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A study of genetic diversity among three endogamous caste populations of Andhra Pradesh using mitochondrial DNA

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Abstract

The objective of this study is to investigate the genetic affinities of Gavara, Kalinga and Turpukapu Caste populations of Andhra Pradesh by using mitochondrial DNA. In this work, hyper variable region of mitochondrial DNA that gives an insight on specific patterns of divergence, migration and evolutionary history is used to trace the genetic diversity of three Caste populations of Andhra Pradesh and also, the genetic affinities with other available Indian populations.

Keywords: Mitochondrial DNA, Haplogroup, Genetic differences, Multi-dimensional analysis

Introduction

Information about the history of our species comes from two main sources: the paleoanthropological record and historical inferences based on current genetic differences observed in humans. Since the 1990s, it has become common to use multilocus genotypes to distinguish different human groups and to allocate individuals to groups (Bam shad et al. 2001).. Studies of Human genetic variation imply that Africa was the ancestral source of all modern humans and that Homo sapiens migrated out of Africa and displaced Homo erectus between 140,000 and 290,000 years ago (Cann et al. 1987).The Human Genome Diversity Project is an international project that seeks to understand the diversity and unity of the entire human species. The Human Genome Diversity Project (HGDP) aims to collect biological samples from different population groups throughout the world, with the aim of building up a representative database of human genetic diversity. The Human genome comprises of actually two genomes: a complex nuclear genome, which account for 99.9995% of total genetic information and a simple mitochondrial genome, which accounts for the remaining 0.0005%. During zygote formation, a sperm cell contributes its nuclear genome, but not its mitochondrial genome to the egg cell. Consequently, the mitochondrial genome of the zygote is determined exclusively by that originally found in the unfertilized egg. The mitochondrial genome is therefore maternally inherited. As a result it does not undergo any genetic reshuffling and thus is intact which makes it a unique tool for studying human origins. Thus everyone carries with them a more or less exact copy

of the mtDNA from their mother and their mother's mother and so forth for countless generations. The term "more or less exact" is the key to scientist solving the mystery of human origins. That's because like all DNA, mtDNA is subject to random mutations over the exons. As these mutations are passed on intact to next generation, they in effect become "tracers" of family. A single type of circular double stranded molecule of 16,569 bases defines human mitochondrial genome.

Mitochondrial DNA (mtDNA) as marker

The mtDNA has no repetitive DNA, spacers or introns. The mtDNA contains 37 genes, all of which are involved in the production of energy and its storage in ATP.The analysis of mitochondrial DNA (mtDNA) has been a potent tool in the understanding of human evolution, owing to its characteristics such as: High copy number 1000-10,000 copies per cell, Apparent lack of recombination as it is a semi-autonomously replicating molecule, High substitution rate almost 10 times greater than nuclear DNA (Brown et al. 1979) and even higher in non-coding control region,. Maternal mode of inheritance ,Semi-autonomously replicating molecule, No repetitive DNA, spacers or introns, Small size of the molecule and simple genome organization and hence easier to study, They serve as "molecular clocks" as they can be used to calculate the divergence time elapsed. However, almost all studies of human evolution based on mtDNA sequencing have been confined to the control region also called the D-loop or the displacement loop, which constitutes less than 7% of the mitochondrial genome.

Mitochondrial DNA control region

Mitochondrial DNA serves as a molecular clock, in that within its structure there is a 1200-base-pair non-coding segment, called the control region that carries the genetic signals needed for replication and transcription."The rate and pattern of sequence substitutions in the mitochondrial DNA (mtDNA) control region (CR) is of central importance to studies of human evolution". The DNA sequence of the control region is termed hyper variable because it accumulates point mutations at approximately 10 times the rate of nuclear DNA. In the human control region, the estimates of the rate of substitution were found to range between 2.8 (Cann et al. 1983) to 5 times (Aquadro & Greenberg 1983) the rate of the rest of the mtDNA. Most of the studies in which control region sequences have been used have focused on intraspecific patterns of variability and phylogenetic relationships of closely related species, a prominent example being the study of human population history. Polymorphic nucleotide sites within this loop are concentrated in two "Hyper variable segments", HVR I (positions 16024-16383) and HVR II positions (Wilkinson-Herbots et al. 1996). Hence HVS I and HVS II data can provide useful insights about inter and intra-specific population variations..

Profiles of castes

Gavara: Gavaras are one of the backward communities or castes of Andhra Pradesh, who live mostly in the north coastal districts i.e. Visakhapatnam, Vizianagaram and Srikakulam.

Turpu Kapu: Turpu in Telugu means east. The Kapus living on the eastern frontier of Andhra Pradesh (Srikakulam, Vizianagaram and Visakhapatnam districts) called themselves Turpu Kapus. The main difference between Kapus and Turpu Kapus is widow marriages are not allowed in Turpu Kapus at any cost, but it is allowed in Kapus.

Kalinga: The Kalingas are essentially Telugus and are found on the borderland between Ganjam (Orissa) and Visakhapatnam (A.P) districts. The word

Kalinga means a native of Kalinga, the name of the sea board of the Telugu country.

Review of literature

The phylogeographic structuring of the human mitochondrial DNA variation has propitiated a genetic approach to study the modern Homo sapiens dispersals throughout the world from a female perspective. Human mtDNA is a non-recombining molecule with maternal inheritance and practically haploid genetics. Differences between mtDNA sequences are only due to mutation.. The entire DNA sequence of the human mitochondrial genome - 16,569 nucleotides was determined.(Anderson et, al., 1981) and has recently been revised (Andrew's et,al.,1999).Variations in the mtDNA sequence have been analyzed in human populations, both in terms of evolution and population dispersal and in terms of the role that mtDNA mutations play in human disease (Torroni et, al., 1995, Ingman et, al., 2001).Huoponen et.al. (2001) studied regarding mitochondrial DNA variation in an aboriginal Australian population. Direct sequencing of the control region hyper variable segment I (HVSI) of the mtDNAs revealed 34 distinct sequences. Phylogenetic analysis of the HVSI sequence data depicted that the Walbiri had ten distinct haplotype groups (haplogroup), or mtDNA lineages.

MtDNA provides a potent tool for studying human evolution, in view of characteristics such as maternal inheritance, high mutation rate (Brown *et al.*, 1979), high copy number and lack of recombination(Elson et al,2001). The information to be gained from polymorphisms is useful for analyzing putatively pathogenic mutations, for constructing mtDNA phylogenies (Torroni & Wallace 1993, Finnilä *et, al.,* 2001) and for tracing population migrations (Wallace 1994, Torroni & Wallace, 1995, Shiela & Van Holst Pellakan et al 2006).Deka et, al.,(1995) analyzed nine hyper variable nuclear DNA (HVR) polymorphisms in the two Samoan groups. Genetic distances indicated minimal population differentiation between the two Samoan groups.Horai et, al., (1996) analyzed Nucleotide sequence of the major noncoding region of human mitochondrial DNA from various races was extended with 72 Native Americans from 16 different local populations (nine populations from Chile, four from Colombia, and one each from Brazil and from Maya and Apache Indians). Comparison of the 482-bp sequences in the 72 Native Americans, 43 different types of mitochondrial DNA sequences were observed. Stoneking et al., (1996) surveyed 898 individuals from 16 tribal populations in India and found 6 individuals with the 9-bp deletion. Sequences of the first hyper variable segment (HVI) of the mtDNA control region from these 9-bp deletion-bearing mtDNAs were compared to those previously reported from Asian and African populations. Phylogenetic analysis indicated three distinct clusters of tribal Indian 9-bp deletion mtDNA types. Herlina et al., (2001) studied 1091 individuals representing 15 ethnic groups was the most extensive mtDNA survey to date of the Indonesian archipelago. Six distinct length polymorphisms in region V were observed within these 15 populations. The 9-bp deletion was found in every population examined at frequencies comparable to those of previously examined East Asian populations and substantially lower than those in most Pacific Island populations. Despite the inclusion of Austronesia-speaking populations and a Papuan-speaking population, there was no statistically significant heterogeneity in the frequency of the 9-bp deletion among the 15 populations ($p = 0.09$).. Watkins et al., (1999) studied the origins and genetic affinities of more than 500 tribal populations in South Asia and compared them to individuals from Asia, Africa and Europe. They observed 9 bp deletions in four South Indian

populations such as Irulas, Yanadis, Siddis and Maria Gonds. Length polymorphisms of the 9 bp were also observed in the Santhals, Khonda, Dora and the Jalaris and different Mundari Mon Khmer speaking populations of Austro Asiatic language group, Thangaraj et al (2005) with respect to different mtDNA haplogroups. Phylogenetic analysis of the mitochondrial control region sequence from individuals with the 9 bp indicated that it had arisen independently in some Indian populations. Recently Thangaraj et al., (2006) unraveled the origin and genetic affinity of Andaman Islanders using complete mtDNA sequences and rigorous in-silico analysis.

Materials and methods

Blood sampling: 10 ml of intravenous blood samples from healthy and unrelated individuals of Gavara Caste (104), Kalinga Caste (100) and Turpu Kapu Caste (100) from Andhra Pradesh, India were collected in vacutainers containing EDTA as an anticoagulant with their informed written consent.

Quantification of DNA: The extracted DNA was quantified by checking in 0.8% agarose gel. Standard protocols for genetic analysis of HVS-1 region of mt DNA including the sequencing are adopted here.

PCR amplification of mtDNA D-loop (HVS1): The HVSI in the D loop region of the human mitochondrial DNA was amplified in order to study the genetic polymorphism. PCR products of 400bp were electrophoresed at 120V in 2% agarose gel. The PCR products were then visualized under UV light in Trans illuminator. On obtaining a single band devoid of any primer-dimer bands the PCR. Products were then sequenced.

Protocol for sequencing the PCR product

PCR products of HVSI (mtDNA -D loop) were directly sequenced using the ABI Prism 3700 DNA analyzer. The amount of dNTPs and the concentration of the primers during the PCR were optimized, leaving no unincorporated dNTPs or excess of primers

Processing of sequence plate: 3ml absolute alcohol was added to 120µl of 3N sodium acetate (pH 5.2) in a tube. The tube was mixed thoroughly. 25µl of the above mixture was added in each well of the plate. The plate was centrifuged at 4000rpm for 20min in Eppendorf (5810R) centrifuge at 25ºC.The plate was then inverted to remove the supernatant. 100µl of fresh 80% ethanol was added to each well and again centrifuged at 4000rpm for 13min.The plate was once again inverted and placing filter paper and giving a pop spin for few seconds at 750rpm removed alcohol. The plate was covered properly with fresh foil. At the time of sequencing, 10µl of 50% HiDye™ formamide was added to all the wells. The sample plates were kept and run in the ABI Prism® 3700 DNA Analyzer (for sequencing).

ARLEQUIN VER 2.000:

Software for Population Genetics Data Analysis-It is population genetic software able to handle large sample of molecular data (RFLP's, DNA sequences, micro satellites), while retaining the capacity of analyzing conventional genetic data (standard multi locus data or more allele frequency data). The analysis ARLEQUIN can perform on the data fall into two main categories: intra-population and inter population methods. The inter-population methods include search for shared haplotype between populations, AMOVA (Analysis of Molecular Variance), pair wise genetic distances, exact test for population differentiation, Assignment test of genotypes.

Results

Analysis of molecular genetic markers

For investigation of the genetic affinities of the three Caste populations (Gavara,

Kalinga and Turpu Kapu) inhabiting at Visakhapatnam district of Andhra Pradesh used the uniparentlly inherited mitochondrial DNA markers, are used here.

Sequence of hypervariable region (HVR-I)

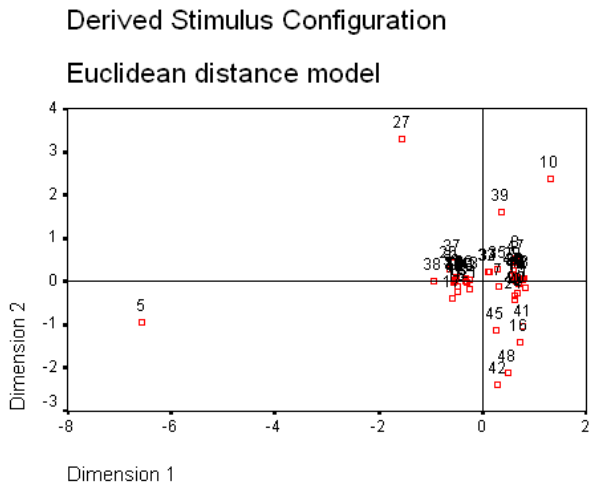
With the sequence of highly polymorphic regions as in the control regions of HVS-1 (nps 16000-16400) of D-loop of mtDNA, most of the population specific neutral mitochondrial variation can be identified at the mutations differentiating the individuals. 304 samples of different individuals have been analyzed with marker HVRI. The HVRI of mtDNA was compared with the Cambridge Reference Sequence (r-CRS) (Andrews et al 1999)

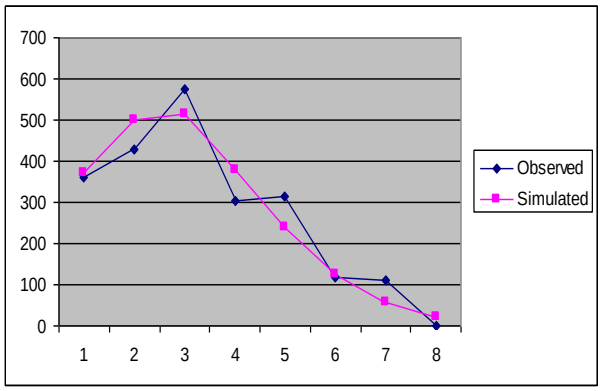
Molecular Diversity and Demographic Expansion

The mismatch distribution of the three Caste populations is obtained by plotting the graph taking pair-wise difference on the X-axis and frequencies on the Y-axis, shown in figure 7. The respected value of the population for plotting the graph is taken from the Arlequin analysis of that population. From the graph it is possible to infer that whether the population is a constant or an expanding one. The mismatch distribution graph of Gavara Caste (Graph-1) and Kalinga Caste (Graph-2) suggests the population is in bottleneck while the Turpu Kapu Caste (Graph-3) showed graph with two peaks which suggests its genetic structure to be in constant gene pool.

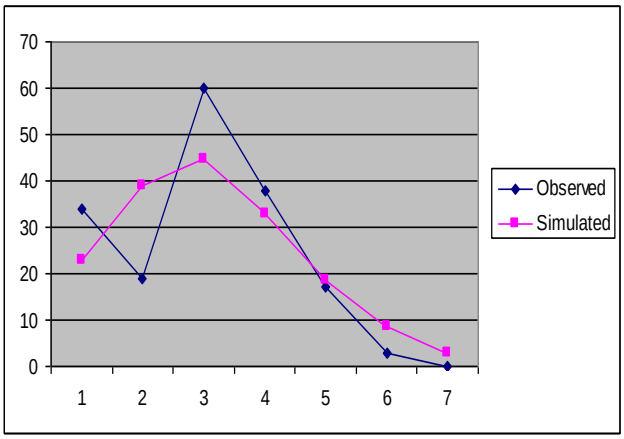
MULTI-DIMENSIONAL ANALYSIS:

Label	Population name	-----	----------		
1	Tharu	2	Buxas	3	Barela
4	Andh	5	Chenchu	6	Koya
7	lambadi	8	Pardhi	9	Thoti
10	Kapu	11	Madiga	12	Mala
13	Relli	14	Yadava	15	Brahmin
16	Habura	17	Santhal	18	Lohra
19	Munda	20	Oraon	21	Chero
22	Bharia	23	Garasias	24	Kanwar
25	Satmani	26	HinduBhils	27	Gond
28	Dungri Bhil	29	Ghasia	30	IrulaNilgiris
31	Oorali	32	Kathodi	33	Dungri Garasia
34	Malayan	35	Ulladan	36	Mawasi
37	Santhal	38	Tanti	39	Jatapu
40	Rathwa	41	Tadvi	42	Kanjad
43	Siddi	44	Jat	45	Mudaga
46	Vishnu	47	Gavara	48	Kalinga
49	Turpu Kapu				

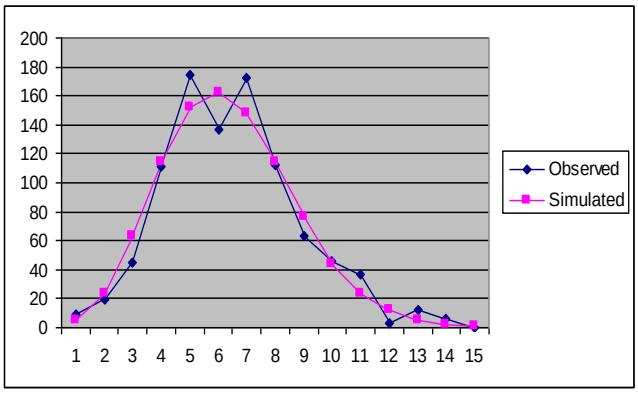




Graph no. 1: The mismatch distribution curve of Gavara Caste of Andhra Pradesh based on the HVR I sequence of mt DNA.



Graph no.2: The mismatch distribution curve of Kalinga Caste of Andhra Pradesh based on the HVR I sequence of mt DNA.



Graph no. 3: The mismatch distribution curve of Turpu Kapu Caste of Andhra Pradesh based on the HVR I sequence of mt DNA.

Multi-Dimensional analysis was done based on the genetic distances values (Fst values) obtained from Arliquine analysis based on HVR-I region sequences. The data from 46 different populations were also used that resides on Indian subcontinent. The results (Fig-9) obtained suggest very close genetic affinity among all Indian population, which formed a cluster. Only Kalinga population showed a bit higher genetic affinity with Habura and Kanjad populations from Uttar Pradesh

Discussion

The objective of this study is to investigate the genetic affinities of Gavara, Kalinga and Turpu Kapu Caste populations of Andhra Pradesh by using mitochondrial DNA. The mtDNA provides clear insights to trace the evolution of the population as they are transmitted uniparentally. Since, mitochondrial DNA markers are inherited uniparentlly and do not undergo any recombination; they are useful for tracing the separate ancestry of paternal and maternal lineage. Hence, in this work, hypervariable region of mitochondrial DNA

that gives an insight on specific patterns of divergence, migration and evolutionary history is used to trace the genetic diversity of three Caste populations of Andhra Pradesh. Also, the phylogenetic analysis of mitochondrial HVR1 region was useful to infer some relatedness of these tribes with other Indian and world populations. According to Seielstad *et al.,* (1999) there are two possible reasons for greater genetic diversity in a population: Firstly, when the population is older and has been accumulating genetic variations for a longer period of time and secondly gene flow is higher from other population. In the mismatch distribution graph, if the curve is of two consecutive peaks of same or different nature, the population is considered to be constant. If the graph shows just a raised straight line, those members follow population bottleneck. If it is bell shaped curve, the population is considered to be expanding. In t mismatch distribution graph based on pair wise differences on mtDNA HVRI sequences we obtained a declining straight line among Gavara Caste (Graph-1) and Kalinga Caste (Graph-2) populations suggesting that the two populations are in bottleneck condition while in case of Turpu Kapu Caste (Graph-3) the simulated curve showed unimodal bell shaped curve while the observed curve showed two peaks that depicts the status of population to be in constant state.

The origins of the culturally and genetically diverse populations of India have been subject to numerous anthropological and genetic studies. It remains unsettled whether the genetic diversity seen between different Indian populations primarily reflects their local long-term differentiation or is due to relatively recent migrations from abroad.

To address the questions concerning the origin, genetic structure and relationship of caste population of Andhra Pradesh with other caste and tribal groups of India, we analyzed 400bp of the hyper variable region of the mitochondrial DNA in 304 individuals belonging to Gavara, Kalinga and Turpu Kapu caste populations of Andhra Pradesh, South India and compared the results with the available data from the Indian sub continent.

Based on the mutations observed in the hyper variable region of mtDNA, haplogroup was assigned to each individual. It was observed that individuals of all three caste populations were falling in macro haplogroup M and N haplogroup. Further, sub-haplogrouping of Gavara Caste individuals showed presence of M, M18, U, U2, JT, HV, N and R haplogroups (Fig-6). R haplogroup was found at very high frequency in all three caste populations. Interestingly Eurasian specific U2 haplogroup was found at much higher frequency in Gavara caste than other two castes. Sub haplogrouping of Kalinga Caste individuals showed most of individuals falling in M, M5, M18, MDG, HV, U2, JT, N and R haplogroups. M macro haplogroup remained major lineage in this population including lineages of M5 and M18. Genetic diversity was found highest among the individuals of Turpu Kapu caste which includes M, M2, M4, M5, M6, M18, MDG, U, U2, R5, R6, HV, N and R lineages (Fig-8). Interestingly, R5, R6, M4 and MDG were found specifically to this caste in present study. This may be the result of high level of admixture with surrounding populations. Recent studies by Thangaraj et al 2005, opened new insights to many unique studies that can be made to found unique patterns of genetic footprints of different maternal and paternal lineages in India. Though aimed primarily at the study of population genetics, nonetheless, mtDNA studies will continue to play an important role in such areas as examining socio-cultural influences on human genetic variation, ancient DNA, certain forensic DNA applications, pharmaco-genomics and in tracing out personal genetic history.

Further Multi-Dimensional analysis was done based on the genetic distances values (Fst values) obtained from Arliquine analysis based on HVR-I region sequences. The data from 46 different populations were also used that resides on Indian subcontinent. The results obtained suggest very close genetic affinity among all Indian population, which formed a cluster. Only Kalinga population showed bit higher genetic affinity with Habura and Kanjad populations from Uttar Pradesh

Conclusions

It was observed that all the individuals were falling in macro haplogroup M and N. Further, sub-haplogrouping of Gavara caste individuals showed presence of M, M18, U, U2, JT, HV, N and R haplogroups (Fig-6). R haplogroup was found at very high frequency in all three-caste populations. Interestingly, Eurasian specific U2 haplogroup was found at much higher frequency in Gavara caste then other two castes. Sub-haplogrouping of Kalinga castes showed most of individuals falling in M, M5, M18, MDG, HV, U2, JT, N and R haplogroups. Genetic diversity was found highest among the individuals of Turpu Kapu caste which includes M, M2, M4, M5, M6, M18, MDG, U, U2, R5, R6, HV, N and R lineages (Fig-8). Interestingly, R5, R6, M4 and MDG were found specifically to this caste in present study. This may be result of high level of admixture with surrounding populations.

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