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Y-Chromosome and Mitochondrial DNA Phylogeny of Poliyar, Malaikuravar and Palliyar Tribes of Tamilnadu, India

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Abstract: The objective of the project was to study the inter genetic diversity within and between Poliyar, Malaikuravar and Palliyar tribal populations of Tamil Nadu and to compare these populations with other populations of India and other parts of the world. 50 Poliyar, 24 Malaikuravar and 20 Palliyar samples were taken for the study. Mitochondrial DNA makers HVR1 and from Y-chromosome, SNPs were analysed. The high frequency of C → T at 16223 locus of HVR1 region suggests that these populations might fall into "M" haplogroup. Median Joining Network analysis reveals that three populations are endogamous as they showed very less haplotypes. In the Neighbour Joining Tree, Poliyar are clustering with Palliyar, palliyan and kadar tribes of TamilNadu. Malaikuravar are clustering with satmani tribal population whereas Palliyar are clustering with palliyan and kadar tribes of TamilNadu. The mismatch distribution graph reveals that population growth is constant in paliyar while it is expanding in case of Malaikuravar. The Poliyar tribes show this tribes going to show the bottle neck. Y-SNP analysis revealed that Poliyar, Malaikuravar and Palliyar, fall into haplogroup VI, VIII and X suggesting that they must have migrated from South India, Pakistan, South Asia and Central Asia, as there haplotypes are found predominantly in the above region. To elucidate their migration routes, sub-haplotyping needed to be done.

Key words: Bottle neck, haplotype, hyper variable region, median joining tree, neighbour joining tree, Polymerase Chain Reaction (PCR)

INTRODUCTION

The evolution and origin of modern human beings is still an unanswered question. Genetic data from such ethnic groups have been used for several decades in the study of human evolutionary history (Cavalli-Sforza, 1998; Jorde *et al*., 1998; Ruiz-Linares, 1999). The major demographic events like migration; population bottlenecks and expansion leave genetic imprints by which gene frequencies are altered (Thangaraj, 2009).

The genetic variation from one person to another reflects the evolution of our species. Evolution is a process of change at the molecular level. This process involves insertion, deletion, and duplication of DNA or substitution of nucleotides. The above process generates polymorphism or variation with, in the species. The discovery of a highly polymorphic region in human genome was a significant advance towards the goal of creating a unique genetic fingerprint, which could be used to identify an individual based solely on genetic sequence.

All genetic variations are caused by mutations, of which there are many different types. The most common and most useful among them are SNPs. The combination of recent molecular age and geographic structures, make mitochondrial DNA and Y-chromosome, a sensitive genetic index capable of tracing the micro evolutionary patterns of novel modern human diversity. Haploid markers from mitochondrial DNA and Y-chromosome have proven invaluable for generating a standard model for evolution of modern humans. By studying NRY and mtDNA polymorphism one can examine phyolgeographic migration mergers and division that accounts for the current spectrum of human variability.

India is a country with rich cultural heritage showing striking diversity in terms of language, marriage practices as well as in their architecture. Geneticists and anthropologists have attempted to elucidate the nature of India's rich diversity for decades. It is generally accepted that the tribal people are the original inhabitants of India (Ray, 1973; Thapar, 1966). The tribal constitute 8.08% of the total population of India (1991 Census of India). There is an estimated 461 tribal communities in India (Singh, 1992). In general, tribes are the most primitive races living in isolation and are considered to be highly inbred

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and of aboriginal origin. It has been hypothesized that Indian subcontinents play a central role in the migration of early Homo sapiens, from Africa to Asia and to Pacific (Nei and Roychoudhury, 1993; Cavalli-Sforza *et al*., 1994). Based on palaeontological evidences (Stringer and Andrews, 1988) and mtDNA data, (Cann *et al*., 1987; Vigilant *et al*., 1991) anatomically modern humans are believed to have arisen in Africa and subsequently migrated to the rest of the world beginning around 100,000 years ago. India is presumed to be path of this "Out of Africa" and subsequent migratory events, thus it is possible that these migrants were the ancestors of the original inhabitants of the Indian subcontinents.

MTDNA provides a valuable locus for DNA typing in certain circumstances. The high number of nucleotide polymorphisms or sequence variants in the two hyper variable fractions of the non-coding control region can allow discrimination among individuals.

Until recently, the Y chromosome seemed to fulfill the role of juvenile delinquent among human chromosomes-rich in junk, poor in useful attributes, reluctant to socialize with its neighbors and inescapable tendency to degenerate. The properties of Y chromosome read like a list of violations of the rulebook of human genetics. However it is because of this disregard for the rules that Y chromosome proves to be such a superb tool for investigating human evolution. The availability of the near complete sequence and new polymorphisms, gives a highly resolved phylogeny and insights into its mutation processes which throws further light on human evolution. Tamil Nadu has a great cultural & population diversity of tribal community. Among these diverse populations, Poliyar from Kodaikanal, Palliyar from Virudhunagar district and Malaikuravar from Theni district are chosen for the present study.

The aim of the present study is to bring the Poliyar, Palliyar and Malaikuravar populations of Tamil Nadu of India on the molecular genetic map of the world. The objectives of the study were:

- To analyze the sequence variation at hyper variable region I (HVR I) of mtDNA D-loop.
- To construct haplotypes from the available data and compare the relationship among individuals by network analyses (MJ network).
- To study genetic data from mtDNA HVR-I sequences of the chosen population (within the population) and draw the graph of mismatch distribution.
- To construct genetic (Neighbor joining) trees with the help of Arlequin and other softwares using mtDNA HVR-I sequence.
- To study single nucleotide polymorphism (SNP) at nucleotide site of mtDNA in 10400, 10398 (15F).
- To study Molecular Diversity and Demographic Expansion.
- To study the population genetic structure inferred by AMOVA.
- To study the various polymorphic sites of Y chromosome.

Understanding variation within a given genomic region is of interest as it is a consequence of dynamics of the genome which is due to the impact of evolutionary forces (viz. mutation, selection, genetic drift and recombination). It is thus a way to understand distribution and pattern of variation found in the genome. On the other hand, when genetic variation is being analyzed in random individuals from specific population, it offers the possibility to trace back their origin. The goal of this study may be to reconstitute evolutionary history of specific population as a subject of study.

MATERIALS AND METHODS

Population samples: 10 mL of intravenous blood from 94 healthy and unrelated male individuals from three endogamous Dravidian-speaking tribal populations of southern India, belonging to Poliyar, Malaikuravar and Palliyar tribal populations of Tamil Nadu were collected in tubes containing EDTA as an anti-coagulant with their informed written consent with necessary information like Name, Age of the donor, Place of collection, diseases (both genetic and other, if any), family pedigrees etc. The present study was completed at Center for Cellular and Molecular biology, Hyderabad, India from January to June 2004. The tribal groups are confined to hilly tracts and valleys of the Kodaikanal Districts of Tamil nadu and consist of Poliyar (n = 50), Palliyar (n = 20) and Malaikuravar (n = 24).

DNA isolation and amplification of mitochondrial DNA: DNA was isolated using the organic method (Gill *et al*., 1987). The extracted DNA was quantified by the spectrophotometer method followed by checking in 0.8% agarose gel (Maniatis *et al*., 1989).The hyper-variable region I (HVR I) and selected coding regions of the mtDNA were amplified from 10 ng of template DNA using 10 pM of each primer, 100 μM dNTPs, 1.5 mM MgCl2 and 1 U of Taq DNA polymerase. Generally, 35 cycles of reaction was performed with 30 sec denaturation at 94ºC, 1 min annealing at 58ºC and 2 min extension at 72ºC. Annealing temperature and time were slightly modified for few sets of primers. The reactions were carried out in MJ Research thermal cycler (PTC-200).

Y-chromosomal markers: Six Y-chromosome biallelic polymorphic markers viz M9, M45, M89, M69 M173, and M175 were analysed to construct the Y-chromosome phylogeny of the studied populations according to Chromosome Consortium (2002). The PCR cycles were set-up with an initial denaturation of 5 min at 95ºC,

followed by 30-35 cycles of 30 sec at 94ºC, 30 sec at the primer-specific annealing temperature (52-60ºC), and 45 sec. at 72ºC, and final extension of 7 min at 72ºC. Polymerase chain reaction consisted of 10mL PCR reaction mixture and included 1.0 mL PCR buffer (10X), 1.0 mL $MgCl_2$ (25 mM), 0.8mL deoxynucleotide tri-phosphates (10 mM), 0.5 pM of each primer, 1unit of Taq DNA polymerase (1 unit/mL) and 20 ng of genomic DNA. Thermal cycling conditions were as follows:

- Initial denaturation = 95ºC for 5 min 1 cycle
- Denaturation = 94ºC for 1 min 35 cycles
- Annealing = 55ºC for 1 min 35 cycles
- Extension = 72ºC for 1 min 35 cycles
- Final extension = 72ºC for 10 min 1 cycle
- 4ºC hold for 5-10 min

The PCR amplicons: The PCR products were checked by electrophoresis in a 2% agarose gel containing ethidium bromide (0.5 mg/mL) and the bands visualized under UV illumination and photographed.

Sequencing of the PCR products: PCR products were directly sequen using Dideoxy chain termination method (Sanger and Coulson, 1975). The Sequencing was carried out in ABI PRISM® 3730 DNA Analyzer. The sequence data were edited as required using Sequencing Analysis Software™ (Applied Biosystems, USA) and sequences were aligned using Autoassembler version 2.0 software (Applied Biosystems, USA) for identification of mutations/polymorphisms.

Analysis of HVR-I sequences: The HVR-I sequences so obtained were assembled with the consensus mitochondrial sequence (Anderson *et al*., 1981) using 'auto-assembler' software. The HVR-I sequences aligned at the position 16000-16400 depending on the length of each sequence. The mutation sites were noted and fed into 'Network' software.

Sequence analysis: The sequences were checked, edited, aligned and assembled with the updated CRS (Anderson *et al*., 1981; Andrews *et al*., 1999) using the AutoAssembler software (Perkin Elmer). The muation sites were filled into the Network software along with the bases in Anderson text at those sites to obtain median-joining tree of Ramgarhia Sikh population. Clustal X program was used to align the sequences along with the published sequences of other Indian and World populations. The output file was used for PHYLIP and ARLEQUIN softwares to generate 'Analysis of Molecular Variance (AMOVA), pairwise genetic distances and Neighbor-Joining (N-J) tree which forms the part of inter-population analysis.

Y-chromosome single nucleotide polymorphism analysis: A total of Six Y-chromosome markers were studied, namely, M9, M89, M45, M69, M173 and M175. The sites looked for in each of them are given below:

The M9 region of the Y-chromosomal DNA was amplified and the 68th position in the sequence was looked for the presence of 'C' (ancestral) or 'T' (derived). The M45 region of the Y-chromosomal DNA was amplified and the 109th position in the sequence was looked for the presence of 'G' (ancestral) or 'A' (derived). The M89 region of the Y-chromosomal DNA was amplified and the 347th position in the sequence was looked for the presence of 'C' (ancestral) or 'T' (derived). The M69 region of the Y-chromosomal DNA was amplified and the 222nd position in the sequence was looked for the presence of 'T' (ancestral) or 'C' (derived). The M173 region of the Y-chromosomal DNA was amplified and the 191 positions in the sequence were looked for the presence of 'A' (ancestral) or 'C' (derived). The M175 region of the Y-chromosomal DNA was amplified to look for the 5 bp deletion at interval 84-88 in the sequence.

RESULTS AND DISCUSSION

Mitochondrial DNA D-loop sequence variation in HVR I: The HVR-I region of mtDNA was sequenced and compared with the revised Cambridge reference sequence (Andrews *et al*., 1999). The Table 1 showing mutated sites of HVR1 region in Poliyar. The Table 2 showing mutated sites of HVR1 region in Palliyar. The Table 3 showing mutated sites of HVR1 region in Malaikuravar. The Table 4 showing a comparison between various populations of world at different position of mutation in mtDNA HVR1. The HVR-I mutations at different nucleotide positions with respect to the above reference sequence are shown in Table 4. Out of the 316 sites studied for polymorphism, Poliyar tribe showed

Table 1: Mutated sites of HVRI region in poliyar population

	Position					
Anderson sequence	16187 c	16223 c	16274 g	16319 g	16320 c	16362 t
PO754	*	T	*	*	*	*
PO763	*	T	*	*	*	*
PO766	*	T	*	*	*	*
PO767	*	T	*	*	*	*
PO768	*	T	*	*	*	*
PO774	*	T	*	*	*	*
PO777	*	T	*	*	*	*
PO778	*	T	*	*	*	*
PO784	*	T	*	*	*	*
PO785	T	T	A	A	T	C
PO786	*	T	*	*	*	*
PO788	*	T	*	*	*	*
PO789	*	T	*	*	*	*
PO790	*	T	*	*	*	*
PO791	*	T	*	*	*	*
PO794	*	T	*	*	*	*
PO797	*	T	T	*	*	*

*: indicates no mutation

Table 2: Mutated sites of HVRI region in palliyar population

	Position						
Anderson sequence	16051 a	16205 a	16222 c	16269 c	16273 g	16318 g	16351 t
PA801	G	C	*	*	*	*	*
PA804	G	C	*	*	*	*	*
PA805	G	C	*	*	*	*	*
PA806	G	C	*	*	*	*	*
PA809	G	C	*	*	*	*	*
PA811	G	C	*	*	*	*	*
PA814	G	C	*	*	*	*	*
PA815	G	C	*	*	*	*	*
PA816	G	C	*	*	*	*	*
PA817	*	*	T	*	*	*	*
PA818	*	*	T	T	A	A	C
PA819	*	*	T	*	*	*	*

*: indicates no mutation

polymorphism at 6 sites, Malaikuravar tribe showed polymorphism at15 sites whereas Palliyar tribe at 7 sites.

Out of the 6 polymorphic sites studied in the Poliyar tribe, transitions were observed at 6 sites, and no transversion were observed in Poliyar tribe. Transitions were observed at nucleotide position 16187, 16223, 16274, 16319, 16320, and 16362 .In these tribal samples, no A to C or G to C substitution was observed.

Out of the 15 polymorphic sites studied in the Malaikuravar tribe, Transitions were observed at 13 sites, whereas transversion was observed at 2 sites. There were totally 6 A to T substitutions at 16318 position and only o tribe.

Out of the 7 polymorphic sites studied in the Palliyar tribe, transitions were observed at 6 sites, whereas transversion was observed at only one site. There were totally 9 A to C substitutions were observed at 16205 position.

There were 9 insertions in Palliyar tribe at the positions of 16188 and 9 deletions were observed in Palliyar tribe at the position of 16191. There was no ne A to C substitution at 16206 position observed in this insertion or deletion in Poliyar and Malaikuravar tribes. In the comparative analysis to the specific mutation sites, these the tribes confirm themselves to the Indian population. The ratio of pyrimidine transition to purine transition is 3.82.

Computational analysis of mitochondrial data: A median joining network on the basis of Roehl's algorithm was drawn for the three tribes using HVR I sequences. The network was based on the variation observed when compared with the reference sequence (i.e.) the Anderson sequence. In the above analysis, all the individuals of three tribes were scattered evenly. Figure 1 showing median joining tree of Poliyar, Malaikuravar and Palliyar constructed using HVR1 sequences.

In Poliyar tribe of Tamil Nadu, single major haplotype was observed in PO754 which contain 16 individuals and rest of 1 is unique haplotype. In Malaikuravar tribe of Tamil Nadu, three major haplotypes were observed in MA825, MA823 and MA821. MA825 contain 4 individuals, MA823 contain individuals and MA821 contain 2 individuals and the rest of 5 individuals are unique haplotypes.

In Palliyar tribe of Tamil Nadu, single major haplotypes were observed in PA801, PA817 which contain 9 individuals. In PA817 contain 2 individual and rest of 1 is unique haplotype. Neighbor joining trees werealso constructed with the data obtained from the sequence of HVR I region of Poliyar, Malaikuravar and Palliyar tribes along with individuals from Indian population and rest of world using ARLEQUIN/PHYLIP software. Figure 2 showing Neighbour joining tree of Poliyar,Malaikuravar and Palliyar with 42 other Indian populations (constructed using HVR1 sequences).

The populations studied in individuals formed 2 comparatively large clusters in the neighbor joining of

Table 3: Mutated sites of HVRI region in malai kuravar

	Position														
Anderson sequence	16051 a	16138 a	16154 t	16206 a	16223 c	16230 a	16256 c	16275 a	16284 a	16309 a	16311 t	16318 a	16325 t	16327 c	16398 g
MA821	*	*	*	*	*	*	*	*	*	G	*	T	*	*	*
MA822	*	*	*	*	*	*	*	*	*	G	*	T	*	*	*
MA823	*	G	*	*	T	*	*	*	*	*	*	*	C	*	*
MA825	*	*	*	*	T	*	*	*	G	*	*	*	*	T	A
MA826	*	G	*	*	T	*	*	*	*	*	*	*	C	*	*
MA827	*	*	*	*	T	*	*	*	G	*	*	*	*	T	A
MA828	*	*	*	*	*	*	*	*	*	G	*	T	*	*	*
MA830	*	*	*	*	T	*	*	*	G	*	*	*	*	T	A
MA833	G	.	C	C	*	G	*	*	*	G	C	*	*	*	*
MA834	*	*	*	*	*	*	*	*	*	*	*	T	*	*	*
MA835	*	*	*	*	T	*	*	*	G	*	*	*	*	T	A
MA837	*	*	*	*	T		T	G	*	*	*	*	*	*	*
MA838	*	*	*	*	*	*	*	*	*	G	*	T	*	*	*
MA840	*	*	*	*	*	*	*	*	*	G	*	T	*	*	*
MA841	*	*	*	*	*	*	*	*	G	*	*	*	*	T	A

*: indicates no mutation

Table 4: A comparison between various populations of world at different position of mutation in mtDNA HVR-I

Populations	16223 C→T	16294 C→T	16311 T→C	16319 G→A	16344 C→T	16357 T→C
Andamans	98	10	50	57	38	57
Africa	91	45	76	1	1	0
Australia	51	2	15	1	0	0
India (Others)	70	42	2	27	0	0
Island SE Asia	42	2	27	2	0	1
English	6	4	21	2	0	0
Western samao	0	0	0	0	0	0
China	59	0	14	14	9	5
Poliyar	17	0	0	1	0	0
Malai kuravar	8	0	1	0	0	0
Palliyar	3	0	0	0	0	0

Table 5: AMOVA results of poliyar, malaikuravar and palliyar population

Group→ ↓	With Indian populations
Percentage of variation	
Among populations	17.27
Within the populations	82.73

Indian populations where Onge tribe was taken as outgroup. Onge was considered to be the most primitive tribe and so taken as outgroup. Kheers, Irulas, Dungrigarasias, Saharias, Yerukulas, Bhilalas, Brahmins, Kattunaickens, Bharias, Oraons Dungribhils, Kanwars, Mawasis were formed in one cluster. The second cluster comprises of Haviks, Bharelas, Brahmins, Rabri, Kols, Nicobarese, Bhils, Koli, Kathodi, Badugas and Thakurs. Kadars, Sugalis, Jarwas, Gonds were formed in one small cluster.

The Poliyar and Palliyar tribe population share similarities with tribes of Kadar, and Palliyan, etc., The Malaikuravar population are very near to Satmani population.

Analysis of molecular variance (AMOVA): AMOVA results of Poliyar, Malaikuravar and Palliyar population were obtained by using 'Arlequin' software indicated the percentage of variation among and within the Indian populations. Forty-two other Indian populations were considered in this analysis and the percentage variation is given in Table 5.

Mismatch Distribution Curve of Poliyar and Malaikuravar Population: Mismatch distribution is defined as the distribution of the observed number of differences between pairs of haplotypes. The mismatch distribution of Poliyar, Malaikuravar and Palliyar population were obtained by plotting the graph taking pairwise differences on the X-axis and their frequency on the Y-axis. The values of observed, simulated and model frequency were given by Arlequin software. This

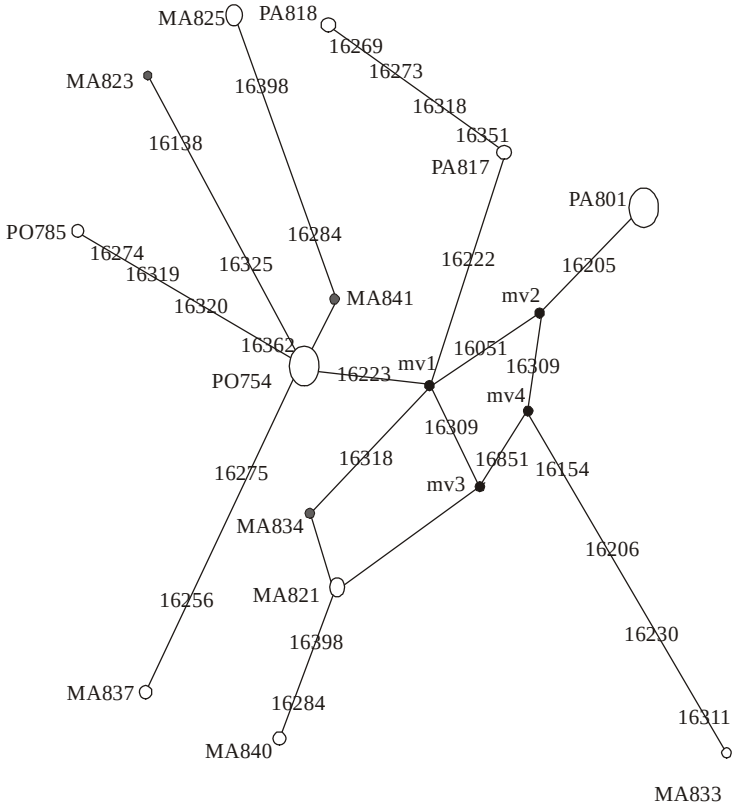

Fig. 1: Median joining tree of poliyar, malaikuravar and palliyar tribes were constructed using HVR-I sequences

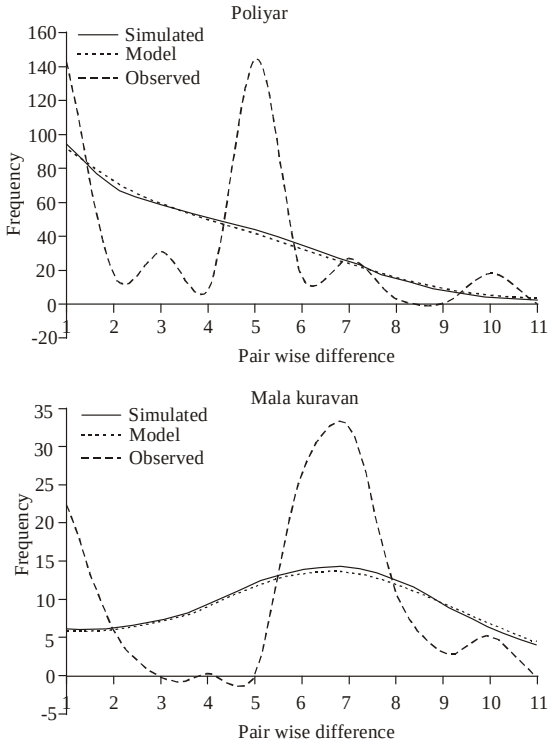

Fig. 2: Mismatch distribution for poliyar and malaikuravartribal populations

distribution is usually uni-modal in populations having passed a recent demographic expansion and usually multi-modal in samples drawn from populations at demographic equilibrium. The mismatch graph of above-mentioned population is shown in Fig. 3.

Y-chromosome markers: The 95 male individuals of Poliyar Malaikuravar and Palliyar population were analyzed with biallelic and SNP markers from the non-recombining position of Y-chromosome. The polymorphic markers M9, M45, M89, M69 M173, and M175 were analyzed according to (YCC) Chromosome Consortium (2002) nomenclature, presented in Fig. 4.

Biallelic and SNP markers:

M9: The 94 samples of PCR amplified products of M9 were sequenced. The C→G substitution was looked at the 68th position of the sequence. The C is ancestral allele and G is mutated allele. 77 out of 95 samples from Poliyar Malaikuravar and Palliyar showed M9 'C' and 18 samples were M9 'G'.

M89: The 77 samples that showed M9 'C' were proceeded with M89 marker to look for C→T substitution. The PCR amplified products of M89 were sequenced. The C→T substitution was looked for at the 347th position of the sequence. 66 samples showed C→T substitution i.e.

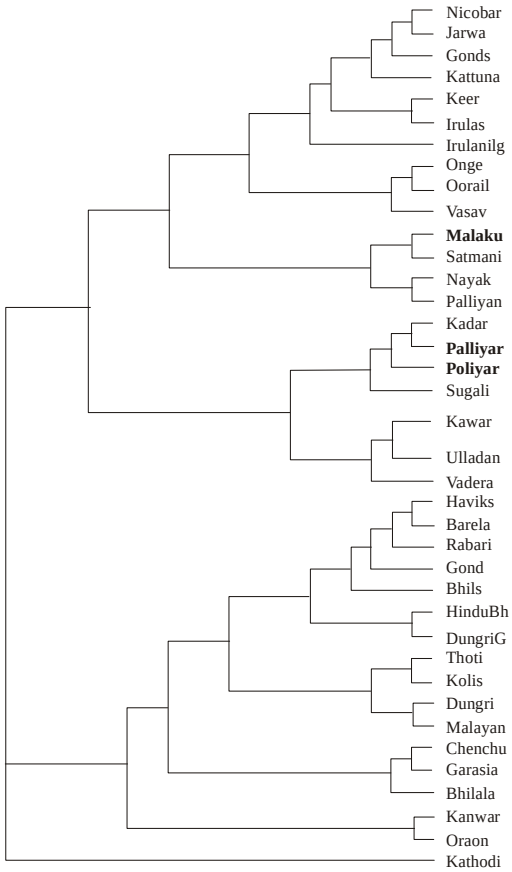

Fig. 3: Neighbour-joining tree of poliyar, malaikuravar and palliyar tribal populations with 42 other indian populations (constructed using HVR-I sequences)

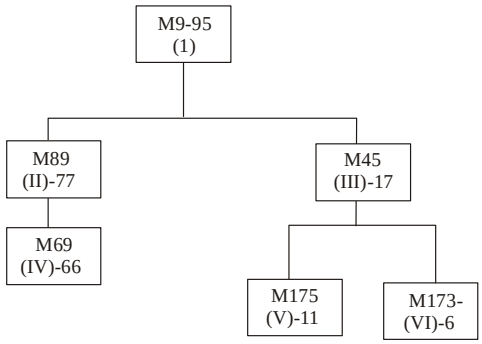

Fig. 4: Y-chromosome phylogeny of poliyar, malaikuravar and palliyar population constructed by following YCC nomenclature for 94 male samples:

M89 'T' and 11 samples showed C→C i.e. M89 'C' (i.e. ancestral state).

M45: The 17 M9 'G' samples were proceeded with M45 marker. The PCR amplified products of M45 were sequenced. The G→A substitution was looked for at the

Table 6: SNPs analyzed for poliyar population

	SNPs					
Reference sequence	M9 G/C	M89 C/T	M45 G/A	M69 T/C	M173 A/C	M175 TTCTCTT CTC DEL (+)
PO749	C	T	*	T	*	*
PO750	C	T	*	C	*	*
PO751	C	C	*	T	*	*
PO752	G	*	G	*	*	+
PO753	C	T	*	T	*	*
PO754	C	T	*	T	*	*
PO755	C	C	*	C	*	*
PO756	C	T	*	T	*	*
PO757	G	*	G	*	*	+
PO758	G	*	A	*	C	*
PO759	C	T	*	T	*	*
PO760	C	C	*	C	*	*
PO761	C	T	*	T	*	*
PO762	C	T	*	T	*	*
PO763	G	*	G	*	*	+
PO764	C	T	*	T	*	*
PO765	G	0	G	*	*	+
PO766	C	C	*	T	*	*
PO767	C	T	*	C	*	*
PO768	C	T	*	T	*	*
PO769	C	T	*	T	*	*
PO770	G	*	A	*	C	*
PO771	C	C	*	C	*	*
PO772	C	T	*	T	*	*
PO773	C	T	*	C	*	*
PO774	C	T	*	T	*	*
PO775	G	*	G	G	*	+
PO776	C	C	*	C	*	*
PO777	C	T	*	T	*	*
PO778	C	T	*	T	*	*
PO779	G	*	G	*	*	+
PO780	G	*	G	*	*	+
PO781	C	C	*	T	*	*
PO782	C	T	*	T	*	*
PO783	C	T	*	T	*	*
PO784	G	*	A	*	C	*
PO785	C	T	*	T	*	*
PO786	C	T	*	C	*	*
PO787	C	T	*	T	*	*
PO788	G	*	A	*	C	*
PO789	C	C	*	C	*	*
PO790	G	*	G	*	*	+
PO791	C	T	*	T	*	*
PO792	G	*	G	*	*	+
PO793	C	T	*	C	*	*
PO794	G	*	G	*	*	+
PO795	C	T	*	T	*	*
PO796	C	T	*	T	*	*
PO797	C	C	*	C	*	*
PO798	G	*	A	*	A	*
PO799	C	T	*	T	*	*

*: indicates no mutation

Table 7: SNPs analyzed for palliyar population

	SNPs					
Reference sequence	M9 G/C	M89 C/T	M45 G/A	M69 A/T	M173 A/C	M175 TTCTCTTC TC DEL (+)
PA800	C	T	*	C	*	*
PA801	C	T	*	T	*	*
PA802	C	T	*	C	*	*
PA803	C	T	*	T	*	*
PA804	C	C	*	C	*	*
PA805	C	T	*	T	*	*
PA806	C	T	*	T	*	*
PA807	C	T	*	T	*	*
PA808	C	T	*	T	*	*
PA809	G	*	G	*	*	+
PA810	C	T	*	T	*	*
PA811	C	C	*	C	*	*
PA812	C	T	*	T	*	*
PA813	C	T	*	T	*	*
PA814	C	T	*	C	*	*
PA815	C	T	*	T	*	*
PA816	G	*	A	*	C	*
PA817	C	T	*	T	*	*
PA818	C	T	*	T	*	*
PA819	C	T	*	T	*	*

*: indicates no mutation

Table 8: SNPs analyzed for malai kuravar population

	SNPs					
Reference sequence	M9 G/C	M89 C/T	M45 G/A	M69 A/T	M173 A/C	M175 TTCTCTT CTC DEL (+)
MA820	C	T	*	T	*	*
MA821	C	T	*	T	*	*
MA822	C	T	*	C	*	*
MA823	C	T	*	T	*	*
MA824	C	T	*	T	*	*
MA825	C	T	*	T	*	*
MA826	C	T	*	T	*	*
MA827	C	T	*	T	*	*
MA828	C	T	*	T	*	*
MA829	C	T	*	C	*	*
MA830	C	T	*	T	*	*
MA831	C	T	*	T	*	*
MA832	C	T	*	T	*	*
MA833	C	T	*	T	*	*
MA834	C	T	*	C	*	*
MA835	C	T	*	T	*	*
MA836	C	T	*	T	*	*
MA837	C	T	*	T	*	*
MA838	C	T	*	T	*	*
MA839	C	T	*	T	*	*
MA840	C	T	*	T	*	*
MA841	C	T	*	T	*	*
MA842	C	T	*	T	*	*
MA843	C	T	*	T	*	*

*: indicates no mutation

109 position of the sequence. Out of 17 samples analysed for this marker, 6 samples showed M45 ‘A’. Remaining 11 samples showed M45 ‘G’.

M69: The 66 samples that showed C→T transition with M89 were proceeded with M69 marker to look for T→C transition at position 222 of the sequence. The PCR amplified products of M69 were sequenced. Out of 66 samples, 19 showed T→C transition and 47 samples showed ‘T’ (i.e. ancestral state).

M173: The 6 sample that showed M45 ‘A’ was proceeded with M173 marker to look for A→C substitution. The PCR amplified products of M173 were sequenced. The A→C substitution was observed at the 191 position of the sequence. 5 samples were M173 ‘C’ and a single sample was M173 ‘A’.

M175: The 11 samples that showed M45 'G' were proceeded with M175 marker to look for 5bp (TTCTC) deletion at position 84 - 88. The PCR amplified products of M175 were sequenced using ABI Prism 3700 automated DNA analyser. All 11 sequences analysed for the particular locus deletions of sequence region TTCTC were observed.

The Table 6 showing SNPs analysis of Poliyar population. Table 7 showing SNPs analysis of Palliyar population. Table 8 showing SNPs analysis of Malaikuravar population.

DISCUSSION

Mitochondrial DNA and Y-chromosome have been used extensively in the study of modern human origins and assigning phylogenetic relationships. A combined study of both Y-chromosome and mtDNA markers are useful to provide knowledge about paternal and maternal migrations and genetic diversity within and between the populations. The mutation rate of animal mtDNA is higher than that of nuclear DNA, mtDNA is a powerful tool for tracking ancestry through females and has been used in this role to track the ancestry of many species back hundreds of generations (Brown *et al*., 1979). The present study has been done using the same approach to obtain genetic relationships of Poliyar ,Malaikuravar and Palliyar population with other Indian and to find the intra-population diversity that exists within the populations.

The Poliyars are mostly distributed in the kodaikanal districts of Tamil nadu was considered to be one of the unique tribe prevailing in India whereas Malaikuravar tribe represent a dominant tribal population in the Theni district of Tamil nadu and Palliyar tribes are distributed in Virudhu nagar and Theni districts of Tamil nadu. Examination of HVR I region in mtDNA revealed 6 polymorphic sites in Poliyar tribe with transition at 6 sites and no transversion. Examination of HVR I region in mtDNA revealed 15 polymorphic sites in Malaikuravar tribe with transition at 13 sites and transversion at 2 site. Examination of HVR I region in mtDNA revealed 7 polymorphic sites in Palliyar tribe with transition at 6 sites and transversion at only one site. Mutations in one of the less highly conserved regions of human mtDNA e.g., the D-loop region or Hypervariable region (Cann *et al*., 1984; Aquadro and Greenberg, 1983) has been the main stream region for the evolutionary studies as it codes the individual and population specific sequences.

Median joining network constructed with mtDNA sequences of HVR I gave prominent outlook on these tribal populations. Only one haplotype in Poliyar tribe, it was inferred that the genetic divergence in this population was very low. All those populations are scattered evenly, when compared with Poliyar and Malaikuravar, showed comparatively less scattered population structure presented in Fig. 1. MJ network analysis revealed that two clusters, which could be assumed of having high level of intra cluster polymorphism. Indo-European-speaking people from West Eurasia entered India from the Northwest and diffused throughout the subcontinent. They purportedly admixed with or displaced indigenous Dravidic-speaking populations. Subsequently they may have established the Hindu caste system and placed themselves primarily in castes of higher rank (Michael *et al*., 2001).

The Neighbour joining tree of HVR I region of Indian population revealed two big clusters, in which Poliyar tribe was found in first cluster whereas Malaikuravar in the second cluster and Palliyar in third cluster. In first cluster Poliyar was along with kadar, Palliyan, Palliyar sub group. Sugali Kanwar and Ulladen were found to be the most basal population in the first cluster. In cluster two, Malaikuravar was grouped with Satnami, Naidu and Nayak tribes. In third cluster Palliyar was along with kadar, Poliyar, Palliyan tribes sub group presented in Fig. 3.

The Indian mtDNA gene pool appears to be more closely related to the east Eurasian gene pool (including central, east and Southeast Asian populations) than the west Eurasian one (including European and Caucasian populations). Within India, northeastern tribes are quite distinct from other groups; they are more closely related to East Asians than to other Indians. This is consistent with linguistic evidence in that these populations speak Tibeto-Burman languages of East Asian origin. Otherwise, analyses of molecular variance suggested that caste and tribal groups are genetically similar with respect to mtDNA variation (Cordaux *et al*., 2003).

On analyzing 370 bp of the first hypervariable region of the mitochondrial DNA (mtDNA) control region in 752 individuals from 17 tribal and four non-tribal groups from the Indian subcontinent diverse histories of tribal populations from India, Cordaux *et al*. (2003) found common ancestry of Indian and western-Eurasian mitochondrial DNA lineages making the perspective of Aryan's entry before 4000 years. But Basu *et al.* (2003) argue that the formation of populations by fission that resulted in founder and drift effects have leftheir imprints on the genetic structures of contemporary populations of India and the upper castes show closer genetic affinities with Central Asian populations, Sun *et al*. (2006) found seven novel basal M haplogroups (viz. M34-M40) and yet another five singular branches of the M phylogeny. They inferred a rapid mode of modern human dispersal along the Asian coast after the initial "Out-of-Africa" event after comparing the matrilineal components among India, East Asia, Southeast Asia, and Oceania at the deepest level.

Information on DNA haplotype divergence is incorporated into an analysis of variance format, derived from a matrix of squared-distances among all pairs of haplotypes. This analysis of molecular variance (AMOVA) produces estimates of variance components. Thus they developed a tool derived from ANOVA

(Analysis of Varience). This is helpful in calculating the divergence time of population from its ancestral one (Excoffier *et al*., 1992).

Mismatch distribution of both Poliyar and Malaikuravar tribes revealed some interesting results. Mismatch graph in Poliyar revealed multimodal peaks from which it can be assumed that there was a possibility of bottleneck in the population at earlier level. In case of Malaikuravar tribal population broad Multimodal peak was obtained which may be due to people may be moving out to different places and there is inflow of people with new mutations into the existing gene pool presented in Fig. 2.

The SNP data gave a comprehensive view of where these three tribal populations belong to the phylogenetic tree. Haplogroup I and II belong to Africa, III, IV and V belong to Africa and Non Africans, Haplotype VI belong to Northern India and Pakistan origin, Haplogroup VII of North East Asia, Haplotype VIII of Central Asia and Haplotype IX and X of North Asia. The confirmatory analysis of these populations with the following markers: M9, M89, M45, M69, M173, M175 revealed that these tribal populations belong to out of Africa haplotypes (Underhill *et al*., 2001). 20 individuals of Poliyar tribe fall in Haplogroup VI, 20 individuals in Haplogroup VII, 6 individuals in Haplogroup VIII and the rest 5 individuals in the most evolved Xth Haplogroup. In case of Malaikuravar tribe 10 individuals shared Haplogroup VI, 9 individuals shared Haplogroup VIII and 5 in Haplogroup X. 10 individuals of Palliyar tribe fall in Haplogroup VI, 5 individuals in Haplogroup VII, and the rest 5 individuals in the most evolved Xth Haplogroup.

From the above results obtained it clearly shows that People settled in India have been migrated from different parts of Asia. Poliyar and Palliyar tribe implicates that it's an admixture of all these populations. Malaikuravar tribe implicates that these population have higher frequency of SNP corresponding to the people of Southern India. Later they migrated to south forming an admixture with the local inhabitants, who might have probably migrated from Central Asia.

Ramana *et al*. (2001) analyzed Y-SNPs of caste and tribal population of India, in which they found the haplotypes H4, H4A, H5A and H16 in the caste population but not in tribal population and they also found H11 haplotype which is found only in tribal populations. But the haplotype H4, H4A, H5A and H16, in siddi s (tribal population) reveals that the siddis have assimilated considerable non-African admixture.

It was pointed out that macro haplogroups M, N, and R are universally distributed in Eurasia but differentiated into distinct haplogroups in East Asia, Oceania, Southeast Asia, and the Andaman Islands in particular (Macaulay *et al*., 2005; Thangaraj *et al*., 2005).

Watkins *et al*. (2008) found among 4 caste population of South India Y-SNPs exhibiting higher affinities towards European population and that of HVR-I showed affinities towards East Asia. This work means to say that the paternal lineage for the South Indians is from Europeans meaning the Aryan men's entry into India as well as Indian populations, even in South Indian populations.

The Y-chromosomal data consistently suggest a largely South Asian origin for Indian caste communities and therefore argue against any major influx, from regions north and west of India, of people associated either with the development of agriculture or the spread of the Indo-Aryan language family (Sahoo and Kashyap, 2006).

Two rival models based primarily on mtDNA and Ychromosome data have been proposed to explain the genetic origins of tribes and castes in South Asia. One model suggests that the tribes and castes share considerable Pleistocene heritage, with limited recent gene flow between them (Kivisild *et al*., 2003), whereas an exact opposite view concludes that caste and tribes have independent origins (Cordaux *et al*., 2004).

Another analogous debate concerns the origins of the hypothetical proto-Elamo-Dravidian language, which is thought to be the precursor of Tamil. It has been proposed that the proto-Elamo-Dravidian language spread eastward from southwest Persia into South Asia with agriculture (Mc-Alpin, 1981), and the argument is bolstered by the existence of a solitary Dravidian-speaking group, the Brahui, in Pakistan (Renfrew, 1996).

The linguistic evidence, however, is compromised by uncertainty regarding whether word borrowing is responsible for the observed linguistic affinities (Blazek and Boisson, 1992). A study of mtDNA-haplogroup frequencies in southwestern and central Asia reported that the Brahui gene pool was more similar to that of Indo-Iranian speakers from southwest Asia than to that of Dravidian populations of India (Quintana-Murci *et al*., 2004).

Major population movements, social structure, and caste endogamy have influenced the genetic structure of Indian populations. Genetic variation between South Indian castes from Tamil Nadu is low (RST = 0.0096). Tamil caste Y-chromosomes and STR alleles are more similar to Europeans than to eastern Asians, and genetic distance estimates to Europeans are ordered by caste rank. In contrast, Tamil caste mtDNA shows greater similarity to eastern Asians than to Europeans (Watkins *et al*., 2008).

The haplogroup O-M95 had originated in the Indian Austro-Asiatic populations ~65,000 yrs BP (95% C.I. 25,442-132,230) and their ancestors carried it further to Southeast Asia via the Northeast Indian corridor. Subsequently, in the process of expansion, the Mon-Khmer populations from Southeast Asia seem to have migrated and colonized Andaman and Nicobar Islands at a much later point of time. (Vikrant *et al*., 2007)

Genetic differentiation among Tamil castes is low (RST = 0.96% for 45 autosomal short tandem repeat (STR) markers), reflecting a largely common origin.

Nonetheless, caste- and continent-specific patterns are evident. For 32 lineage-defining Y-chromosome SNPs, Tamil castes show higher affinity to Europeans than to eastern Asians, and genetic distance estimates to the Europeans are ordered by caste rank (Watkins *et al*., 2008).

Kivisild *et al*. (2003) and Cordaux *et al*. (2003) emphasize that the combined results from mtDNA, Y-chromosome and autosomal markers suggest that Indian tribal and caste populations derive largely from the same genetic heritage of Pleistocene southern and western Asians and have received limited gene flow from external regions since the Holocene.

The sharing of some Y-chromosomal haplogroups between Indian and Central Asian populations is most parsimoniously explained by a deep, common ancestry between the two regions, with diffusion of some Indian-specific lineages northward. The Y-chromosomal data consistently suggest a largely South Asian origin for Indian caste communities and therefore argue against any major influx, from regions north and west of India, of people associated either with the development of agriculture or the spread of the Indo-Aryan language family (Sahoo and Kashyap, 2006).

Wells *et al*. (2001) report a very high frequency (approaching 50%) of Haplogroup L in South India appear to have been due to extrapolation from data obtained from a sample of 84 Kallars, a Tamil-speaking warrior caste of Tamil Nadu, among whom 40 (approx. 48%) displayed the M20 mutation that defines Haplogroup L. Subsequent studies of various Indian populations have shown this high frequency of Haplogroup L among the Kallars to be an anomaly in the region; Haplogroup L Y-chromosomes rarely comprise even 25% of the Y-chromosome diversity among any Indian population.

CONCLUSION

The combined use of mtDNA and Y chromosome markers taken for the study of two tribal populations namely Poliyar, Malaikuravar and Palliyar tribes of TamilNadu lead to the final conclusions.

Tribal population Poliyar, Malaikuravar and Palliyar tribes were genetically distinct. Poliyar tribal population and Malaikuravar shows uniform expansion in population size. Those Poliyar, Malaikuravar and Palliyar populations were evenly scattered. Malaikuravar were highly inbred population initially, but now due to socialization endogamy is less practiced. Those Poliyar, Malaikuravar and Palliyar tribes were migrated from parts of Asia and hence they are of Asiatic tribal populations. Poliyar and Palliyar tribes are genetic relatedness with kadar and palliyan tribes of TamilNadu. Malaikuravar tribes are genetic relatedness with Satnami, Naidu and Nayak. Their relatedness with other tribal populations of India needs to be investigated Further. All those tribes are endogamous populations.

Although the tribes are genetically distinct from each other, present study revealed that there is a recent admixture among them. This may be due to the migration of tribes slowly from their original habitats towards the urban areas for their social and economic betterment. Though they follow their cultural and ritual practices, a gradual change was observed these days. Although the tribes are genetically distinct from each other, present study revealed that there is a recent admixture among them. This may be due to the migration of the tribes slowly from their original forest habitats towards the urban areas for their social and economic betterment. Though they strictly follow their cultural and ritual practices a gradual change was expected as is observed in the present study. Study on the same tribal population, which live in interior forest would help in assessing the evolutionary status of the population, further, the rate of out-breeding and as well as the inflow of gene pool from other population and their migration can be assessed.

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