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Identification of South Indian Muslims by Sequencing the Control Region of Mitochondrial DNA

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ABSTRACT

Mitochondrial DNA (mtDNA) analysis has proven to be an excellent tool for studying the genetic ancestry of many populations .It is also helpful for forensic investigations because of its unique qualities, such as a high mutation rate, maternal mode of inheritance, a high quantity of copies in cells, and control region with specific genetic markers. Therefore, the present study is conducted to establish high-quality forensic data, as well as to assign predominant haplogroups by studying variations generated from mitochondrial DNA control regions among the south Indian Muslims. To this aim, 5ml blood samples were collected from 60 healthy unrelated Muslim individuals of Srirangapatna town in Karnataka state, South India. DNA extracted from the blood sample was amplified, and the sequence of the control region of mtDNA was determined by the Sanger method. Using these sequence data, 48 different haplotypes and 113 polymorphic positions were defined. Of the 48 haplotypes assessed, 40 were unique, and eight were observed in more than one individual. Diversity indices such as genetic diversity, power of discrimination, and random match probability were 0.9870, 0.9705, and 0.0294, respectively. The mean of pairwise differences was estimated at 14.671751 +/- 6.659951 and nucleotide diversity at 0.019229 +/- 0.009680. Consequently, the low random match probability and high genetic diversity were obtained from the present data, while previous studies suggest a high heterogeneity in the Indian Muslim population. The haplogroup pattern and its frequency were indicative of the composition of South Asian (52%), West Eurasian (28%), and West Asian (20%) genetic content in this population. The diversity indices and phylogenetic findings confirm the high potential of mtDNA control region polymorphisms in forensic investigation casework and phylogenetic studies.

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Introduction

Today, India is composed of many ethnic populations with complex cultural diversity. The vast majority of these ethnic populations are Hindu, which provides 80% of the whole population and is socially divided into castes and sub-castes. Muslims contribute 14% of the total population, and the rest are Christians and Sikhs (https://www.census2011.co.in/religion.php). At present, Muslims are the second largest population in India. The Muslim community may have evolved through two major conjectures: (i) military expansions and migration of merchants from Middle Eastern countries, Turkey, Iran, and Arabia, who may have various levels of genetic admixture with the local population.; and (ii) religious conversion that resulted in the spread of Islam throughout India (Roychoudhury *et al*., 2001; Gutala *al*., 2006; Khan *et al*., 2007; Terreros *et al*., 2007; Eaaswarkhanth *et al*., 2008; Eaaswarkhanth *et al*., 2010). It has been stated

that most Indian Muslims belong to indigenous non-Muslim ethnic groups and represent the descendants of local Hindu converts (Balgir and Sharma, 1988; Aarzoo and Afzal, 2005; Eaaswarkhanth *et al*., 2009). Alternately, the Indian Muslim groups may exhibit a high genetic affinity to Middle Eastern or Central Asian migrants. The present study was designed to test these various hypotheses by analyzing mtDNA variations of the control region. Analyses of the control region of mitochondrial DNA have become a pivotal tool for forensic identification, human migration, and phylogenetic studies due to its high substitution rates. The mt-DNA control region is divided into three segments, which display a high level of mutability among individuals and so-called hypervariable regions. Hypervariable region I (HVRI) ranges from nucleotide positions (np) 16024 to 16365, hypervariable region II (HVRII) extends from nucleotide positions 73-340, and hypervariable region III (HVRIII) spans nucleotide positions 438-574 (Samehsalari and Reddy, 2019). Many mtDNA studies in India were carried out mostly on tribes and castes (Cordaux *et al*., 2003; Palanichamy *et al*., 2004; Metspalu *et al*., 2004; Rajkumar *et al*., 2005; Chandrasekar *et al*., 2009; Palanichamy *et al*., 2015; Sylvester *et al*., 2018); however, only two studies have been conducted to date to assess the mtDNA ancestry of Indian Muslims (Terreros *et al*., 2007; Eaaswarkhanth *et al*., 2010). Due to the lack of research, mtDNA information is still limited for a broad overview of the genetic composition of Indian Muslims. Therefore, the present study is conducted to determine mtDNA control region (HVRI and HVII) variations to evaluate the maternal genetic ancestry of Southern Indian Muslims and to provide additional tools for forensics analysis and phylogenetic studies.

Materials and Methods

Population and DNA extraction

Five ml of whole blood was obtained from 60 maternally unrelated and healthy individuals (30 males and 30 females) from the Muslim population of Srirangapattana town, South India. The Institutional Ethical Committee of the University of Mysore generated ethical approval for this study.DNA was extracted from whole blood samples using a QIA am DNA mini kit (Qiagen, Hilden, Germany) as described by the manufacturer. To assess quality and quantity of the extracted genomic DNA, all isolated DNA samples were electrophoresed using a 1% agarose gel.

PCR amplification and sequencing

Two segments of control region HVRI (position 16,024 to 16,365) and *HVRII* (position 73 to 340) were amplified by the following primers: *HVIF* (5′ TAATACACCAGTCTTGTA), *HVIR* (5′ GGATATTGATTTCACGGA), *HVIIF* (5′ GATCACAGGTCTATCACC), and *HVIIR* (5′ CTGGTTAGGCTGGTGTTA). PCR amplification of hypervariable region I (HVRI) was carried out in a 40 µl reaction mixture with cycling conditions 95 °C for 3 minutes, 95 °C for 30 s, 49 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min for 35 cycles as mentioned previously by Mohsenpour *et al.*, (2021). The amplification of hypervariable region II (*HVRII*) was performed under conditions of initial denaturation at 95 °C for 3 min followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 53 °C for 30 s, extension at 72 °C for 45 s, and a final extension at 72 °C for 5 min. The PCR products were sequenced using both forward and reverse primers by the Sanger technique.

Data analysis

The 60 mtDNA sequences generated from this study were aligned to compare with the revised Cambridge Reference Sequence (rCRS; Andrews *et al*., 1999) using the cluster W analysis tool in the bioEdit version.7.2.6. The forensic parameters were calculated by the methods described previously (Samehsalari and Reddy, 2019; Samehsalari and Chandrasekar, 2021). The random match probability (p) was estimated by the equation $P= \sum X^2$, where *x* refers to the observed haplotype's frequency. The discrimination power (DP) was also defined using the equation $DP= (1-\sum X^2)$, where *x* is the frequency of each observed mtDNA haplotype (Tajima, 1989). The genetic diversity (h) was determined following $h= n(1-\sum X^2)/(n-1)$, where *n* is the sample size and *x* refers to the observed haplotype's frequency (Stoneking *et al*., 1991). The rest of the statistical parameters, such as nucleotide diversity and mean number of pairwise differences, were calculated using Arlequin version 3.5.2 software (Excoffier and Lischer, 2010). Mitochondrial haplogroups of the present population were determined via the electronic databases involving the global human mitochondrial DNA phylogenetic tree at http://www.phylotree.org, as well as the mtDNA haplogroup prediction tool published by Mitomap (http://www.mitomap.org/MITOMASTER/webHome).

Results and Discussion

The present paper discusses the mtDNA control region of 60 unrelated individuals of the Muslim population in Srirangapattana town of Karnataka state, South India. Cann et al. (1987) used mtDNA sequences to study the genetic differences and migration patterns of the human population through female inheritance. They reported their mtDNA

analysis from 147 people from five different geographic regions, including Africa, Asia, Australia (aboriginal), Europe, and New Guinea (aboriginal). All these mitochondrial DNAs stem from one woman postulated to have lived about 200,000 years ago, probably in Africa (Cann *et al.*, 1987). Barik et al. (2008) analyzed ten mtDNA samples from Andaman islands and identified only two Andaman-specific M31 and M32 lineages, which are also present in all the Great Andaman tribes and Onge of Andaman islands to reveal the earliest settlers' antiquity and population structure of Andaman islanders. About 70 individuals from Punjab were examined for some mtDNA polymorphisms to study pre-Caucased and Caucasoid genetic features of the Indian populations (Passarino *et al.*, 1996). Forty individuals from Finland, 37 individuals from Sweden, and 48 individuals from Tuscany were studied for the classification of European mtDNAs (Torroni *et al*, 1996); it concluded that 99% of mtDNAs were subsumed within ten mtDNA haplogroups (H, I, J, K, M, T, U, V, W, and X). Two mtDNA macro-haplogroups (M and N) that arose from the African haplogroup L3 encompass virtually all mtDNAs outside Africa (Torroni *et al*., 1994; Chen *et al*., 1995; Quintana-Murci *et al*., 1999; Alves-Silva *et al*., 2000). The current Indian mtDNA gene pool was shaped by the initial settlers and was galvanized by minor events of gene flow from the east and west to the restricted zones (Chandrasekar *et al*., 2009). Overall, haplotype diversity in Indian tribals ranged from 0.671 to 0.995, and nucleotide diversity from 0.005 to 0.023 (Cordaux *et al*., 2003).

Diversity indices

The results of the present study have reported 48 haplotypes and 113 variable sites from the complete control region (HVRI, HVRII, and HVRIII) for 60 individuals. Out of the 48 haplotypes detected, 40 were unique, and eight were found in more than one individual. It was determined that 113 polymorphic sites were distributed in 91 positions with only transition, 11 sites with only transversion, and 11 with indels.

The molecular diversity indices calculated for three hypervariable regions (HVRI, HVRII, HVRIII) in the south Indian Muslim population are available in Table 1. The results depict the greater informativeness of the HV1 region when compared to the other two hypervariable regions and show an estimate of high genetic diversity of 0.9870, a sufficiently Low random match probability of 0.0294, and a higher power of discrimination of 0.97053 when the combination of the three data sets (HVRI, HVRII, and HVRIII) is analyzed. The molecular diversity parameters of the south Indian Muslim population were also compared with Indian subpopulations that were previously studied (Supplement 1). For comparison purposes, only mutations in the HVRI of the control region were considered.

Based on comparative analysis, the Kashmiri population displayed the highest genetic diversity (0.9919) and lowest random match probability (0.01123) (Rakha *et al*., 2016). The random match probability of the present sample (0.03444) was lower than that of the Southeast (0.048641), North (0.07580), and South (0.36051) tribal populations of India. In contrast, genetic diversity demonstrated a higher value (0.9819) than that of Indian tribes and less than that of the Kashmiri Muslim population (Kumar *et al*., 2008; Chaubey *et al*., 2008; Sylvester *et al*., 2018; Verma *et al*., 2018). The high genetic diversity and low random match probability obtained from the present study and previous Indian Muslim population reports (Eaaswarkhanth *et al*., 2010; Rakha *et al*., 2016) confirm the Muslim population heterogeneity in this country.

Table 1. Molecular diversity indices of mtDNA control region (16024-574) sequences for 60 unrelated Indian Muslims.

Parameters	HVI	HVII	HVIII*	Combined data**
Genetic diversity (h)	0.9819	0.9542	0.89939	0.9870
Random match probability (P)	0.03444	0.06166	0.1155	0.029462
Nucleotide diversity(π_n)	0.018565 +/- 0.009838	0.008250 +/-0.004900	0.010071 +/-0.006230	0.019229 +/- 0.009680
Mean number of pairwise differences (π)	6.776271 +/-3.237789	2.805085 +/- 1.502346	2.255932 +/-1.258400	14.671751 +/- 6.659951
Power of discrimination (PD)	0.9655	0.93834	0.8844	0.970538
Number of haplotypes	42	31	18	48
Number of polymorphic sites	65	31	17	113

*= Samehsalari and Chandrasekar 2021; **= Combined data(HVI,HVII,HVIII)

mtDNA haplogroup distribution

The assignment of haplogroups to each individual was evaluated according to the haplotypes identified in the mtDNA control region of the South Indian Muslim population and using PhyloTree Build 17 (www.phylotree.org). Identified haplotypes and detected haplogroups for each individual are shown in Supplement 2. There are a total of six major haplogroups into which 60 samples were classified. The more frequent haplogroup among the population under study was M (52%), followed by haplogroups JT (20%), R (16.67%), U (8.33%), N5, and HV (1.7% each), as showed in Tables 2 and 3.

Table 2. Frequency distribution of South Asian haplogroups among the Indian Muslims.

Haplogroups	Frequency	Percentage %
M*	3	5
M2	6	10
M3	2	3.3
M4'67	1	1.7
M5	3	5
M6	2	3.3
M14	1	1.7
M18	1	1.7
M30	1	1.7
M35	6	10
M49	1	1.7
M57	1	1.7
M65	2	3.3
M66	1	1.7
Total	31	52

It is expected that the majority of Indian Muslim individuals have deep rooting in-situ expansion of mtDNA haplogroup M, which is most abundant in India according to earlier studies (Sun *et al*., 2006; Thangaraj *et al*., 2005; Terreros *et al*., 2007; Eaaswarkhanth *et al*., 2008; Kumar *et al*., 2008; Chandrasekar *et al*., 2009; Eaaswarkhanth *et al*., 2010; Rakha *et al*., 2016; Rej *et al*., 2017).

Table 3. Frequency distribution of West Eurasian and West Asian haplogroups among the Indian Muslims.

Haplogroups	Frequency	Percentage%
N5	1	1.7
R*	2	3.3
R5	2	3.3
R7	2	3.3
R8	4	6.7
JT	12	20
HV14	1	1.7
U2	2	3.3
U4'9	1	1.7
U7	2	3.3
Total	29	48

The frequency of the JT haplogroup was found to be high in the Arabian Peninsula and Iran (Abu-Amero *et al*., 2008; Derenko *et al*., 2013). Alternately, the significant percentage of haplogroup JT (20 %) in the south Indian Muslim population confirms the past gene flow from Iranian and Arabian immigrants during the Islamic religion expansion. According to historical evidence, these immigrant Muslims have married the local Hindu population and produced a new hybrid genetic pool in contemporary Indian Muslims (Eaaswarkhanth *et al*., 2010).

Distribution of west Eurasian haplogroups (R, U, N5, and HV) in the present population suggests that these haplogroups may have originated from Indo-Aryan migration or migrations of proto-Dravidian farmers who spread to India from the eastern horn of the Fertile Crescent, as it was reported in Kivisild *et al*. (1999a,b), Palanichamy *et al*. (2015), and also other historical data.

Haplogroup diversity identified in the studied population indicates a highly admixed mtDNA genetic pool consisting of 52% of South Asian haplogroups (M*, M2, M3, M4’67, M5, M6, M14, M18, M30, M35, M49, M57, M65, and M66), 28% of West Eurasian haplogroups (N5, R5, R7, R8, HV14, U2, U4’9, and U7), and 20 % of West Asian haplogruops (JT). Therefore, according to haplogroup distribution, it can be perceived that South Indian Muslims display a higher affinity to local Indian populations than to the West Eurasian and West Asian groups.

Conclusion

MtDNA analysis data in this study confirms that the Muslim population of South India displays a heterogeneous origin, with a high percentage of South Asian haplogroups followed by haplogroups from West Eurasia and West Asia. The higher frequencies of South Asian haplogroups indicate that most Indian Muslims from the present population share a higher degree of mtDNA similarity with the indigenous Indian regional populations. In addition, the present study provides accurate estimates of random match probability and the power of discrimination, which are essential in routine forensic examinations and identification purposes.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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