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7. Sequence-length variation of mtDNA HVR-I C-stretch in the Muslim population of South India

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Abstract

The aim of this study was to evaluate variations of mitochondrial DNA hypervariable I region C-stretch among 60 unrelated Indian Muslim population of Shrirangapatna town, Karnataka state, south India. The mtDNA hypervariable I region was amplified and sequenced by the Sanger sequencing method. It revealed a total of 8 different C- stretch haplotypes resulting from 8 variable sites. The most frequent haplotype occurred thirty seven times and it is also common in other populations. A statistical estimate for the studied population defined a genetic diversity of 0.569 and random match probability of 0.44. The power of discrimination was found to be 0.56 and the rest of the statistical parameters such as mean of pair-wise differences and nucleotide diversity were calculated as 1.104520 +/- 0.733189 and 0.073635 +/- 0.054209 respectively. These results imparted that the C-stretch in mtDNA HVS-I region could be a valuable genetic marker in forensic investigation of the Muslim population in south India, and the findings from the Fst genetic distance matrix, neighbor-joining tree and multidimensional scaling plot indicated that C-stretch could be applied to molecular evolution and population genetic studies as well.

***Key words**: mtDNA, HVRI, C-stretch, Muslim population*

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Introduction

Every human cell contains a ''second'' genome, exists in the cell's energy-producing organelle, the mitochondrion. Each mitochondrion has many copies of its own genome, and there are several hundred to several thousand mitochondria per cell (Butler 2011). Three aspects of the mitochondrial DNA genome make it a valuable tool in many fields including evolutionary anthropology, genetic genealogy, population history and forensic science (Bender et al. 2000; Adachi et al. 2014). First, its high copy number, which increases the probability of recovering mtDNA in the face of molecular damage that may affect forensic or ancient biological samples. Second, it contains nearly high levels of polymorphisms, which allow individuals to be differentiated. Third, the maternal inheritance of mitochondria is characteristic that has enabled investigators to identify missing persons and trace population migration patterns as well (Bender et al. 2000; Kouvatsi et al. 2001; Kobilinsky et al. 2007; Hughes 2012; Lembring 2013). The mitochondrial genome is a circular double-stranded molecule of 16,569 base pairs in length and it contains the coding region that codes for 37 genes. There is control region or noncoding region that generally referred to as displacement loop (D loop) consisting of approximately 1100 base pairs, it is highly polymorphic and contains three hypervariable regions (HVRI, HVRII, HVRIII) (Samehsalari and Reddy 2018). The highest degree of polymorphism in HVRI has made it the most popular region of the mtDNA to analyze. mtDNA hypervariable region I (HVRI) contains a C-continuous tract, known as C-stretch has a sequence of 13 bp and span nucleotide positions 16180 to 16193(Chen et al. 2009). Due to slipping of the DNA polymerase during replication, the C-stretch evolves much faster than other segments of mtDNA, and mutations in this region have been demonstrated extensively among unrelated individuals(Lee et al. 2004; Lutz et al. 2004; Lee et al. 2006; Chen et al. 2009).Therefore, the C-stretch might be highly significant in forensic identification and population genetic studies. In this study, we evaluated the mtDNA HVR-I C-stretch sequence-length variation in 60 unrelated southern Indian Muslims to explore the significance of these findings to forensic and population genetics studies.

Materials and methods

Population and location

Muslim population under the study inhabited Srirangapatna town which is located at 12.41° N 76.7° E on the south east of Mandya district in Karnataka state at the Southern region of India. The city of Srirangapatna consists of about 25 thousand population according to the 2011 census. Hindus provide

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74% of the whole population and are the most religious network in the city followed by Muslims which contribute 24% of the total population and the rest are Christians and Sikhs (Shankar and Uma 2012; Samehsalari and Reddy 2019). The origin of all participants was belonging to a Muslim population who had inhabited Srirangapatna town for more than three generations.

Sample collection

Blood samples were collected from 60 unrelated Indian Muslim of Shrirangapatana. All samples were taken after written informed consent approved by the Mysore University Ethics Committee was obtained.

DNA extraction

DNA was isolated using a QIA am DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

PCR amplification and sequencing

The mtDNA hypervariable region I (HVRI) was amplified by polymerase chain reaction (PCR) using the primers HVIF (TAATACACCAGTCTTGTA) and HVIR (GGATATTGATTTCACGGA).Polymerase chain reaction (PCR) was performed in a 40-µl reaction volume using GeneAmp PCR System 9700 (Applied Biosystems). The thermal cycling conditions were 95 °C for 3min followed by 35 cycles of 95 °C for 30 s, 49 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 10 min. The PCR products were then sequenced using the Big Dye cycle sequencing ready reaction kit (Applied Biosystems) and fluorescent amplimers were detected through ABI3730 DNA analyzer (Applied Biosystems)

Statistical analysis

The edited data were aligned with the revised Cambridge Reference Sequence (rCRS) (Andrews et al. 1999) using clustal W software in the bioEdit version 7.2.6. The population diversity indices were estimated by Arlequin software ver. 3.5.2 (ExcoffierandLischer 2010). The probability (P) of two randomly selected individuals from a population having identical haplotype types was $P=\sum X^2$ (Stoneking et al. 1991), the genetic diversity (h) was calculated based on the following formula: $h= n(1-\sum X^2)/(n-1)$ and the discrimination power (DP) was also defined using the equation $DP= (1-\sum X^2)$ (Tajima 1989), in which n stands for the sample number and X is the frequency of each C-

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stretch haplotype. A comparative analysis between our haplotype data and the previously published data (Eaaswarkhanth et al. 2010; Irwin et al. 2010; Pankratov et al. 2016; Rakha et al. 2016;Rej et al. 2017) was performed by using Arlequin software ver.3.5.2 (Excoffier et al. 2010). According to the Fst genetic distances a phylogenetic tree from allele frequency data was constructed based on Neighbor Joining (NJ) method using POPTREE2 software (Takezaki et al. 2010)and a multidimensional plot was performed using SPSS software ver. 16.0.

Results and discussion

After performance sequence alignment against the Revised Cambridge Reference Sequence, variations from the rCRS represented mutations, nucleotide positions of these mutations were determined and subsequently mitochondrial haplotypes were concluded and correlated to the published databases.

In the HVS-I region, we defined a total of 8 different c stretch haplotypes resulting from 8 variable sites. Among them three types were unique and the most common haplotype (AAAACCCCC TCCCC) was consistent with the Anderson sequence(Table 1). Transitions (50%) were approximately more than transversions (37.5%) and a lower number of insertions (12.5 %) were defined (Table2). The genetic diversity values of HVS-I C-stretch haplotype in the studied population was 0.569. The probability of random match (p) of two individual's sharing same haplotype was 0.44. A statistical estimate of the results for this population showed a discrimination power (DP) of 0.56, a Mean number of pairwise differences (π) of 1.104520 +/- 0.733189 and other Statistical parameter such as nucleotide diversity (π_n) was calculated as 0.073635 +/- 0.054209 (Table 3).it can be concluded that use of the mtDNA HVR-I C-stretch sequence-length variations along with other polymorphic sites within in HVRI region will increase the power of discrimination in forensic case work. C stretch polymorphisms present in this study and some world populations are inferred in (Table 4), C- stretch sequence variation (haplotype) number 1 was found in all populations, haplotype number 2 and 7 were unique for the present study and sequence number 3, 4, 5, 6 and 8 were common markedly with Indian Muslim populations. The Fst genetic distance matrix of six populations is shown in (Table 5). All pairwise Fst comparisons between the studied population and other five populations were significant with values ranging between 0.062 and 0.158. There were no significant differences of Indian Muslim vs central Asian (*Fst*=0.007, *P*>0.05), Northeast Indian vs East Eurasian (*Fst*=0.007, *P*>0.05), Northeast Indian vs indicates Kashmir & Pakistani (*Fst*=0.011, *P*>0.05). Based on the Fst

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value (Table5), a multidimensional scaling plot (Fig.1) and an unrooted NJ tree (Fig.2) of six populations were constructed and its robustness was assessed through 1000 bootstrap replicates. The results from genetic matrix, phylogenetic tree and multidimensional plot indicate that the genetic distances between the studied population and Indian Muslim population were relatively close, which could be expected as the Muslim population belongs to a same language family and these two populations are closely related to the Central Asian. Due to geographical distance, Northeast Indians and Kashmir & Pakistanis relatively far from the population under study.

The sequence structure of different C- stretch detected in this study is depicted in (Fig.3).

Conclusion

We have obtained genetic data relating to the variation of the C-stretch in mtDNA HVS-I region in 60 unrelated Muslims from south India. This study indicates that the C-stretch could be an additional forensic investigation marker in human identification and could be applied as a useful genetic marker in population genetics and molecular evolutionary studies as well.

Abbreviations

mtDNA: Mitochondrial DNA; HVR: Hypervariable region; C:Cytosine; D-loop: Displacement loop; PCR: Polymerase chain reaction; rCRS: Revised Cambridge Reference Sequence; MDS: Multidimensional scaling

Competing interests

The authors declare that they have no competing interests.

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Table 1 Distributions of the sequence variations in the region from 16180 to 16193(C-stretch) in HVI

No.of Haplotype	Sequence variations	Frequency	Percentage
Hap-1	AAAACCCCCTCCCC (rCRS)	37	0.617
Hap-2	AAAACCCCCCCCCCC	3	0.05
Hap-3	AAACCCCCCCCCCC	13	0.217
Hap-4	AAAACCCCCTCCTC	2	0.0333
Hap-5	AAAACCCTCTCCCC	2	0.0333
Hap-6	AAAATCCCCTCCCC	1	0.0167
Hap-7	AAAACCCCATCCCC	1	0.0167
Hap-8	AACCCCCCCCCCCC	1	0.0167
Total	8	60	1.0007

Table 2 Nucleotide substitutions, insertions in the region from 16180 to 16193(C-stretch) in HVI

Mutation type	Number of positions	Total number of mutations
Transition		
T→C	1	17
C→T	3	4
Total Transversion	4	21
A→C	2	14
C→A	1	1
Total Insertion	3	15
+C	1	3
Total	1	3

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Table 3 Diversity measures in the region from 16180 to 16193(C-stretch) in HVI

Parameters	**HVI**
Genetic diversity (h)	0.569
Random mach probability (P)	0.44
Nucleotide diversity(π_n)	0.073635 +/- 0.054209
Mean number of pairwise differences (π)	1.104520 +/- 0.733189
Discrimination of power(PD)	0.56

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Table 4 Sequence-length variation of mtDNAhypervariable I region C-stretch (np 1680 to 1693) in different human populations

C-Stretch sequence variations	Central Asian[a]	East Eurasian[b]	Northeast Indian[c]	Kashmiri and Pakistani[d]	Indian Muslim[e]	Present study
1.AAAACCCCCTCCCC(rCRS)	78	34	180	289	375	37
2.AAAACCCCCCCCCCC	-	-	-	-	-	3
3.AAACCCCCCCCCCC	-	-	-	-	16	13
4.AAAACCCCCTCCTC	-	1	-	-	11	2
5.AAAACCCTCTCCCC	1	-	-	-	-	2
6.AAAATCCCCTCCCC	-	-	-	4	5	1
7.AAAACCCCATCCCC	-	-	-	-	-	1
8.AACCCCCCCCCCCC	-	-	-	-	8	1
9.AAAACCCCCACCCC	3	-	-	-	-	-
10.AAAACCCCCCCCCC	4	2	-	3	23	-
11.AAAACCCCCTCCCT	1	1	-	5	5	-
12.AAAACCTCCCCCCC	3	-	-	-	-	-
13.AAACCCCCCCCCCCC	7	-	-	-	-	-
14.AAGGCCCCCTCCCC	1	-	-	-	-	-
15.AAAACCCTCCCCCC	1	-	-	-	-	-
16.AAAACCTCCTCCCC	-	-	1	-	-	-
17.AAACCCCCCCCCC	-	-	12	-	-	-
18.AAAACCTCCCCCCC	-	-	1	2	2	-

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19.AAAGCCCCCCCCC	-	-	1	-	-	-
20.AGAACCCCCTCCCC	-	-	1	1	7	-
21.AAAACCCCTTCCCC	-	-	-	4	2	-
22.AAAACTCCCTCCCC	-	-	-	5	1	-
23.AAAACCCCCCCCTC	-	-	-	4	-	-
24.AAAAACCCTTCCCC	-	-	-	-	1	-
25.AAACCCCCTCCCCA	-	-	-	-	6	-
26.AAAACCCTGCCCCC	-	-	-	-	3	-
27.AAAATTCCCCCCCC	-	-	-	-	1	-
28.AAAGTCCCCTCCCC	-	-	-	-	1	-
29.AAACCCTCCCCCCC	-	-	-	-	1	-
30.AAAACTCCCCCCCA	-	-	-	-	2	-
31.AAACCCCCCTCCCC	-	-	-	-	1	-
Total	99	38	196	317	471	60

[a]Irwin et al. 2010; [b]Pankratov et al. 2016; [c]Rej et al. 2017; [d]Rakha et al. 2016; [e]Eaaswarkhanth et al. 2010

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Table 5 Pairwise Fst value between six populations based on HVS-I C-stretch haplotype

Populations	Central Asian	East Eurasian	Northeast_Indian	Kashmiri&Pakistani	Indian Muslim	Muslim of sout Indian*
Central_Asian	—					
East Eurasian	0.018	—				
Northeast_Indian	0.046	0.007**	—			
Kashmiri&_Pakistani	0.035	-0.006	0.011**	—		
Indian Muslim	0.007**	0.010	0.041	0.030	—	
Muslim of sout Indian*	0.075	0.131	0.158	0.149	0.062	—

"*"Indicates population of present study; **p>0.05

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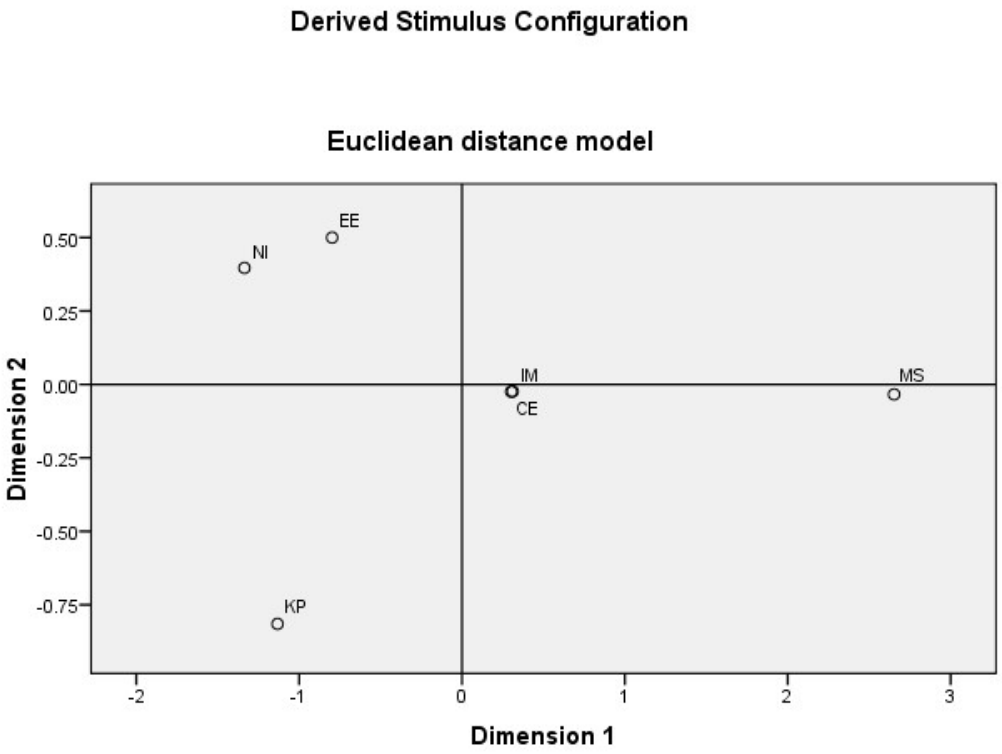

Fig. 1 Multidimensional scaling plot(MDS)of six populations based on Fst distance given in Table 5

"MS" indicates Muslim population of South India (the present study), "IM" indicates Indian Muslim,"CE" indicates central Asian, "EE" indicates East Eurasian, "NI" indicates Northeast Indian, "kp" indicates Kashmir & Pakistani

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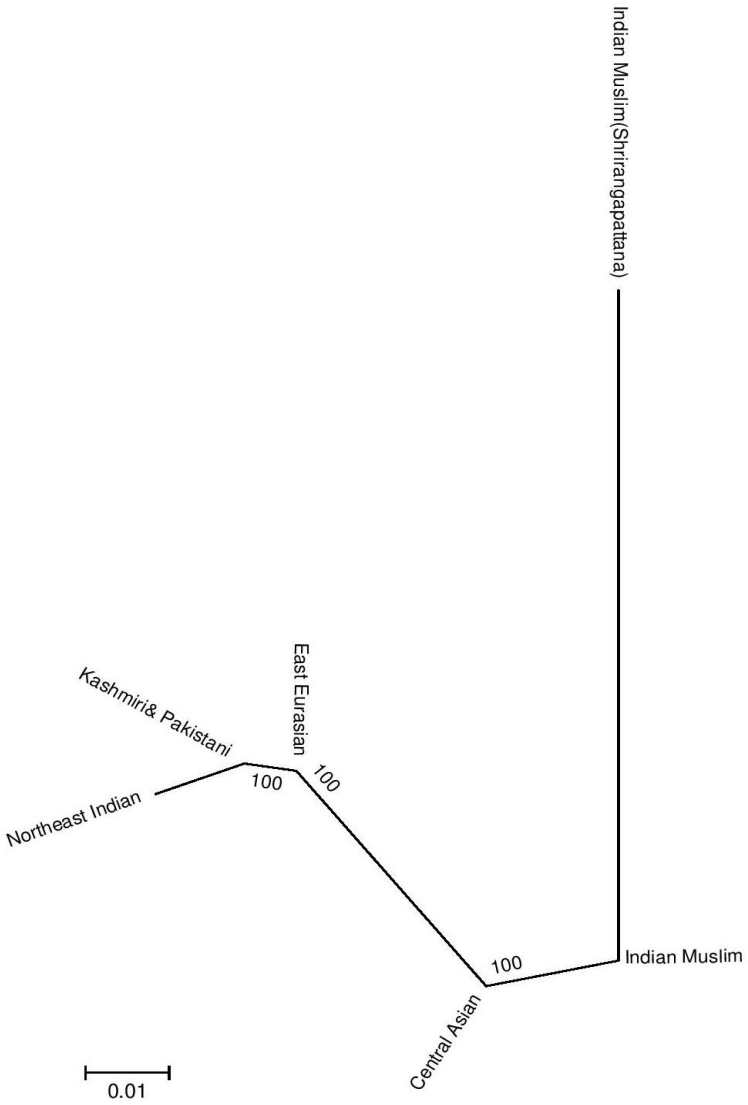

Fig. 2 NJ tree of six populations based on Fst distance given in Table 5, Indian Muslim of Shrirangapattana indicates present study

Citation : Koohyar Mohsenpour, Adimoolam Chandrasekar & Somayyeh Samehsalari (2021). Sequence-length variation of mtDNA HVR-I C-stretch in the Muslim population of South India, **The Researchers** – June 2021, 7(1):60-75. doi - 10.21276/tr.2020.7.1.AN7

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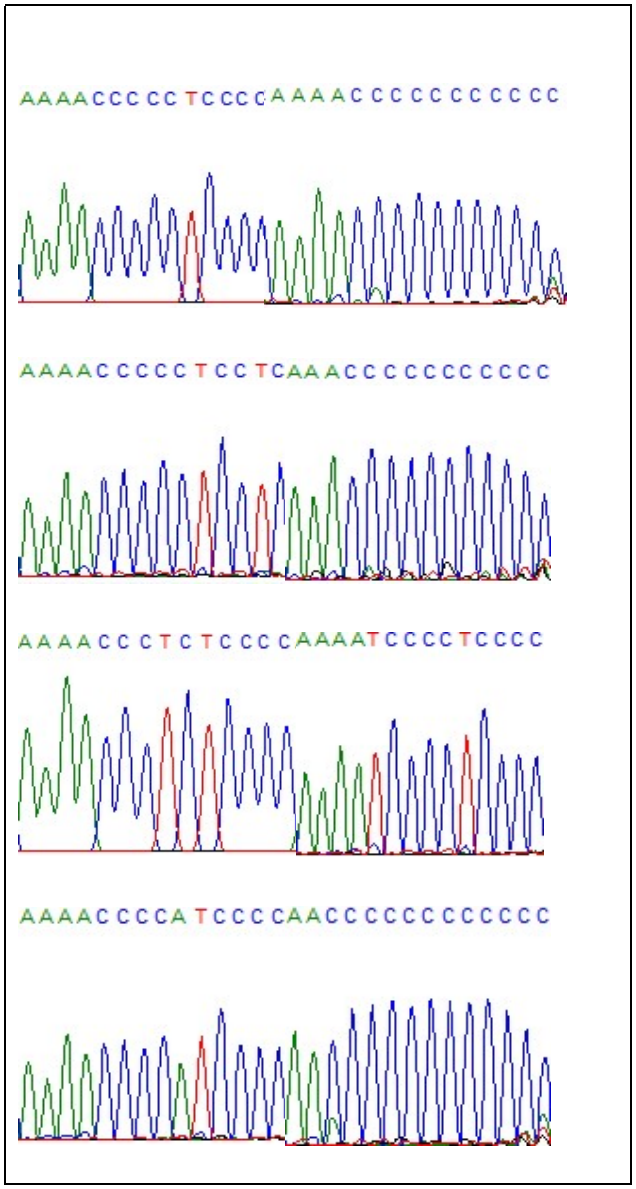

Fig. 3 Different kinds of C-stretch (position 16180 to 16193) sequence types

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