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Detection of novel polymorphisms in the mitochondrial DNA D-Loop hypervariable region HVI from 400 healthy unrelated individuals from central and North-central Iraq

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The mitochondrial DNA (mtDNA) is a small circular genome located within the mitochondria in the cytoplasm of the cell and a smaller 1.2 kilobase pair fragment, called the control region (D-loop). The aims of this research were to study this region by using the Sanger sequencing technique and establish the degree of variation characteristic of a fragment. FTA® Technology (FTA™ paper DNA extraction) was utilized to extract DNA. PCR products were purified by EZ-10 spin column then sequenced and detected by using the ABI 3730xL DNA Analyzer. Novel polymorphisms discovered at positions 16037, 16075, 16104 and 16201 in future may be suitable sources for identification purpose.

Key words: D-loop, frequency, north-central Iraq, mitochondrial DNA, polymorphism.

INTRODUCTION

Mitochondrial DNA (mtDNA) 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 (Stoneking and Soodyall, 1996; Kraytsberg et al., 2004; Imad et al., 2015a). mtDNA is a small circular genome located within the mitochondria in the cytoplasm of the cell. The mitochondrial genome can be divided into two regions: 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 kilobase pair fragment, called the control region. It is found to be highly polymorphic and harbors three hypervariable regions (HV), HV1, HV2 and HV3 (Helgason et al., 2003; Ingman and Gyllensten, 2003; Ukhee et al., 2005). mtDNA molecules contain 37 genes coding for 22 tRNAs, two rRNAs and 13 mRNAs (Brown et al., 1993; Giulietta et al., 2000; Young, 2009; Imad et al., 2014a). In modern population genetics research, studies based on mtDNA and Y-chromosome DNA are an excellent way of illustrating population structure while tracing uni-parental inheritance and ancestry since mtDNA is maternally inherited while the Y-chromosome is paternally inherited.

mtDNA is therefore inherited from generation to

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

Primer name	Primer sequence (5' - 3') (Forward; F, Reverse; R)	Region amplified	Fragment size (bp)	PCR Product length
HVI-1	F: 5'- TCGTACATTACTGCCAGCCA -3'	16094-16113	20	103
	R: 5'- CATGGGGAGGGGGTTTTGAT -3'	16196-16177	20	
HVI-2	F: 5'- AAACCCCCTCCCCATGCTTA -3'	16181-16200	20	117
	R: 5'- AGGGTGGGTAGGTTTGTTGG -3'	16297-16278	20	
HVI-3	F: 5'-TCACCCATCAACAACCGCTA -3'	16068-16087	20	133
	R: 5'- TAAGCATGGGGAGGGGGTTT -3'	16200-16181	20	
HVI-4	F: 5'-TTCGTACATTACTGCCAGCCA-3'	16093-16113	20	100
	R: 5'- GGGAGGGGGTTTTGATGTGG -3'	16192-16173	20	
HVI-5	F: 5'-TCATGGGGAAGCAGATTTGGG-3'	16029-16049	21	91
	R: 5'- TCATGGTGGCTGGCAGTAAT -3'	16119-16100	20	
	R: 5'-GTCTGTGTGGAAAGTGGCTGT-3'	277-257	21	

generation through the maternal line. Mechanisms for this include simple dilution (an egg contains 100,000 to 1,000,000 mtDNA molecules, whereas a sperm contains only 100 to 1000), degradation of sperm mtDNA in the fertilized egg, and at least in a few organisms, failure of sperm mtDNA to enter the egg. Most mitochondria are present at the base of the sperm's tail, which is used for propelling the sperm cells; sometimes the tail is lost during fertilization. Also, unlike nuclear DNA, where there is a shuffling of the chromosomes at every generation, the mitochondrial DNA does not recombine with any other DNA type and remains intact from generation to generation (Brown, 2000; Brown, 2002; Dobbs et al., 2002; Imad et al., 2014b). mtDNA from all mammalian is very similar, with the order and position of the genes being the same.

Genetic analyses in population studies of the mitochondrial genome can be done either by sequencing the mtDNA or through the use of restriction fragment length polymorphisms (RFLPs) (Guntheroth et al., 1986; Pastore, 1994). 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. These polymorphisms allow scientists to compare mtDNA from crime scenes to mtDNA from given individuals to ascertain whether the tested individuals are within the maternal line (or another coincidentally matching maternal line) of people who could have been the source of the trace evidence.

MATERIALS AND METHODS

Sample collection

Population sample collected from 400 healthy unrelated volunteer donors, recruited from central and north-central Iraq, where the samples were collected from donors after they have been displaced to the center of Iraq because of the bad security situation in the provinces at north of the center stricken.

mtDNA extraction and amplification

DNA was extracted from all dried blood samples on FTA cards following the manufacture's procedure as described in Whatman FTA Protocol BD01 except that the Whatman FTA purification reagent was modified to half the volume reference (Imad et al., 2014c). 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 time, the disc 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 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 HVI region was carried out using five sets of primers (Table 1). A portion of a noncoding region encompassing positions from 16024 to 16365 for HVI was amplified in accordance with the Anderson reference sequence (Anderson et al., 1981). 20 µL of Master Mix was added into PCR tube. Change the pipette tip and add 20 µL of Primer Mix into PCR tube. Add 10 µL of extracting DNA into the PCR tube after changing the pipette tip again. Allow all the liquid settles 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. The location of the tube on the grid after putting the mixture in the thermal cycler 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 which is the cycling protocol for amplification of mtDNA PCR.

Purification, cycle sequencing and sequence analysis of mitochondrial DNA

mtDNA PCR products were purified by EZ10-spin column DNA clean up kit 100 prep. The DNA sequencing of the PCR products

was done using the BigDye TM Terminator and utilizing POP-7 polymer (Applied Biosystems). The separation of the cycle sequencing products was carried out. Detection was by using the ABI 3730xL DNA Analyzer, cap array size of 96 and cap array length of 50. The reference sequence described by Anderson et al. (1981) was compared to the data observed. Within the mtDNA coding region, sequencing results were studied from a consensus sequence derived from multiple sequence results. Data were analysed by Sequencher™ 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 x_i is the frequency of i-th mtDNA type (Gu, 2001).

The probability of two randomly selected individuals from a population having identical mtDNA types is:

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

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 non-coding region of mtDNA. The study enabled identification of 117 different haplotypes and 30 polymorphic nucleotide positions (Table 2). Within these 30 variations, the most frequent variant (H1) was consistent with the Anderson sequence, transitions between T and C, transitions between A and G and only and transversions. This fact is consistent with abundant literature data revealing significant domination of transitions over transversions (Brown et al., 1982; Yang and Yoder, 1999; Imad et al., 2015b).

Eight polymorphic positions (16037, 16041, 16067, 16075, 16104, 16144, 16201 and 16234) showed trans-versions (Table 2) and 4 are novel (Table 3); the number of analyzed markers was 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.

Genetic diversity for the analysed DNA fragment was calculated according to the formula: D= 1-$\sum p^2$ and recorded 95.4%. The calculated value of the genetic diversity should be understood as high in the context of noncoding function of the analysed DNA fragment. The relatively high gene diversity and a relatively low random match probability were observed in this study.

Comparative analysis of our results with previously published Iraqi data revealed significant differences in varying patterns (Nadia et al., 1999). This observation supports the hypothesis that different SNP-type polymorphisms can be strongly associated with a given population. Haplotypes detected in this study group have been compared with other global populations: German (n = 200) (Lutz et al., 1998), US Caucasian (n = 604), Africa (n = 111), Malaysia (n = 195) (Budowle et al., 1999) and India (n = 98) (Mountain et al., 1995) (Table 4).

Walsh et al. (1991) and Tang (2002) showed that the polymorphisms of mtDNA coding area are less than that of mtDNA control region. Therefore, more efficient polymorphic sites should be used to provide an improved discrimination power for forensic mtDNA testing (Imad et al., 2014). As forensic markers, they should be phenotypically neutral to avoid landing investigators into serious situations of medical genetic privacy and ethnics, especially for mtDNA coding area whose mutation is often correlated with an increased risk of some disease. With the whole mtDNA sequences being researched, we are optimistic that the polymorphisms sites within mtDNA coding area will be useful in combination with SNPs in the control region in order to increase the discrimination power of mtDNA (David et al., 2013; Imad et al., 2014).

Conclusion

Sequence analysis of the noncoding region of mtDNA (HVI) conducted on a population of 400 unrelated individuals enabled identification of 117 different haplotypes. The novel polymorphic positions 16037, 16075, 16104 and 16201 described in future may be suitable sources for identification purpose.

Conflict of interests

The author(s) did not declare any conflict of interest.

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Table 2. Variable positions and haplotypes for HVI.

Anderson	16037	16038	16041	16048	16063	16067	16069	16071	16075	16083	16092	16104	16124	16140	16144	16145	16147	16148	16153	16162	16163	16171	16179	16183	16201	16209	16217	16224	16234	16247	No. of Individual
	A	A	A	G	T	C	C	C	T	C	T	C	T	T	T	G	C	C	G	A	A	A	C	A	C	T	T	T	C	A	
H1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	210
H2	G		-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	1
H3	-	G	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	G	5
H4	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	C	-	-	-	1
H5	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	G	-	-	-	-	-	-	1
H6	G	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	1
H7	-	-	G	-	-	-	-	-	-	-	-	-	C	-	-	-	-	T	-	-	-	-	-	G	-	-	-	-	-	-	1
H8	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	C	-	-	1
H9	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	1
H10	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	G	-	-	-	-	-	-	-	-	3
H11	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	2
H12	G	-	-	A	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	1
H13	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	1
H14	-	-	-	-	-	T	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	1
H16	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	T	-	-	-	-	-	2
H17	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	2
H18	-	-	G	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H19	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H20	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
H21	-	-	-	-	-	-	T	-	C	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	1
H22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	1
H23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	T	-	3
H24	-	-	-	-	-	-	-	-	-	T	C	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H25	T	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	1
H26	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H27	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H28	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	T	-	-	-	-	-	4
H29	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	2
H30	-	-	-	A	-	-	-	-	C	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	G	2
H31	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H32	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	1
H33	-	-	-	-	C	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H34	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Table 2. Contd.

H35	-	-	G	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	6
H36	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	1
H37	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	C	-	-	-	2
H38	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	C	-	-	-	1
H39	-	-	-	-	-		-	-	-	-	-	-	C	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	1
H40	-	-	-	-	-		T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H41	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	1
H42	G	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	5
H43	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
H44	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	3
H45	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	T	C	-	-	-	-	3
H46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
H47	-	-	-	-	-	-	-		C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	T	-	3
H48	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	1
H49	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	1
H51	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	1
H52	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	C	-	-	1
H54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	1
H55	G	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
H56	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	-	-	-	-	1
H57	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T		1
H58	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		G	1
H59	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	-	-			1
H60	-	-	G	-	-	-	-	-	-	-	-	-	C	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H61	-	G	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	1
H62	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	C	-	-	-	1
H63	-	-	-	A	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	1
H64	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G	-	1
H65	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	2
H66	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	2
H67	-	-	-	-	-	-	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	2
H68	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H69	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	G	-	-	-	-	-	-	2
H70	-	-	-	A	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H71	-	-	-	-	-	T	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	3

Table 2. Contd.

H72	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	1
H73	-	-	-	-	-	-	-	T	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H74	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	1
H75	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	1
H76	-	-	-	-	C	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	1
H77	-	-	G	-	-	-	-	-	-	-	-	-	-	C	-	-	T	-	-	-	-	-	T	-	-	-	-	-	-	-	1
H78	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
H79	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	G	-	G	-	-	-	-	-	-	-	-	2
H80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	2
H81	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	C	-	-	-	4
H82	-	-	-	A	-	-	-	-	-	-	C	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H83	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	1
H84	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H85	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	G	1
H86	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	1
H87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	T	-	-	-	-	-	1
H88	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	C	-	-	-	-	1
H89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	6
H91	-	-	-	-	C	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H92	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	C	-	-	1
H93	-	-	C	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	1
H94	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	1
H95	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	G	-	-	-	-	-	-	-	-	1
H96	-	-	-	-	-	-	-	T	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	1
H97	-	-	-	A	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
H98	-	-	-	-	C	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	3
H99	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	C	-	G	2
H100	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	T	-	-	-	-	-	-	-	-	-	-	-	-	2
H101	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-		-	-	-	-	-	G	-	-	-	-	-	-	-	-	2
H102	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	-	-	-	-	1
H103	-	-	-	-	-	-	-	-	-	-	C	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H104	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	1
H105	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	1
H106	-	-	-	-	-	-	-	-	-	-	-	-	C	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H107	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	1
H108	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	C	-	-	1

Table 2. Contd

H109	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	1
H110	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	1
H111	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	1
H3112	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	C	-	-	-	1
H113	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H114	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	1
H115	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	C	-	-	1
H116	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	2
H117	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	C	-	-	-	1

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

Position	Mutation	Type of mutation	Presence in Mitomap	Frequency	Frequency %
16037	Transition	A-G	Presence	0.0425	4.25
16037	Transversion	A-T	New*		
16038	Transition	A-G	Presence	0.0425	4.25
16041	Transition	A-G	Presence	0.0375	3.75
16048	Transition	G-A	Presence	0.0525	5.25
16063	Transition	T-C	Presence	0.0555	5.55
16067	Transition	C-T	Presence	0.0325	3.25
16069	Transition	C-T	Presence	0.0375	3.75
16071	Transition	C-T	Presence	0.04	4.00
16075	Transition	T-C	Presence	0.0325	3.25
16075	Transversion	T-A	New*		
16083	Transition	C-T	Presence	0.03	3.00
16092	Transition	T-C	Presence	0.03	3.00
16104	Transition	C-T	Presence	0.02	2.00
16104	Transversion	C-G	New*		
16124	Transition	T-C	Presence	0.035	3.50
16140	Transition	T-C	Presence	0.0425	4.25
16144	Transition	T-C	Presence	0.0325	3.25
16144	Transversion	T-A	Presence		
16145	Transition	G-A	Presence	0.0455	4.55
16147	Transition	C-T	Presence	0.0325	3.25
16148	Transition	C-T	Presence	0.0575	5.75
16153	Transition	G-A	Presence	0.04	4.00

Table 3. Contd.

16162	Transition	A-G	Presence	0.0275	2.75
16163	Transition	A-G	Presence	0.0125	1.25
16171	Transition	A-G	Presence	0.03	3.00
16179	Transition	C-T	Presence	0.0475	4.75
16183	Transition	A-G	Presence	0.025	2.50
16201	Transition	C-T	Presence	0.0425	4.25
16201	Transversion	C-G	New*		
16209	Transition	T-C	Presence	0.0275	2.75
16217	Transition	T-C	Presence	0.0425	4.25
16224	Transition	T-C	Presence	0.035	3.50
16234	Transition	T-C	Presence	0.0305	3.10
16247	Transition	T-C	Presence	0.035	3.50
Genetic diversity*	$D= 1-\sum p^2 = 0.954$		= 95.4 %		

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

Table 4. Comparisons of the characteristics across D-loop region in different human population groups.

Population	Iraq[1]	India[2]	Malaysia[3]	Africa[4]	German[5]	US Caucasian[6]
Sample size	400	98	195	111	200	604
No. of variant sites	30	83	149	97	153	233
A→G	104	233	473	323	330	1112
G→A	155	66	81	78	55	219
T→C	110	145	461	382	308	1007
C→T	115	117	321	486	199	688
A→T	2	1	2	0	4	2
A→C	0	23	81	15	5	47
G→T	0	0	0	18	0	1
G→C	0	0	3	0	1	6
C→A	0	0	30	17	11	12
C→G	9	4	1	6	19	6
T→A	2	7	5	0	1	0
T→G	0	0	3	0	0	0
Insertion	0	168	322	140	291	983
Deletion	0	0	28	6	6	14

Note: % of transitions and transversions were calculated as number of observations divided by total substitutions.[1]This study; [2]Mountain et al. (1995); [3]Budowle et al. (1999); [4]Budowle et al. (1999); [5]Lutz et al. (1998); [6]Budowle et al. (1999).

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