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Research Report

Mitochondrial DNA $(CA)_n$ dinucleotide repeats in Muslims from South India.

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Abstract – We have studied the variability of the CA repeats in HVIII region of mitochondrial DNA among 60 unrelated Muslims of Srirangapatna town, Karnataka state in South India. MtDNA HVIII region was amplified and sequenced by Sanger sequencing method. A total of four alleles [$(CA)_4$ to $(CA)_7$] were detected and $(CA)_5$ rCRS repeat was the most frequently defined repeat followed by $(CA)_4$ 28%, $(CA)_6$ 16%, $(CA)_7$ 15%. A statistical estimate indicated a high genetic diversity of 0.7218 and a low random mach probability of 0.2903, and the discrimination power calculated was 0.7097. Compared with those obtained in other explored world populations our data support the significance application of HVIII CA repeat polymorphism of mitochondrial DNA in the genetic differentiation of human populations and forensic investigation casework.

Keywords: Mitochondrial, HVIII, $(CA)_n$ repeats, Muslim, South India

Introduction

The mitochondrial DNA (mtDNA) is a small circular genome located within the mitochondria in the cytoplasm of the cell (Butler 2011). MtDNA contains approximately 16,569 base pairs and most of those codes for 37 gene (22 tRNAs, 2 rRNAs and 13 mRNAs) products used in cellular energy production. There is also a 1122bp "control" region that does not code for any gene products and is so referred to sometimes as the "non-coding" region which is highly polymorphic and contains three hypervariable regions (HVRI, HVRII, HVRIII) (Butler 2011; Samehsalari and Reddy 2018) Although the most extensive mtDNA variations between individuals are found within the two segments of control region HVRI which ranges from position 16,024 to 16,365 and HVRII extends from position 73 to 340, occasionally a third portion of the control region, known as HVRIII (np 438 to 574) can provide more information regarding a tested sample (Samehsalari and Reddy 2018). It is noteworthy that CA dinucleotide repeats between nucleotide position 514 and 523 in HV3 (third hypervariable region) show length variability like STRs (short tandem repeats) in nuclear DNA and can be useful as supplementary polymorphic segment to identify human remains in forensic casework (Szibor et al. 1997; Chung et al. 2007; Szibor et al. 2007; Hurst 2007). This study aimed to evaluate the variability of the CA repeat in HVIII region among Southern Indian Muslims to explore the significance of these findings to forensic investigations.

Materials and methods

Population and location

Muslim population under the study inhabited in Srirangapatna town which is located near the city of Mysore in Karnataka state at the Southern region of India. Srirangapatna town is located at 12.41° N 76.7° E on the southeast of Mandya district (Shankar and Uma 2012).

The city of Srirangapatna consists of about 25 thousand population according to 2011 census. Amongst them around 12 thousand (49%) are male and 13 thousand (51%) are female. Hindus provide 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). Origin of all participants was belonging to Muslim family who had inhabited in Srirangapatna town for more than three generations.

DNA samples

Blood samples were collected from 60 unrelated Muslim individuals after obtaining written consent which approved by the ethical committee of University of Mysore. DNA was extracted using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. To assess quality and quantity of DNA, all isolated DNA samples were electrophoresed to observe quantity and quality of the extracted genomic DNA, using a 1% agarose gel. The concentration of the extracted DNA and level of protein contamination were detected through spectrophotometer at absorption of 260 nm and 280 nm (with diluted DNA 1 in 5) using an Eppendorf®1 Biophotometer.

PCR amplification and genotyping

The following set of PCR primers was used to amplify HVIII region (position 438-574) of mtDNA: HV3F (5'-GAGCCCGTCTAAACATTTTCAG-3') and HV3R (5' CAGCACTTAAACACATCTCTGC-3'). PCR amplification was carried out in 40 ml reaction mixture subjected to 35 cycles of 95 ℃ for 3 min, 95 ℃ for 30 s, 58 ℃ for 30 s, 72 ℃ for 45 s, and a final extension at 72 ℃ for 10 min. The products were directly sequenced by capillary electrophoresis (using the Big Dye cycle sequencing ready reaction kit Applied Biosystems), on both strands.

Statistical Analysis

Sequences were aligned and compared to the Revised Cambridge Sequence (rCRS) (Andrews et al. 1999) using clustal W software in the bioEdit version 7.2.6. The probability of two randomly selected individuals with the identical mtDNA types (random match probability ($_P$)) was defined by equation $P=\sum X^{2,}$ where X is the frequency of each observed mtDNA haplotype (Stoneking et al. 1991). Genetic diversity (h) for the analyzed DNA fragment was calculated according to the following formula: $h=n\ (1-\sum X^2)/\ (n-1)$, n is the sample size and X is the frequency for each observed haplotype and the discrimination power (DP) was also estimated using the equation $DP=\ (1-\sum X^2)$, where X is the frequency of each observed haplotype (Tajima 1989).

Results and discussion

Allele frequencies, and sequence structures of CA dinucleotide repeats are depicted in Table 1. The results revealed four different [$(CA)_4$ to $(CA)_7$] dinucleotide repeat sequences, 36 out of 60 individuals have indel changes from reference sequence [$(CA)_5$ rCRS] dinucleotide repeats. $(CA)_5$, the threshold sequence, is the most frequently detected repeats in 40% Indian Muslims followed by $(CA)_4$ 28%, $(CA)_6$ 16%, $(CA)_7$ 15%.

Table 1. The frequencies and sequence structures of the CA dinucleotide repeats in the mtDNA HVIII detected in 60 Indian Muslims from the South India

Number of CA repeats	Sequence structure	Number of individuals	Frequency %
5	CACACACACACCGC(rCRS)	24	40
4	CACACACACCGC	17	28.33
6	CACACACACACACCGC	10	16.67
7	CACACACACACACACCGC	9	15
Total		60	

rCRS: revised *Cambridge Reference Sequence*

Table 2. Numbers and percentages of $(CA)_n$ dinucleotide repeat in mtDNA HVIII region in different human populations

$(CA)_n$ dinucleotide repeat	Tunisians (a)	Philippines (b)	Egyptians (c)	Pakistani (d)	Iranians (e)
$(CA)_{5\ rCRS}$	31(70.09%)	53(48.62%)	22(62%)	213(67.19%)	55(69.62%)
$(CA)_3$	-	1(0.9%)	-	-	-
$(CA)_4$	12(27.9%)	55(50.45%)	13(37.14%)	104(32.80%)	16(20.25%)
$(CA)_6$	-	-	-	-	8(10.12%)
$(CA)_7$	-	-	-	-	-
Total	N=43	N=109	N=35	N=317	N=79

Table 2 continued

$(CA)_n$ dinucleotide repeat	Romanians (f)	Germans (g)	Indians (h)	Indians (Present study)
$(CA)_{5\ rCRS}$	320(76.92%)	312(78.78%)	14(14%)	24(40%)
$(CA)_3$	-	-	3(3%)	-
$(CA)_4$	22(5.28%)	42(10.60%)	49(49%)	17(28.33%)
$(CA)_6$	54(12.98%)	33(8.33%)	14(14%)	10(16.67%)
$(CA)_7$	20(4.80%)	9(2.27%)	20(20%)	9(15%)
Total	N=416	N=396	N=100	N=60

N: sample size; The frequency percentage is shown in bracket

References: (a): Costa et al. 2009; (b): Gunnarsdóttir et al. 2011; (c): Kujanova et al. 2009; (d): Rakha et al. 2016; (e): Zarei and Rajabi-Maham 2016; (f): Irwin et al. 2007; (g): Szibor et al. 2007 ; (h): Sylvester et al. 2018

Numbers and percentages of $(CA)_n$ dinucleotide repeat polymorphisms obtained in different world populations are presented in Table 2. $(CA)_5$ repeat was the most abundant detected repeats in Tunisians (70.09%), Egyptians (62%), Pakistani (67.19%), Iranians (69.62%), Romanians (76.92%), Germans (78.78%) and Southern Indians (40 % in the present Muslims sample); while $(CA)_4$ repeat observed as the most frequent allele in Philippines (50.45%) and Southern Indians (49 % in an Urali Kuruman tribal sample). However, a rare dinucleotide repeat $(CA)_3$ was only detected in Philippines (0.9%) and in Southern Indians (3% in an Urali Kuruman tribal sample). Hence it could be specific to South Asiatic populations. It has to be mentioned also that the dinucleotide repeat $(CA)_6$ and $(CA)_7$ are only present in Indian, Iranian (CA_7 not observed) and European populations reflecting possible historical movements shared between these populations.

Table 3. Diversity measures of $(CA)_n$ dinucleotide repeat in mtDNA HVIII region in different human populations

Parameters	Tunisians	Philippines	Egyptians	Pakistani	Iranians	Romanians	Germans	Indians	Indians (present study)
Genetic diversity(h)	0.4120	0.513	0.480	0.442	0.470	0.3875	0.3617	0.6866	0.7218
Random mach probability(p)	0.5975	0.4910	0.533	0.559	0.5358	0.6135	0.6393	0.3202	0.2903
Power of discrimination (PD)	0.4025	0.509	0.467	0.441	0.4642	0.3865	0.3607	0.6798	0.7097

Diversity measures of $(CA)_n$ dinucleotide repeat in mtDNA HVIII region estimated in Southern Indians and in other world human populations are recapitulated in Table 3. The genetic diversity (h) estimated in the two samples from southern India (0.68 in the Urali Kuruman tribal sample and 0.72 in the present Muslims sample) is higher than those found in the other explored world populations (estimated between 0.36 and 0.51). This relatively high genetic diversity would reflect the influence of the geographical location and the history of South India that, situated at the "crossroads" of trading pathways, would have long been characterized by interaction and exchange with other world populations.

The probability of random mach (p) of two individuals showing the same mtDNA haplotype is of 0.2903 in the present sample (Southern Muslims Indians) and 0.3202 in the other southern Indian sample; while it is estimated between 0.49 and 0.64 in the other presented populations. In the present sample (Southern Muslims Indians) the calculated power of discrimination is 0.7097 and 0.6798 in the other Indian sample; while it was computed between 0.36 and 0.50 in the other presented world populations.

Although there are some distinctions in genetic diversities and allele distributions among geographically different populations, it can be easily concluded that use of length polymorphism in CA dinucleotide repeats in the HVIII region along with sequence polymorphism in HVI and HVII regions will increase the power of discrimination in forensic case work. The sequence structure of $CA)_n$ dinucleotide detected in this study is given in (Fig.1).

Conclusion

In this paper we have studied the variability of the CA repeat in HVIII region at np 514-523 of mitochondrial DNA in 60 Indian Muslims from South India. The comparative analysis of our data with those obtained in other explored world populations shows the important utility of indels variability of these CA dinucleotide repeats in genetic differentiation of human populations and forensic investigation casework.

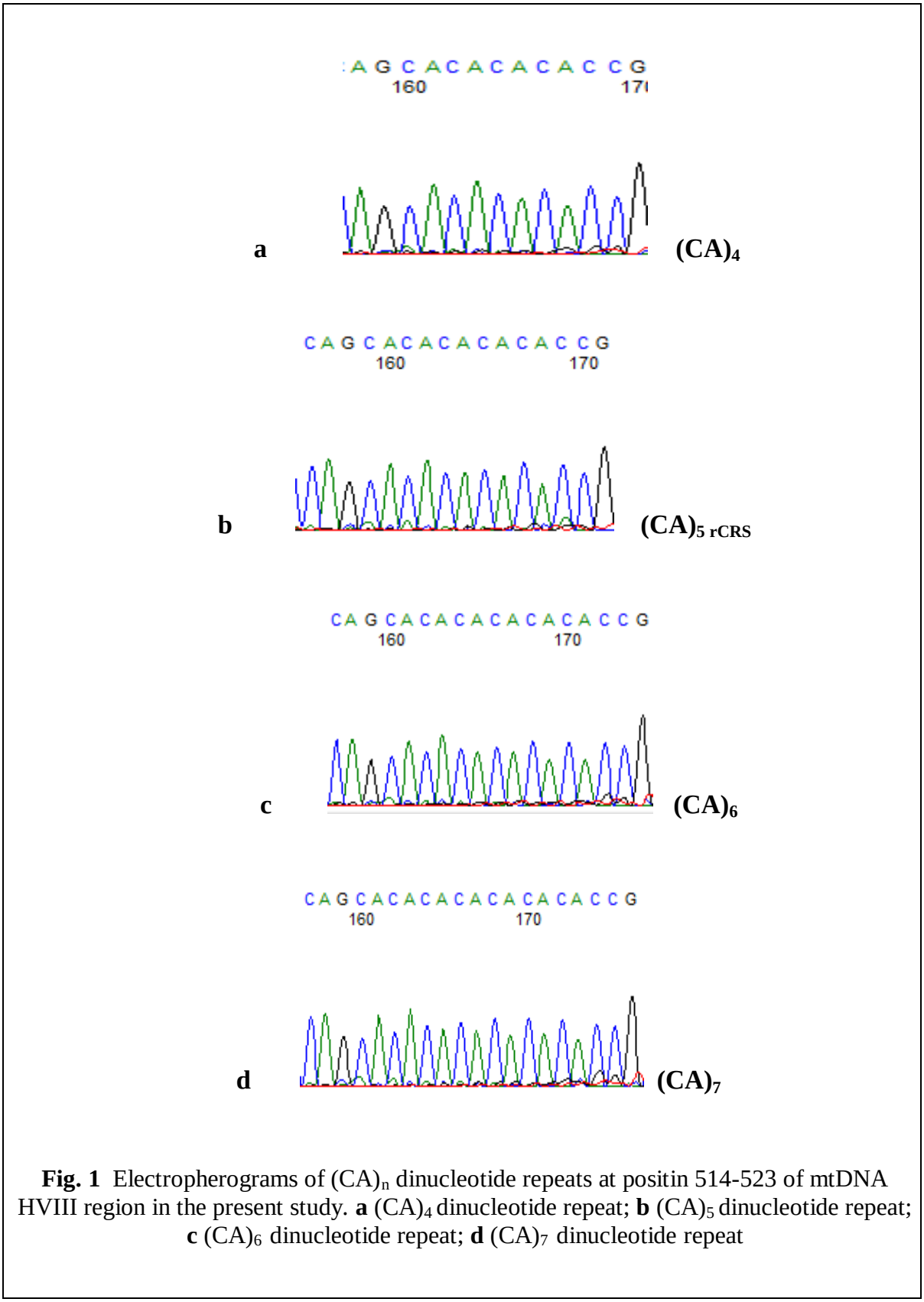

Fig. 1 Electropherograms of $(CA)_n$ dinucleotide repeats at positin 514-523 of mtDNA HVIII region in the present study. **a** $(CA)_4$ dinucleotide repeat; **b** $(CA)_5$ dinucleotide repeat; **c** $(CA)_6$ dinucleotide repeat; **d** $(CA)_7$ dinucleotide repeat

Conflicts of Interest: The authors declare no conflict of interest

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