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Full Length Research Paper

Analysis of mtDNA hypervariable region II for increasing the discrimination power from Middle and South of Iraq

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Mitochondrial DNA is a useful genetic marker for answering evolutionary questions due to its high copy number, maternal mode of inheritance, and its high rate of evolution. The aims of this research were to study the mitochondria noncoding region by using the sanger sequencing technique and establish the degree of variation characteristic of a fragment FTA® Technology (FTA™ paper DNA extraction) utilized to extract DNA. A portion of a non-coding region encompassing positions 37 to 340 for HVII was amplified in accordance with the Anderson reference sequence. PCR products were purified by EZ-10 spin column then sequenced and detected by using the ABI 3730*xL* DNA analyzer. New polymorphic positions G92C, C113G, C150G, T156A, C194G, C198G, G207C, G225C and G228C are described and may in future be suitable sources for identification purpose. The data obtained can be used to identify variable nucleotide positions characterized by frequent occurrence most promising for identification variants.

Key words: D-loop, HVII, Middle and South of Iraq, mitochondrial DNA.

INTRODUCTION

The mitochondrial DNA (mtDNA) is a small circular genome located within the mitochondria in the cytoplasm of the cell. The mitochondrial genome can be divided into two sections: a large coding region, which is responsible for the production of various biological molecules involved in the process of energy production in the cell, and a smaller 1.2 kb pair fragment, called the control region. It is found to be highly polymorphic and harbors three hypervariable regions (HV), HV1, HV2 and HV3 (Kraytsberg et al., 2004). Mitochondrial DNA comprising of 37 genes coding for 22 tRNAs, two rRNAs and 13 mRNAs are a small circle of DNA (Helgason et al., 2004). Mitochondrial DNA does not recombine and thus there is no change between parent and child, unlike nuclear DNA. MtDNA is only passed on from mother to child and this is an important fact (Brown et al., 1993; Giulietta et al., 2000). There is more sequence divergence in mitochondrial than in nuclear DNA (Giulietta et al., 2000; Stoneking et al., 2002).

Genetic analyses in population studies of the mitochon-

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drial genome can be done either by sequencing the mtDNA or through the use of restriction fragment length polymorphisms (RFLPs) (Young, 2009). RFLPs utilize restriction enzymes that can recognize the presence or absence of specific polymorphic DNA regions, and cut sites in the coding region of the mtDNA. Mitochondrial DNA is a useful genetic marker for answering evolutionary questions due to its high copy number, maternal mode of inheritance, and its high rate of evolution. In modern population genetics research, studies based on mitochondrial DNA (mtDNA) and Y-chromosome DNA are an excellent way of illustrating population structure while tracing uni-parental inheritance and ancestry—mtDNA is maternally inherited while the Y-chromosome is paternally inherited.

The aim of this study was to sequence the portion of the noncoding region of mtDNA in order to ascertain the degree of variation present in this fragment and to find those particular polymorphic positions that fulfill the conditions necessary for their future application in the identification process.

MATERIALS AND METHODS

Sample collection, mitochonderial DNA extraction and amplification

Population sample was collected from 380 healthy unrelated volunteer donors, recruited from Middle and South of Iraq. DNA was extracted from all dried blood samples on FTA cards following the manufacturer's procedure as described in Whatman FTA Protocol BD01 except that the Whatman FTA purification reagent was modified to half the volume (Dobbs et al., 2002).

A 1.2 mm diameter disc was punched from each FTA card with a puncher. The discs were transferred to new Eppendorf tubes and washed three times in 100 µl Whatman FTA purification reagent. Each wash was incubated for 5 min at room temperature with moderate manual mixing and the reagent was discarded between washing steps. The discs were then washed twice in 200 µl Tris-EDTA (TE) buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0), the buffer was discarded and the discs were left to dry at room temperature for 1 h. Amplification of HVII region was carried out using five sets of primers

A portion of a noncoding region for HVII was amplified in accordance with the Anderson reference sequence (Table 1). 20 µL of Master Mix was added into a PCR tube and 20 µL of Primer Mix also added. To the same PCR tube, 10 µL of extracting DNA was added after changing the pipette tip again. All the liquid were allowed to settle at the bottom of the tube, and not elsewhere. Check the volume in the PCR tube using the PCR tube with 50 µL in it. 95°C hold for 10 min, 30 cycles of: 94°C for 30 s, 52.5°C for 30 s, 65°C for 1 min. 72°C hold for 10 min. 4°C hold, ∞ infinity is the cycling protocol for amplification of mtDNA PCR.

Purification, cycle sequencing and sequence analysis of mitochonderial DNA

Purification of mitochonderial DNA by EZ10-spin column DNA cleanup kit 100 prep. The DNA Sequencing of the PCR products was done using the BigDye TM Terminator. Utilizing POP-7 polymer (Applied Biosystems) polymer lot number 1206453. The separation of the cycle sequencing products was carried out. Detection was by using the ABI 3730xL DNA analyzer, cap array size 96, cap array length 50. The reference sequence described by Anderson et al. (1981) was compared to the data observed. Within the coding region Mitochondrial DNA, sequencing results are studied from a consensus sequence derived from multiple sequence results. Data were analysed by Sequencher™ (SEQUENCHER™ 4.7 User Manual for Windows © 1991-2007) and aligned with the Anderson sequence (Anderson et al., 1981) using sequence Navigator software.

Statistical analysis

Genetic diversity for the analyzed DNA fragment was calculated according to the formula:

$$h=(1-\sum x_i^2)n/(n-1)$$

Where; n is sample size and xi is the frequency of i-th mtDNA type (Gu, 2001).

The probability of two randomly selected individuals from a population having identical mtDNA types was calculated.

$$(P=\sum x_i^2)$$

Where, p = frequencies of the observed haplotypes (Jones, 1972).

RESULTS AND DISCUSSION

The basic aim of this work was to assess the degree of variation characterizing a selected segment of the noncoding region of mtDNA of human populations from Iraq. The study enabled identification of 107 different haplotypes and 38 polymorphic nucleotide positions (Table 2).

The most frequent variant (H1) was consistent with the Anderson sequence. Substitutions determined during the study are transitions and transversion. This fact is consistent with abundant literature data revealing significant domination of transitions over transversions (Brown et al., 1982; David et al., 2013; Imad et al., 2015a; Mohammed and Imad, 2015). Sixteen (16) polymorphic positions G92C, A95T, C113G, C150G, T156A, T173A, G185C, C186G, A188G, C194G, T195A, C198G, G203C, G207C, G225C and G228C have transverse substitution (Table 3). All the other substitutions determined during the analysis are transitions. The number of analyzed markers has been increased to compensate for the increasing number of profiles in the databases in order to minimize accidental matches between unrelated individuals. Progression of new technology is therefore very slow and the use of SNPs has sometimes met a reluctant reception (Imad et al., 2015b and Mohammed et al., 2015.

Genetic diversity for the analysed DNA fragment was calculated according to the formula: D= 1-$\sum p^2$ and recorded 0.950%. The calculated value of the genetic diversity should be understood as high in the context of

Table 1. Primer sequence, region amplified, fragment size (bp) and PCR product length for HVII.

Primer name	Primer sequence (5' - 3') (forward; F, reverse; R)	Region amplified	Fragment size (bp)	PCR Product length
HVII-1	F: 5'- CACGCGATAGCATTGCGAGA -3'	76-95	20	189
	R: 5'- GTGGCTGTGCAGACATTCAA -3'	264-245	20	
HVII-2	F: 5'- AGCACCCTATGTCGCAGTATC-3'	108-128	21	158
	R: 5'-AGTGGCTGTGCAGACATTCAA-3'	265-245	21	
HVII-3	F: 5'- GAGCACCCTATGTCGCAGTAT-3'	107-127	21	160
	R: 5'-AAGTGGCTGTGCAGACATTCA-3'	266-246	21	
HVII-4	F: 5'- GCGATAGCATTGCGAGACG -3'	79-97	19	200
	R: 5'-TGTCTGTGTGGAAAGTGGCTG-3'	278-258	21	
HVII-5	F: 5'- CGCGATAGCATTGCGAGAC -3'	78-96	19	200
	R: 5'-GTCTGTGTGGAAAGTGGCTGT-3'	277-257	21	

Table 2. Variable positions and Haplotypes for HVII.

Anderson	85	89	92	93	95	113	114	131	143	146	150	151	156	173	178	182	183	185	186	188	189	194	195	198	200	203	207	210	217	225	228	235	239	242	247	248	250	257	No. of individuals
	G	T	G	A	A	C	C	T	G	T	C	C	T	T	A	C	A	G	C	A	A	C	T	C	A	G	G	A	T	G	G	A	T	C	G	A	T	A	
H1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	220
H2	-	-	A	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	1
H3	-	-	-	G	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	3
H4	-	-	-	-	-	T	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	A	-	-	-	-	-	-	G	1
H5	-	-	A	G	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H6	-	-	-	-	-	T	-	-	-	C	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	1
H7	-	-	-	-	G	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	G	-	-	-	-	-	-	1
H8	-	-	-	-	-	-	T	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	2
H9	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	A	-	-	-	-	-	-	-	-	1
H10	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H11	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	3
H12	-	-	-	-	-	-	-	-	A	-	-	T	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H13	-	C	-	-	-	-	-	C	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	2
H14	-	C	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	-	-	-	2
H15	-	-	-	-	-	-	-	-	A	-	-	-	-	-	G	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H16	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	G	-	G	4
H17	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Table 2. Contd.

H18	-	-	-	-	-	-	-	C	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	2
H19	-	-	A	-	-	-	-	-	-	-	-	-	-	C	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H20	-	-	-	-	G	-	-	-	-	-	-	-	C	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	3
H21	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H22	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	C	-	-	-	C	-	2
H23	-	-	-	-	-	T	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H24	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H25	A	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	1
H26	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	G	-	-	3
H27	-	-	-	-	-	-	-	-	A	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	3
H28	-	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H29	-	-	-	-	-	T	-	-	-	C	-	-	-	-	-	-	-	-	-	G	-	T	-	-	-	-	A	-	-	-	-	-	-	-	A	-	-	-	1
H30	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	1
H31	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	A	-	-	-	3
H32	-	-	-	-	-	-	-	-	-	C	-	T	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H33	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	C	-	-	-	C	-	1
H34	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	1
H35	-	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	1
H36	-	-	-	-	-	-	-	C	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	2
H37	-	-	-	-	-	-	-	-	-	-	T	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	2
H38	-	-	-	-	G	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H39	-	C	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	1
H40	-	-	-	-	-	T	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H41	A	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
H42	-	-	A	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	1
H43	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	1
H44	-	-	-	G	-	-	-	-	-	-	-	-	C	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	1
H45	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H46	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	1
H47	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H48	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	G	1
H49	-	-	-	-	-	-	-	C	-	-	-	-	C	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H50	-	-	A	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H51	-	-	-	-	-	-	-	-	A	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	1
H52	A	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	C	-	3
H53	-	C	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	3
H54	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	2
H55	-	-	-	-	-	T	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	2
H56	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H57	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H58	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Table 2. Contd.

H59	A	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	1
H60	-	-	-	G	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	T	-	-	-	A	-	-	-	-	-	-	-	-	-	G	-	-	1
H61	A	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H62	-	-	-	-	G	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	T	-	-	-	-	3
H63	-	-	-	G	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	1
H64	-	-	-	-	-	-	T	C	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	1
H65	-	-	-	-	-	-	-	-	-	C	-	-	C	-	-	-	-	-	T	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	1
H66	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	C	-	-	-	-	-	1
H67	-	-	A	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	2
H68	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	1
H69	-	-	-	G	G	-	-	-	-	-	-	-	-	-	G	T	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H70	-	-	-	-	-	-	-	-	-	C	-	-	C	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	3
H71	-	C	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	G	2
H72	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H73	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H74	-	-	-	-	-	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	1
H75	A	-	A	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	A	-	-	-	4
H76	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	G	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	1
H77	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	2
H78	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	G	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H79	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	3
H80	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	1
H81	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	2
H82	-	C	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	1
H83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	G	-	-	2
H84	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	1
H85	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	3
H86	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	3
H87	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H88	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	G	1
H89	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	1
H90	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	3
H91	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	1
H92	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	1
H93	A	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	G	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H94	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	1
H95	-	-	-	-	-	-	-	-	-	-	-	-	C	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	1
H96	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	G	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	1
H97	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H98	-	C	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	C	-	-	-	-	-	-	-	C	-	1
H99	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	1

Table 2. Contd.

H100	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	1
H101	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	1
H102	A	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H103	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H104	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	G	-	-	2
H105	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	2
H106	-	C	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	1
H107	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	1
Total	-	-	A	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	380

Table 3. Types of mutations in variable positions for HVII.

Positions	Mutation	Type of mutation	Presence in Mitomap	Frequency	Frequency (%)
85	Transition	G-A	Presence	0.043	4.3
89	Transition	T-C	Presence	0.0375	3.75
92	Transition	G-A	Presence	0.05	5
92	Transversion	G-C	New*		
93	Transition	A-G	Presence	0.0375	3.75
95	Transversion	A-T	Presence	0.0405	4.05
113	Transition	C-T	Presence	0.045	4.5
113	Transversion	C-G	New		
114	Transition	C-T	Presence	0.0475	4.75
131	Transition	T-C	Presence	0.0425	4.25
143	Transition	G-A	Presence	0.05	5
146	Transition	T-C	Presence	0.0325	3.25
150	Transition	C-T	Presence	0.035	3.5
150	Transversion	C-G	New		
151	Transition	C-T	Presence	0.045	4.5
156	Transition	T-C	Presence	0.0375	3.75
156	Transversion	T-A	New		
173	Transition	T-C	Presence	0.0325	3.25
173	Transversion	T-A	Presence		
178	Transition	A-G	Presence	0.0225	2.25
182	Transition	C-T	Presence	0.015	1.5
183	Transition	A-G	Presence	0.0475	4.75
185	Transition	G-A	Presence	0.015	1.5
185	Transversion	G-C	Presence		

Table 3. Contd.

186	Transition	C-T	Presence	0.0175	1.75
186	Transversion	C-G	Presence		
188	Transition	A-G	Presence	0.03	3
188	Transversion	A-G	Presence		
189	Transition	A-G	Presence	0.01	1
194	Transition	C-T	Presence	0.015	1.5
194	Transversion	C-G	New		
195	Transition	T-C	Presence	0.025	2.5
195	Transversion	T-A	Presence		
198	Transition	C-T	Presence	0.0125	1.25
198	Transversion	C-G	New		
200	Transition	A-G	Presence	0.0175	1.75
203	Transition	G-A	Presence	0.03	3
203	Transversion	G-C	Presence		
207	Transition	G-A	Presence	0.025	2.5
207	Transversion	G-C	New		
210	Transition	A-G	Presence	0.015	1.5
217	Transition	T-C	Presence	0.015	1.5
225	Transition	G-A	Presence	0.015	1.5
225	Transversion	G-C	New		
228	Transition	G-A	Presence	0.025	2.5
228	Transversion	G-C	New		
235	Transition	A-G	Presence	0.0405	4.05
239	Transition	T-C	Presence	0.0275	2.75
242	Transition	C-T	Presence	0.0225	2.25
247	Transition	G-A	Presence	0.035	3.5
248	Transition	A-G	Presence	0.045	4.5
250	Transition	T-C	Presence	0.02	2
257	Transition	A-G	Presence	0.0225	2.25
Genetic diversity*		D= 1-$\sum p^2$ = 0.95	= 95 %		

New*: new polymorphic positions; Genetic diversity* Genetic diversity for the analyzed DNA fragment was calculated according to the formula: D = 1-$\sum p^2$.

noncoding function of the analysed DNA fragment. The relatively high gene diversity and a relatively low random match probability were observed in this study. Holland et al. (2011) showed that the polymorphism of mtDNA coding area is less than that of mtDNA control region. Therefore, more efficient poly-morphic sites should be used to provide an improved discrimination power for forensic mtDNA testing (Nadia et al., 2011; Imad et al., 2015c; Muhanned et al., 2015).

As forensic markers, they should be phenotypic neutral to avoid landing investigators into serious situations of medical genetic privacy and ethnics, especially for mtDNA coding area whose mutation often correlated with an increased risk of some disease. With the whole mtGenome sequences being researched, we are optimistic that the polymorphism sites within mtDNA coding area will be useful in combination with control region SNPs so as to increase the discrimination power of mtDNA.

Conclusion

Sequence analysis of the noncoding region of mtDNA (HVII) conducted on a population of 380 unrelated individuals enabled identification 107 different haplo-types. New polymorphic positions G92C, C113G, C150G, T156A, C194G, C198G, G207C, G225C and G228C are described may be in future be suitable sources for identification purpose.

Conflict of interests

The authors did not declare any conflict of interest.

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