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# Studies on genetic diversity of kongu vellalar population using mitochondrial DNA and Y- chromosome markers

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## Abstract

The genetic diversity of Kongu Vellalar population of Salem District Tamil Nadu was studied using mt DNA and Y-chromosomal biallelic SNP markers.In this study, 400 base pair of the HVR-1 region and selected coding regions of the mitochondrial DNA (mtDNA), and 8 Y chromosome SNPs were analyzed in 96 Kongu Vellalar caste population of Tamil Nadu, and compared the results with the available data from the Indian subcontinent. It was observed that all the individuals of Kongu Vellalar caste population were falling in macrohaplogroup M and N. Further, subhaplogrouping of "M" revealed that Kongu Vellalar caste population was falling in haplogroup M*, M35 and M5. On the other hand at the Y chromosome haplogroup level 29% of the studied Kongu Vellalar caste population falls in Indian specific haplogroup M82-H1a. Our study concluded that there might be an admixture of this population with the surrounding Austro-Asiatic populations.

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## Introduction

Evidence of ancient human dispersals and settlement in South Asia is preserved in the genomes of its inhabitants, in the form of randomly accumulating mutations, which are passed down through the generations(Petraglia and Allchin, 2007). The analysis of mitochondrial mtDNA has been a potent tool for the better understanding of human evolution and control region sequences (hypervariable segments) of mtDNA are mainly focused on intraspecific patterns of variability and phylogenetic relationships of closely related species of human population (Cavalli-Sforza, 1994). The mtDNA can also be used to trace the maternal ancestry(Giles Richard *et al.*,1980). Geographic distribution of human population variation of mtDNA can be highly informative to define the potential range of expansion and migration routes in the distant past. The mitochondrial haplogroup M is denoted first as an ancient marker of East-Asian origin and has been found at high frequency in the populations of India and Ethiopia (A haplogroup is a group of haplotypes that share some sequence variations)(Mark Jobling and Chris Tyler-Smith 2003). Its variation and geographical distribution suggested that more than 50, 0000 years ago Asian haplogroup M separated from eastern-African haplogroup M. likewise the properties of Y chromosome are akin to a list of violations to rulebook of human genetics and the Y chromosome potential tool for the investigation of human evolution ( Mark Jobling and Chris Tyler-Smith 2003) . Much of the extant variation of Y chromosomal DNA will be attributable to the accumulation of neutral mutations combined with the effects of migration.

The social structure of the Indian population is highly dominated by the Hindu caste system still. The origin of caste system in India is matter of debate. Previous genetic studies on Indian castes and tribes failed to achieve a consensus on Indian origins and affinities (Bamshad *et al* 2001). A few studies reported that closer affinity of Indian castes with either the Europeans or the Asians. The genetic differentiation of caste and tribal populations, and the North Indian invasion of Indo-European speaking nomads are pushing the Dravidian tribes of southern peninsula (Bamshad *et al.,* 2001 and Basu *et al*., 2003). More studies are essential for the better understanding of the genetic structure of the diverse Indian populations, where many questions are remain unanswered. Y-Chromosome as well as mitochondrial DNA are inherited uniparentlly and do not undergo any recombination, Due to this unique properties these are used for tracing the separate ancestry of paternal and maternal lineage. In this study Y- chromosomal biallelic SNP markers and mt DNA were used to trace the genetic diversity of Kongu Vellalar population of Salem District Tamil Nadu.

## Methods and Materials

*DNA isolation*

About 5 - 10 ml of blood samples from healthy unrelated individuals from Kongu vellalar caste populations (n=96) were collected from Salem district of Tamil Nadu, South India. DNA was isolated from the samples using the standard protocol (Thangaraj *et al*., 2002)

*Amplification of mitochondrial DNA*

The hypervariable regions (HVR I and HVR II) and selected coding regions of the mtDNA were amplified using MJ Research thermal cycler (PTC-200).

*Y-chromosomal markers*

Sixteen Y- chromosome biallelic polymorphic markers viz M9, M45, M82, M172, M11, M175, M122 and M124 were amplified and studied to construct the Y-chromosome phylogeny of the selected populations according to Y-Chromosome Consortium nomenclature (Jobling and Tyler Smith, 2003). The PCR amplicons along with GS500 LIZ size standard were analyzed using the ABI 3730 DNA Analyser (Applied Biosystems, Foster City, CA). The raw data was analyzed using the GeneMapper v3.7 software program (Applied Biosystems, Foster City, CA).

*Sequencing of the PCR products*

PCR products were directly sequenced using BigDye™ terminator cycle sequencing kit (Applied Biosystems) in ABI Prism 3730 DNA Analyzer following manufacture's protocol. The individual mtDNA sequences were judged against the rCRS [6] using Auto Assembler - ver 2.1 (Applied Biosystems, Foster City, USA). The sequences were aligned using CLUSTAL X (Jeanmougin *et al.*, 1998), and mutation data were scored with MEGA ver 3.1 (Kumar *et al.*, 2004). Mitochondrial haplogroups were assigned to all samples (Thangaraj *et al.*, 2002).

**Results**

*Analysis of molecular genetic markers*

For tracing the ethnic origin of the Kongu Vellalar population from Salem district of Tamil Nadu, around 96 individuals whose were bloody un related was selected for the analysis of uniparentlly inherited mtDNA and Y-chromosome markers.

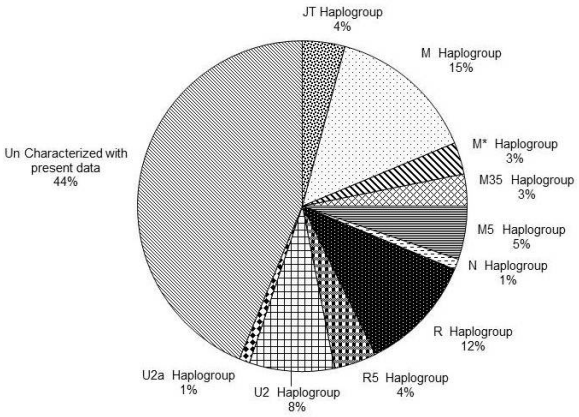


**Fig. 1.** Pie diagram showing the percentage distribution Mt-DNA lineages in Kongu vellalar population of Tamil Nadu.

*Mitochondrial DNA markers*

Sequence of Hypervariable Region (HVR-I)

HVRI region and partial coding sequence of the mtDNA was analyzed in order to resolve the origin and genetic structure of the population with reference to the other Indian populations. The marker in the non-recombining portion of the mtDNA was expected to provide insights into the origin and history of the maternal lineages of these populations. 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. 96 samples of different individuals were undergone analysis of mtDNA analyzed with marker HVRI. The HVRI of mtDNA was compared with the Cambridge Reference Sequence (r- CRS) (Andrews *et al.*, 1999). The sequence variations at different nucleotide positions of the population with respect to the reference sequence was shown in the Fig. 1

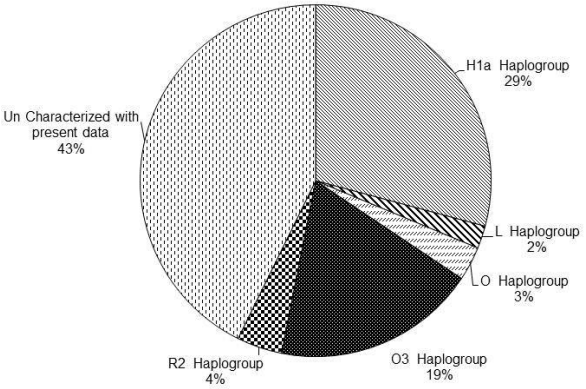


**Fig. 2.** Pie diagram showing the percentage distribution of Y- SNP typing and haplogroups of Kongu vellalar population of Tamilnadu.

Haplogrouping of mtDNA haplotypes

The Kongu Vellalar populations of Tamil Nadu were analyzed for the HVR-1 region of mtDNA. All the samples which do not match with CRS (Andrews *et al.*, 1999). at the position of 16223 nucleotide fall in 'M' haplogroup whereas samples which matches with CRS at the same nucleotide position falls in 'N' haplogroup, most of the mtDNA lineages were showing Indian specific lineage of M and its derivatives in Kongu Vellalar population (27%). Only one sample was falling in to the R haplogroup. The haplogroups were assigned based on the defining mutations reported by various authors, and observed mtDNA haplotypes in Kongu Vellalar populations were shown in Fig. 1. Further

details analysis was more likely required to resolve the data further to categories the unclassified lineage.

**Table 1.** List of mutations observed in 23F region (non-coding region) of Kongu vellalar population.

| S. no. | 23F | 15F | Haplogroup |
|---|---|---|---|
| **1** | Rpt | Rpt | UC |
| **2** | Rpt | Rpt | UC |
| **3** | Rpt | Rpt | UC |
| **4** | 16086 | Rpt | R |
| **5** | 16129-154 | Rpt | U2a |
| **6** | Rpt | Rpt | UC |
| 7 | 16172-234-266-309 | Rpt | R |
| **8** | 16172-222-223-234-309 | 10238-388-398 | N |
| **9** | 16166-172-256 | Rpt | R |
| **10** | 16093 | Rpt | R |
| **11** | 16210-213-223-320 | Rpt | R |
| **12** | 16223-304-311 | Rpt | M35 |
| **13** | 16093 | Rpt | R |
| **14** | Rpt | Rpt | UC |
| **15** | Rpt | Rpt | UC |
| **16** | Rpt | Rpt | UC |
| **17** | 16126 | Rpt | JT |
| **18** | Rpt | Rpt | UC |
| **19** | Rpt | Rpt | UC |
| **20** | 16051-093 | Rpt | U2 |
| **21** | Rpt | Rpt | UC |
| **22** | 16051-093 | Rpt | U2 |
| **23** | 16126-287 | Rpt | JT |
| **24** | Rpt | 10398-400 | M |
| **25** | 16129-210-213-223 | Rpt | M5 |
| **26** | 16184 | 10398-400 | M* |
| **27** | Rpt | Rpt | UC |
| **28** | Rpt | 10398-400 | M |
| **29** | Rpt | 10268-398-400 | M |
| **30** | 16129-311 | Rpt | R5 |
| **31** | 16223-311 | Rpt | M35 |
| **32** | Rpt | Rpt | UC |
| **33** | Rpt | 10398-400 | M |
| **34** | Rpt | Rpt | UC |
| **35** | Rpt | Rpt | UC |
| **36** | Rpt | Rpt | UC |
| **37** | Rpt | 10398-400 | M |
| **38** | 16086 | Rpt | R |
| **39** | Rpt | Rpt | UC |
| **40** | 16126 | Rpt | JT |
| **41** | Rpt | Rpt | UC |
| **42** | 16093-266 | 10158-398-400 | M35 |
| **43** | 16172-256 | Rpt | R |
| **44** | 16154-184-311-362 | Rpt | U2 |
| **45** | Rpt | 10158-398-400 | M |
| **46** | Rpt | Rpt | UC |
| **47** | Rpt | Rpt | UC |
| **48** | 16304 | Rpt | R5 |
| **49** | Rpt | Rpt | UC |
| **50** | 16093-311 | Rpt | R |
| **51** | Rpt | Rpt | UC |
| **52** | 16129-261-264-292-344 | Rpt | R5 |
| **53** | Rpt | 10398-400 | M |
| **54** | Rpt | Rpt | UC |
| **55** | Rpt | Rpt | UC |
| **56** | Rpt | Rpt | UC |
| **57** | Rpt | 10398-400 | M |
| **58** | Rpt | 10398-400 | M |
| **59** | 16051-093 | Rpt | U2 |
| **60** | 16126 | Rpt | JT |
| **61** | 16051-316 | 10398 | U2 |
| **62** | 16086 | Rpt | R |
| **63** | 16129-172-223 | Rpt | M5 |
| **64** | Rpt | Rpt | UC |
| **65** | Rpt | Rpt | UC |
| **66** | Rpt | 10398-400 | M |
| **67** | 16129-172-234-266 | 10398-400 | M5 |
| **68** | 16086 | 10158-398-400 | M* |
| **69** | Rpt | 10398-400 | M |
| **70** | Rpt | 10398-400 | M |
| **71** | Rpt | Rpt | UC |
| **72** | Rpt | Rpt | UC |
| **73** | 16086 | 10158-400 | M* |
| **74** | Rpt | Rpt | UC |
| **75** | Rpt | 10398-400 | M |
| **76** | Rpt | Rpt | UC |
| **77** | Rpt | Rpt | UC |
| **78** | Rpt | Rpt | UC |
| **79** | 16129-172-222-223-234-309 | Rpt | M5 |
| **80** | Rpt | Rpt | UC |
| **81** | 16075-086-092 | Rpt | R |
| **82** | 16129-154-311 | Rpt | U2 |
| **83** | 16129-213-223 | 10398-400 | M5 |
| **84** | Rpt | Rpt | UC |
| **85** | Rpt | Rpt | UC |

| | | | |
|---|---|---|---|
| **86** | Rpt | Rpt | UC |
| **87** | Rpt | Rpt | UC |
| **88** | Rpt | Rpt | UC |
| **89** | 16129-210-213 | Rpt | R5 |
| **90** | Rpt | Rpt | UC |
| **91** | Rpt | 10398-400 | M |
| **92** | 16129-154-311 | Rpt | U2 |
| **93** | Rpt | Rpt | UC |
| **94** | Rpt | Rpt | UC |
| **95** | 16051-093-316 | Rpt | U2 |
| **96** | Rpt | Rpt | UC |

UC - Uncharacterized with present data
JT - Haplogroup
M - Haplogroup
M* - Haplogroup
M35- Haplogroup
M5 - Haplogroup
N - Haplogroup
R - Haplogroup
R5 - Haplogroup
U2 - Haplogroup
U2a- Haplogroup
UC - Haplogroup

Y- Chromosome markers

The SNP data (Fig. 2) gives a comprehensive view of where this caste belongs to in the World phylogenetic tree. A total of 96 individuals belonging to this caste populations were analyzed with SNP markers for the non-recombining position of Y chromosome. The polymorphic markers analyzed were M9, M11, M45, M82, M122, M124, M172, and M175. The ancestor/derived state of all the mutations were tabulated (in Table 2) and the results were analyzed using Phylogenetic tree constructed by YCC (2002). Based on this reference phylogenetic tree of YCC, Y-SNP Chromosome haplotypes were characterized in respective Y haplogroup.

**Table 2.** Y- SNP typing and haplogroups of the Kongu Vellalar population.

| Sample | M9 | M82 | M175 | M122 | M11 | M172 | M45 | M124 | Haplogroup |
|---|---|---|---|---|---|---|---|---|---|
| **KOV1** | A | | A | | A | | | | UC |
| **KOV2** | D | | A | | A | | | | UC |
| **KOV3** | A | | A | | A | | | | UC |
| **KOV4** | D | | A | | A | | | | UC |
| **KOV5** | A | | D | D | | | | | O3 |
| **KOV6** | A | | D | D | | | | | O3 |
| **KOV7** | A | | A | | A | | | | UC |
| **KOV8** | A | | A | | A | | | | UC |
| **KOV9** | A | | D | D | | | | | O3 |
| **KoV10** | A | | D | D | | | | | O3 |
| **KOV11** | D | | D | D | | | | | O3 |
| **KOV12** | D | | A | | A | | | | UC |
| **KOV13** | D | | A | | A | | | | UC |
| **KOV14** | A | | A | | A | | | | UC |
| **KOV15** | A | | D | | | | | | O |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **KOV16** | A | | D | | | | O |
| **KOV17** | D | | A | | A | | UC |
| **KOV18** | D | | D | D | | | O3 |
| **KOV19** | D | | D | D | | | O3 |
| **KOV20** | | | D | D | | | O3 |
| **KOV21** | | | D | D | | | O3 |
| **KOV22** | D | | A | | A | | UC |
| **KOV23** | | | A | | A | | UC |
| **KOV24** | D | | A | | A | | UC |
| **KOV25** | D | | D | D | | | O3 |
| **KOV26** | D | | D | D | | | O3 |
| **KOV27** | A | | D | D | | | O3 |
| **KOV28** | A | | D | D | | | O3 |
| **KOV29** | A | | D | D | | | O3 |
| **KOV30** | | | D | D | | | O3 |
| **KOV31** | | | A | D | A | | O3 |
| **KOV32** | A | | D | D | | | O3 |
| **KOV33** | D | | D | D | | | O3 |
| **KOV34** | D | | D | | | | O |
| **KOV35** | D | | A | | A | | UC |
| **KOV36** | D | | A | | A | | UC |
| **KOV37** | D | | A | | A | | UC |
| **KOV38** | | D | | | | | H1a |
| **KOV39** | D | D | | | | | H1a |
| **KOV40** | | D | | | | | H1a |
| **KOV41** | | D | | | | | H1a |
| **KOV42** | D | D | | | | | H1a |
| **KOV43** | | D | | | | | H1a |
| **KOV44** | D | D | | | | A | H1a |
| **KOV45** | D | D | | | | | H1a |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **KOV46** | A | D | | | | | | H1a |
| **KOV47** | D | D | | | | | | H1a |
| **KOV48** | | D | | | | | | H1a |
| **KOV49** | D | D | | | | | | H1a |
| **KOV50** | A | D | | | | | | H1a |
| **KOV51** | D | D | | | | | | H1a |
| **KOV52** | | D | | | | | | H1a |
| **KOV53** | D | D | | | | | | H1a |
| **KOV54** | | D | | | | | | H1a |
| **KOV55** | | D | | | | | | H1a |
| **KoV56** | D | D | | | | | | H1a |
| **KOV57** | D | D | | | | | | H1a |
| **KOV58** | D | D | | | | | | H1a |
| **KOV59** | D | D | | | | | | H1a |
| **KOV60** | | D | | | | | | H1a |
| **KOV61** | A | D | | | | | | H1a |
| **KOV62** | D | A | | | A | | | UC |
| **KOV63** | A | D | | | | | | H1a |
| **KOV64** | | D | | | | | | H1a |
| **KOV65** | D | D | | | | | | H1a |
| **KOV66** | A | D | | | | | | H1a |
| **KOV67** | | | | A | | A | | UC |
| **KOV68** | A | | | A | | A | | UC |
| **KOV69** | A | | | A | | A | | UC |
| **KOV70** | A | | | | | D | A | UC |
| **KOV71** | A | | | A | | A | | UC |
| **KOV72** | | | | A | | A | | UC |
| **KOV73** | D | | | D | | A | | L |
| **KOV74** | A | | | A | | A | | UC |
| **KOV75** | D | | | D | | A | | L |

| | | | | | |
|---|---|---|---|---|---|
| **KOV76** | | A | A | | UC |
| **KOV77** | D | A | A | | UC |
| **KOV78** | | | D | D | R2 |
| **KOV79** | A | A | A | | UC |
| **KOV80** | | | D | D | R2 |
| **KOV81** | | | D | A | UC |
| **KOV82** | D | A | A | | UC |
| **KOV83** | | | D | A | UC |
| **KOV84** | D | A | A | | UC |
| **KOV85** | | A | A | | UC |
| **KOV86** | A | | D | D | R2 |
| **KOV87** | D | | D | A | UC |
| **KOV88** | A | A | A | | UC |
| **KOV89** | | A | A | | UC |
| **KOV90** | D | A | A | | UC |
| **KOV91** | | A | A | | UC |
| **KOV92** | A | A | A | | UC |
| **KOV93** | | A | A | | UC |
| **KOV94** | | A | A | | UC |
| **KOV95** | | A | A | | UC |
| **KOV96** | | | D | D | R2 |

H1a - Haplogroup
L - Haplogroup
O - Haplogroup
O3 - Haplogroup
R2 - Haplogroup
UC - Haplogroup

**Discussion**

The present study provided the snapshot of the different lineages prevailing at this population, further detailed analysis at high resolution level will be necessary to trace exact historic event associated with their migration and to significantly solve their closest genetic neighbors. In the previous studies that involved populations from all geographic locations of the Indian subcontinent shows that almost 70% of the Indian mtDNA lineages are belongs to M and its subclads. R and it derivatives forms the rest of the mtDNA pool of India. We focused on macrohaplogroup M, which harbors more than 60% Indian mtDNA lineage (Kivisild.*et al*., 1999, Kivisild *et al*., 2003, Bamshad *et*

*al*., 2003, Metspalu *et al*., 2004, Thangaraj *et al*., 2005). A number of mtDNA studies based on HVS1 in Indian population has been carried out and make available some information about genetic structure of Indian gene pool (Bamshad *et al*., 2003, Metspalu *et al*., 2004, Thangaraj *et al*., 2005).However such studies are based on only short stretch (HVS1) and few RFLP sites, none of the M subhaplogroup have been fully characterized so far. Some India specific haplogroups viz. M2, M4, M5 and M6 are identified by some of the previous studies (Kivisild *et al*., 2003, Bamshad *et al*., 2003, Metspalu *et al*., 2004). Besides these common mtDNA haplogroups, a number of other mtDNA variants in India have not been possible to characterize without full mtDNA genome sequencing as the full sequencing of mitochondrial genome provides the best resolution for phylogenetic studies. Inbreeding is very common in this isolated, highly endogamous population that results in female founder effect.

The Indian origin of these populations is further supported by the Y chromosome study. Presence of Indian specific H1 haplogroup in Y chromosome suggests that their paternal lineage is also Indian. This is further supported by the remaining samples falling in the other Indian specific haplogroups such as R2, O and L. Interestingly, we have found M122-O3 haplogroup at second highest frequency which has been reported to be at high frequency in Austro-Asiatic caste populations and has always been in the controversy of debate questioning Himalaya as the barrier for population migrations

**Conclusion**

It is evident that our investigation of small population can offer no more than snapshot of Indian prehistory from the genetic perspective. In future detailed phylogeographic and phylogenetic analyses of more caste population can reveal some interesting patterns of maternal as well as paternal lineages and genetic footprints in central and Himalayan areas of India. Moreover, recent studies opens new insights too many unique studies that can be made to found unique patterns of genetic footprints of different maternal and paternal lineages in India (Thangaraj *et al*., 2005).

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