or absence (deletion, D) of a 287 bp fragment in intron 16 that leads to three forms of genotypes II, ID, and DD. The I allele produce a fragment in the 490 bp and D allele produce the fragment in the 190 bp (Figure-1) .

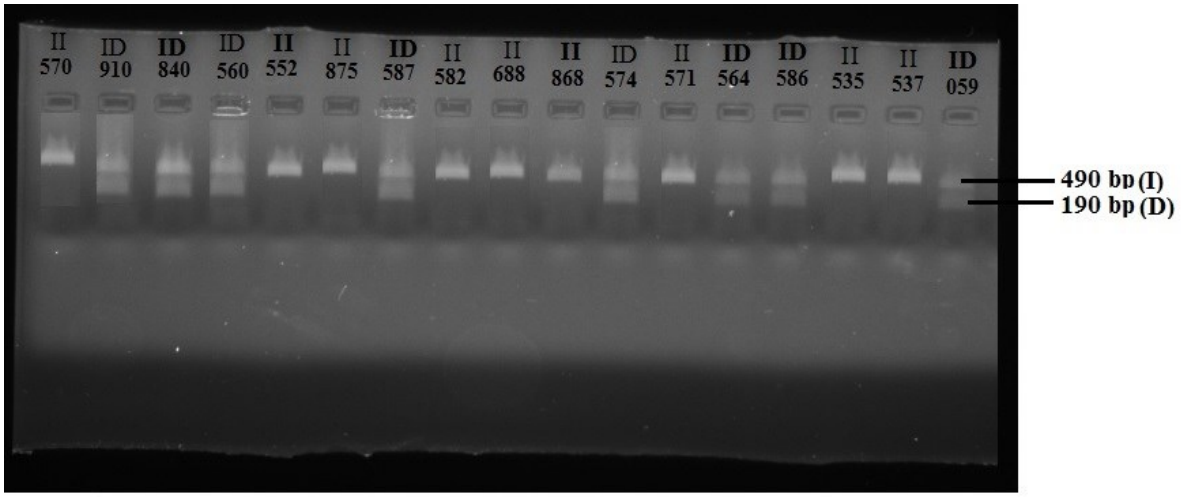

Figure1: Genotyping Analysis of ACE insertion (+) /deletion (-) polymorphism

Statistical analysis:

Hardy- weinberg equation was used for the assement of expected genoype compared with observed genotype frequency for ACE insertion (+) /deletion (-) polymorphism. Two tailed Fisher's exact was done to compare the genotype frequency among the populations using SPSS (v20). The allele and genotype data were collected from the published literature and compared with the present study population. Frequency table was created using excel (windows 2007). The frequency data was converted into genetic distances in Arlequin (v3.5). Dendrogram was constructed using Molecular and Evolutionary Genetics Analysis (MEGA7.0). The genetic distances were also used for Principal Component Analysis (PCA) in Genetic analysis using excel.

RESULTS AND DISCUSSION

Allele Frequency and Genotypic frequency among the selected South Indian Population:

The allele frequency, genotype frequency and heterozygosities for the insertion (+) /deletion (-) alleles of selected South Indian populations such as Paliyar tribes, Pallar and Nadar were studied and the results are depicted in table-1. The study revealed that the genetic variation of Indian population in different ethnic origin. The Hardy-Weinberg equilibrium was applied for population study and there was no significant difference in genotype frequency of Pallar and Nadar. But, Paliyar tribe population showed significant difference and the *p*-value was found <0.0001. The frequency distribution of insertion (+) has been higher in Indian caste and tribal population except Agharia (41.7%), Tanti (43.3%) and Konda redid (44.9%) populations. Comparatively, Insertion (+) allele is higher than the deletion (-) allele in other populations. Krishnaveni *et al.* 2015 reported that the similarity of allele frequency found in Indian populations is due to the influence of social structure. Prevalence of ACE insertion (+) /deletion (-) polymorphism varies across the ethnic groups (Agarwal *et al.* 2004; Patkar *et al.* 2009).

The average heterozygosity (H_T) was observed in North Indian caste population Rajput (0.461), Assamese (0.461) and Nadar (0.464).

Table.1: Allele and Genotype Frequency of South Indian Population

Ethnicity	N	Allele Frequency		Genotype Frequency			HWE P value
		+	-	+/+	+/-	-/-	
Pallar	48	0.613	0.387	0.387	0.452	0.161	NS
Nadar	47	0.633	0.367	0.467	0.333	0.200	NS
Paliyar	52	0.769	0.231	0.692	0.154	0.154	**

**-<0.0001, NS- Not Significant

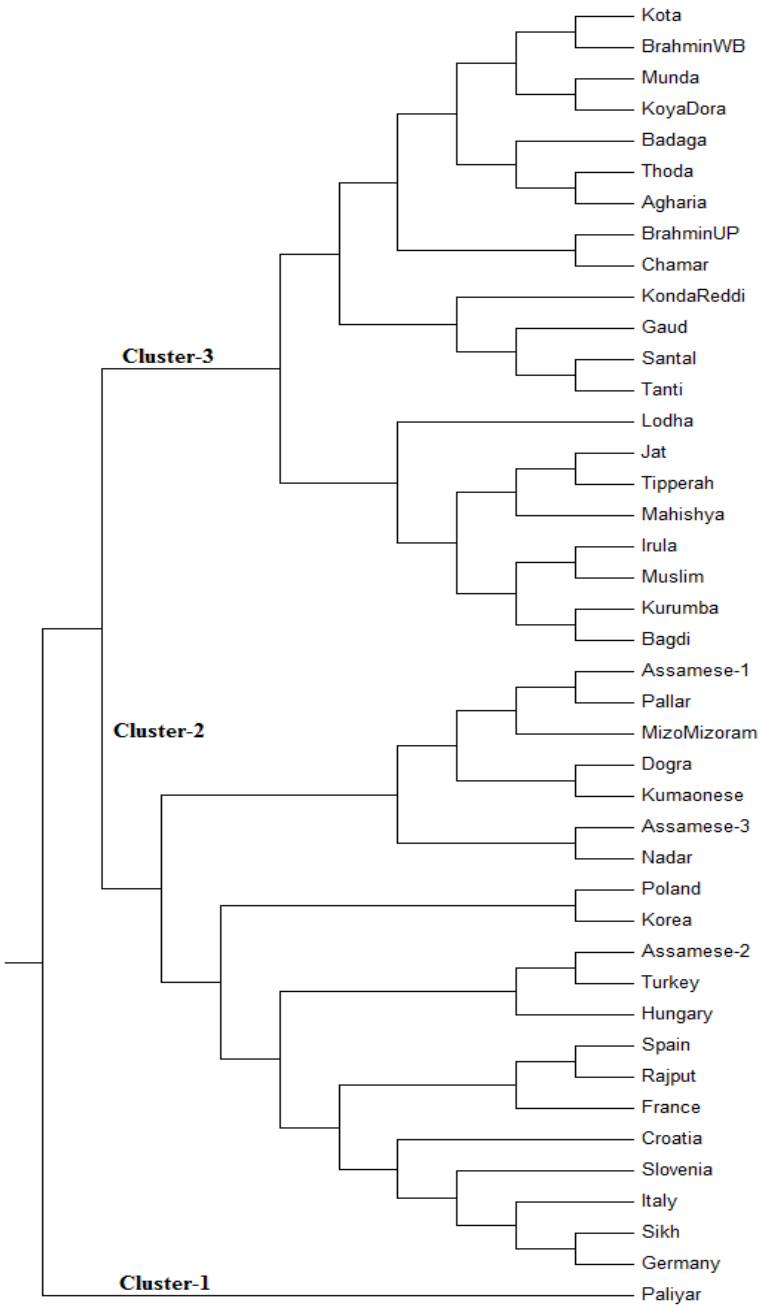

Figure 2: Cluster Analysis for Genomic Diversity of South Indian Population with other Population.

Genomic Diversity of South Indian among Other Population:

The root of UPGMA network showed three major clusters (Figure-2). In the first cluster, Paliyar tribal's of South Indian population stand quite distance from other populations which indicated the genetic distance between other populations. The second cluster showed two branches in the network. In the first branch, Nadar population was clubbed with Assamese3 (AS3) and in the second branch Pallar population was clubbed with Assamese1 (AS1) population. In the third cluster, all the other Indian populations were clubbed in same branch. The south Indian tribal Paliyar were shown in separate cluster.

The Principal Component Analysis for all population showed that the most Indian caste and tribal Population fall under Upper and lower left quadrant (Figure-3). The South Indian Nadar Population fell in lower right quadrant along with Assamese3 and the Pallar population fall in Upper right quadrant along with Indian population. On the other hand, the Paliyar population falls different from the whole population group.

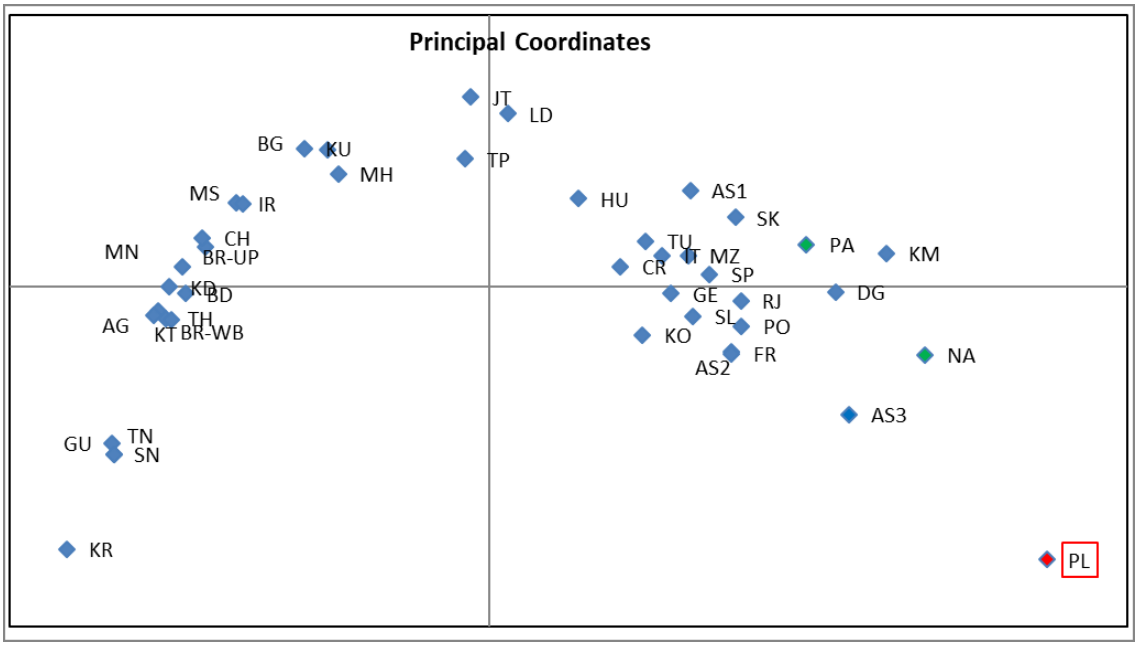

Figure 3: PCA for Genomic Diversity of South Indian among Other Population.

DISCUSSION

The present study provides an evidence of the difference between south Indian caste and tribal endogamous population from the total population group by the frequencies of ACE +/-Polymorphism. The South Indian Paliyar tribe population shown the significant difference in the frequency of ACE +/-Polymorphism and it was strongly proved through phylogenetic tree and principle component analysis. Several investigations have been confirmed an extensive database on genotyping distribution of population based on ethnic background and the influence of ACE +/-Polymorphism in global populations (Pasha *et al.,* 2002 and Basu *et al.,* 2003). It might be due to genetic drift selection pressure identified on these loci .The Alu polymorphism based affinities of the global populations forms a potential genetic data for mapping population migrations, histories, genetic similarities and higher level of prevalence of infectious disease (Chinnaih *et al.,* 2016) Mendelian population of some specific geographical region has several genetic variants in a group of alleles combined with negative or positive effect. ACE gene is one of the candidate genes for complex disorders (Kameih and Panmei 2015). Alu ACE loci are highly polymorphic in India and other world population except Africa (Veeraraju *et al.,* 2001; Majumder *et al.,* 1999). Genetic drift played a significant role in shaping the patterns of genetic variation in Southern tribal population (Viswanathan *et al.,* 2004).

CONCLUSION

The present work concluded that the Paliyar tribe population must have undergone selective pressure. Genetic diversity allows selective pressure on the population for any specific trait. Without the diversity, all the individuals in the population would be identical and if there were a change in the environment that required a responsive change in the population and the particular population would not be able to adapt properly. They would have to rely on mutations cropping up which could potentially take multiple generations to occur.

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Conflicts of interest: The authors stated that no conflicts of interest.

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