Forensic Analysis of Mitochondrial DNA Hypervariable Region HVII (Encompassing Nucleotide Positions 37 to 340) and HVIII (Encompassing Nucleotide Positions 438-574) and Evaluating the Importance of These Variable Positions for Forensic Genetic Purposes

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Abstract

The study aims to detection of mitochondrial hypervariable HVII and HVIII regions. The second objective is to evaluate the importance of these positions for forensic genetic purposes and establish the degree of variation characteristic of a fragment. Blood samples were collected from 270 healthy unrelated male living in middle and south of Iraq. FTA® Technology is utilized to extract DNA. A portion of a noncoding region encompassing positions 37 to 340 for HVII and encompassing positions 438 to 574 for HVIII amplified in accordance with the Anderson reference sequence. By using EZ-10 spin column the PCR products were purified, sequenced and detected by using the ABI 3730*xL* Genetic Analyzer. New polymorphic positions 57, 63 , 101, 469 and 482 are described and may in future be very important for forensic identification purpose. This study showed the importance of the adoption of mitochondria in forensic medicine and criminal diagnosis and sites that have been discovered during the study in a private Iraqi society. Further study on larger number of samples from different Iraqi ethic groups is suggested to confirm the results obtained by this study.

Keywords: Forensic, Frequency, HVII, HVIII, Iraq, polymorphism.

الخلاصة

الغرض الأول من الدراسة الحالية هو الكشف عن مناطق عالية التغاير الثانية والثالثة للمايتوكوندريا. والغرض الثاني هو تقييم أهمية هذه المواقع لأغراض الوراثة العدلية وتثبيت درجة التغايرات الموصوفة للقطع . عينات الدم تم جمعها من 270 متبرع ذكور من وسط وجنوب العراق. تقنية أف تي أي تم استخدامها في تنقية الدنا . المنطقة غير المشفرة والمشتملة على المواقع النيوكليوتيدية من 37 إلى 340 كذلك المنطقة غير المشفرة والمشتملة على المواقع النيوكليوتيدية من 438 إلى 574 تم تضخيمها بالاعتماد على تسلسلات أندرسون . وباستخدام أعمدة (أي زت −10) تم تنقية نواتج التضخيم كما تم الكشف عن تسلسل نواتج التضخيم باستخدام جهاز الدنا انلايزر . مواقع متغايرة جديدة 57 و63 و101 و469 و482 تم وصفها وهي ربما مستقبلا لها أهمية تشخيصية في الطبابة العدلية. هذه الدراسة بينت أهمية اعتماد المايتوكوندريا في الطبابة العدلية والتشخيص الجنائي والمواقع التي تم اكتشافها خلال الدراسة هي خاصة بالمجتمع العراقي. دراسة أخرى لعدد اكبر من العينات ومن مجاميع عرقية مختلفة تم اقتراحها لتثبيت النتائج التي تم الحصول عليها بهذه الدراسة.

الكلمات المفتاحية: قضائي، تردد، مناطق عالية التغاير الثانية والثالثة ، العراق، تعدد الاشكال.

Introduction

The introduction of DNA fingerprinting by an English scientist, Sir Alec Jeffreys in 1985 has had an enormous impact in forensic science (Jeffreys *et al*., 1985). Mammalian cells possess two different types and interdependent genomes, the nuclear genome and mitochondrial genome. Human DNA is basically composed of the `coding' and `non-coding' regions. The`coding' region only makes up about 3% of human genomic DNA. Mitochondria are semi-autonomously functioning organelles containing a resident genome that undergoes replication, translation and transcription of their own DNA.

Mitochondrial DNA Comprising of about 37 genes coding for 22 tRNAs, two rRNAs and 13 mRNAs are a small circle of DNA (Helgason *et al*., 2003). MtDNA is only passed on from mother to child, it does not recombine and therefore there is no change between parent and child, unlike nuclear DNA (Ingman and Gyllensten, 2003; Ukhee *et al*., 2005). There is more sequence divergence in mitochondrial than in nuclear DNA (Brown *et al.,* 1993; Giulietta *et al*., 2000).

Each mitochondrion contains its own DNA, with many copies of the circular mitochondrial DNA in every cell. It is thought that each mitochondrion contains between 1 and 15, with an average of 4 to 5, copies of the DNA (Reynolds, 2000) and there are hundreds sometimes thousands of mitochondria per cell. The result is that there are many thousands of copies of the mitochondrial DNA in every cell. This compares with only two copies of nuclear DNA. The mitochondrion also has a strong protein coat that protects the mitochondrial DNA from degradation by bacterial enzymes. This compares to the nuclear envelope that is relatively weak and liable to degradation. DNA alterations (mutations) occur in a number of ways. One of the most common ways by which mutations occur is during DNA replication. An incorrect DNA base may be added; for example, a C is added instead of a G. This creates a single base change, or polymorphism, resulting in a new form. These single base mutations are rare but occur once every 1,200 bases in the human genome. The result is that the rate of change, or evolutionary rate, of mitochondrial DNA is about five times greater than nuclear DNA (Bar, 2000; Ingman and Gyllensten, 2003). This is important in species testing, as even species thought to be closely related may in time accumulate differences in the mitochondrial DNA but show little difference in the nuclear DNA. A further reason for the use of mitochondrial DNA in species testing, and in forensic science, is its mode of inheritance. Mitochondria exist within the cytoplasm of cells, including the egg cells. Spermatozoa do not normally pass on mitochondria and only pass on their nuclear DNA. The resulting embryo inherits all its mitochondria from its mother (Brown, 2002; Tully, 2004). This polymorphism allows 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.

Genetic studies of middle and south of Iraq by the use of molecular markers of mitochondrial DNA (mtDNA) have attracted the interest of population geneticists (Al-Zahery *et al.,* 2003; Nadia *et al.,* 2011). Sequence analysis of the HV1 and HV2 fragments of mitochondrial DNA (mtDNA) is today a routine method applied to forensic identification in cases where evidence specimens are not suitable for STR analysis.

Materials and Methods

Population

Two hundred seventy healthy, randomly chosen individuals deriving from the middle and south of Iraq provinces (Baghdad, Babil, Diwania, and Basrah). The number and ethnicity of individuals were chosen in order to obtain a population sample to achieve the highest possible representation of the major ethno-religious and tribal groups of the Country living in these central and southern areas.

DNA Extraction and PCR primers

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 (Dobbs *et al.*, 2002). A 1.2mm diameter disc was punched from each FTA card with a puncher. The discs were transferred to new eppendorf tubes and washed three times in 100µl Whatman FTA purification reagent. Each wash was incubated for 5 minutes at room temperature with moderate manual mixing and the reagent was discarded between washing steps. The discs were then washed twice in 200µl 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 hour.

The primers were designed manually by using The Cambridge Reference Sequence. Each primer was diluted to a final concentration of 100 pm/µl and kept at -20 °C for longer storage. A portion of a noncoding region encompassing positions from 37 to 340 for HVII amplified in accordance with the Anderson reference sequence (Anderson *et al.,* 1981) GenBank: J01415. In MtDNA- HVII the portion of DNA was amplified in two primers: the first one is HVII-F (37-58) 5'-CATTCTCATAATCGCCCACGG-3' and the second HVII-R has a position (320-340) 5'-CCCCCCATCCTTACCACCCTC-3'. A portion of a noncoding region encompassing positions from 438 to 574 for HVIII amplified in accordance with the Anderson reference sequence (Anderson *et al.,* 1981) GenBank: J01415. In MtDNA- HVIII the portion of DNA was amplified in two primers: the first one is HVIII-F (438-459) 5'-CAACTAACACATTATTTTCCCC-3' and the second HVIII-R has position (574-555) 5'-AACCCCAAAGACACCCCCCA-3'. PCR reaction was done in 0.2 ml PCR tubes with the following mixtures: 1 µl of each forward and reverse primer (10 pm/µl), 2jl of DNA template (5ng/41) and 46 µl of PCR ReddyRunTM Master Mix. The following PCR condition was used: 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 54 °C for 30 s, 72 °C for 45 s and final extension step at 72 °C for 7 min. PCR products were kept at 4 °C in a separate fridge from the pre- PCR components to avoid contamination.

Sequencing reaction of the PCR product

Purification and sequencing reaction of the PCR product was performed by EZ10-spin column DNA cleanup kit 100 prep EZ-10 spin column purification kits. PCR fragment was sequenced using ABI Prism Big Dye® Terminator Cycle Sequencing Kit on an ABI 377 sequencer. Each sequence obtained was then aligned with the Cambridge Reference Sequence.

Statistical analysis

The pattern of inheritance had made statistical analysis of mtDNA type much easier than any other genetic marker. Since mtDNA presented in each human being is haploid, determination of mtDNA type did not require the prerequisite of Hardy-Weinberg equilibrium for statistical analysis. Genetic diversity 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.

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

Where p – frequencies of the observed Haplotypes (Jones, 1972).

Results and Discussion

Hypervariable region (HVII) sequence variance and mtDNA haplotypes

The study enabled identification of 95 different haplotypes and 28 polymorphic nucleotide Positions in HVII **Table 1** . Among these 28 variations, there were 17(61%) variation between T and C and 11 variations (39%) between A and G. Seven polymorphic positions, 56, 63, 69, 81, 101, 208, and 222 have transverse substitution **Table 2**. All the other substitutions determined during the analysis are transitions. The most frequent variant (H1) was consistent with the Anderson sequence (Brown *et al., 1982*; Guntheroth *et al.*, 1986; Pastore, 1994; Yang and Yoder, 1999).

Hypervariable region (HVIII) sequence variance and mtDNA haplotypes

The study enabled identification of 86 different haplotypes and 16 polymorphic nucleotide Positions in HVIII **Table 3**. Among these 16 variations, there were 11(69%) variation between T and C and 4 variations (25 %) between A and G. and just one position (6 %) between T and A. Three polymorphic positions, 447, 453, and 469 have transverse substitution **Table 4**. Genetic diversity for the analysed DNA fragment was calculated according to the formula: $D= 1-\sum p^2$ and recorded 0.950% and 0.965% for HVII and HVIII respectively. 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 Iraq data (Pastore, 1994; Yang and Yoder, 1999), revealed significant differences in SNP patterns. 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 5**. Walsh et al., (1991) and Tang, (2002) show that the polymorphism of mtDNA coding area is 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 (Walsh *et al.*, 1991). However, mtDNA data on Iraqi population are very limited. This had limited the application of mtDNA in forensic cases and study of mtDNA population genetics in Iraq. In future, development of more multiplexes targeting mtDNA polymorphisms within the control and coding regions might reduce the matching probability of mtDNA type and increased the utility of mtDNA in forensic cases.

Table 1. Hypervariable region (HVII) sequence variance and mtDNA haplotypes.

Anderson	39	41	42	46	49	53	56	57	63	69	70	71	78	81	101	105	127	141	179	196	208	216	220	222	234	275	322	327	No. of Individual
H1*	C	C	T	T	A	G	A	T	T	G	G	G	C	G	G	C	T	C	T	T	T	T	T	C	A	G	G	C	126
H2	-	T	C	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H3	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	3
H4	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	1
H5	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	G	-	-	-	-	1
H6	T	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	1
H7	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	-	1
H8	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H9	-	-	-	-	-	-	T	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H10	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	C	-	-	-	-	-	-	1
H11	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
H12	-	-	-	C	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H13	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	2
H14	T	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	C	-	-	-	-	-	-	1
H16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	4
H17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	C	-	-	-	-	-	-	-	1
H18	-	-	-	-	-	-	-	-	-	A	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H19	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	C	-	-	-	-	-	-	-	-	-	-	-	2
H20	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	3
H21	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	1
H22	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	2
H23	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	T	1
H24	-	-	-	-	-	-	-	-	-	-	A	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H25	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	1
H26	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	3
H27	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
H28	-	-	-	-	-	-	G	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	1
H29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	C	-	-	-	-	A	-	1
H30	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	1
H31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	3
H32	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	1
H33	-	-	-	-	G	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H34	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	T	-	-	-	-	1
H35	-	-	C	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	1

Table 1. ***Continued*.**

	39	41	42	46	49	53	56	57	63	69	70	71	78	81	101	105	127	141	179	196	208	216	220	222	234	275	322	327	
Anderson	C	C	T	T	A	G	A	T	T	G	G	G	C	G	G	C	T	C	T	T	T	T	T	C	A	G	G	C	No. of Individual
H36	T	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H37	-	-	-	C	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H38	-	-	-	-	-	A	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H39	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	2
H40	-	T	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H41	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	2
H42	-	-	C	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
H43	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	1
H44	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	1
H45	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	3
H46	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	2
H47	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H48	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	1
H49	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	1
H50	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	G	-	-	-	1
H51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	2
H52	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	1
H53	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	1
H54	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	2
H55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	1
H56	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	C	-	-	-	-	-	1
H57	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	3
H58	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	A	-	-	1
H59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	3
H60	-	T	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H61	-	-	-	-	-	A	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	1
H62	-	-	-	-	-	-	-	-	-	-	A	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H63	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	C	-	-	-	-	-	-	-	1
H64	T	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	T	2
H66	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	2
H67	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	C	-	-	-	-	-	-	1
H68	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	1
H69	-	-	-	-	-	-	G	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H70	-	T	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Table 1. ***Continued***.

Anderson	39	41	42	46	49	53	56	57	63	69	70	71	78	81	101	105	127	141	179	196	208	216	220	222	234	275	322	327	No. of Individual
H71	C	C	T	T	A	G	A	T	T	G	G	G	C	G	G	C	T	C	T	T	T	T	T	C	A	G	G	C	1
H72	-	-	-	C	-	-	G	-	-	-	-	-	-	-	-	-	C	-	-	-	-	C	-	-	-	-	-	-	1
H73	-	-	-	-	-	A	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
H74	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	1
H75	-	-	C	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	C	-	-	C	-	-	-	-	-	1
H76	T	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H77	-	-	-	-	-	A	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H78	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	1
H79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	2
H80	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	3
H81	-	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H82	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	A	-	1
H83	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	2
H84	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	G	-	-	-	2
H85	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	1
H86	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	1
H87	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	G	-	A	-	1
H88	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
H89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	-	1
H90	-	-	-	-	-	-	-	-	-	-	-	A	-	A	-	-	-	-	-	C	-	-	-	-	-	-	-	-	1
H91	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-	1
H92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
H93	-	-	-	-	-	-	-	-	-	-	-	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	1
H94	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	T	1
H95	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	A	-	-	-	1
Total	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	1
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	280

H : Haplotype ; G: Guanine ; T: Thiamine ; C: Cytosine ; A: Adenine.*

Table 2. Types of mutations in variable positions for HVII .

Positions	Mutation	Type of mutation	Presence in Mitomap	Frequency	Frequency %
39	Transition	C-T	Presence	0.047	4.7%
41	Transition	C-T	Presence	0.053	5.3%
42	Transition	T-C	Presence	0.042	4.2%
46	Transition	T-C	Presence	0.031	3.1%
49	Transition	A-G	Presence	0.049	4.9%
53	Transition	G-A	Presence	0.046	4.6%
56	Transition	A-G	Presence	0.036	3.6%
56	Transversion	A-T	Presence		
57	Transition	T-C	Presence	0.039	3.9%
57	Transition	T-G	New*		
63	Transversion	T-A	Presence	0.030	3%
63	Transition	T-C	New		
69	Transversion	G-C	Presence	0.044	4.4%
69	Transition	G-A	Presence		
70	Transition	G-A	Presence	0.040	4%
71	Transition	G-A	Presence	0.026	2.6%
78	Transition	C-T	Presence	0.046	4.6%
81	Transition	G-A	Presence	0.026	2.6%
81	Transversion	G-C	Presence		
101	Transition	G-A	New	0.036	3.6%
101	Transversion	G-C	Presence		
105	Transition	C-T	Presence	0.036	3.6%
127	Transition	T-C	Presence	0.035	3.5%
141	Transition	C-T	Presence	0.025	2.5%
179	Transition	T-C	Presence	0.027	2.7%
196	Transition	T-C	Presence	0.035	3.5%
208	Transversion	T-A	Presence	0.019	1.9%
208	Transition	T-C	Presence		
216	Transition	T-C	Presence	0.036	3.6%
220	Transition	T-C	Presence	0.032	3.2%
222	Transition	C-T	Presence	0.024	2.4%
222	Transversion	C-G	Presence		
234	Transition	A-G	Presence	0.023	2.3%
275	Transition	G-A	Presence	0.023	2.3%
322	Transition	G-A	Presence	0.012	1.2%
327	Transition	C-T	Presence	0.045	4.5%
		Genetic diversity*	D= 1-$\sum p^2$ = 0.964 = 96.4 %		

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 3. Hypervariable region (HVIII) sequence variance and mtDNA haplotypes..

	444	447	449	453	456	458	469	471	482	485	493	494	504	508	533	534	
Anderson	A	C	T	T	C	C	C	T	T	T	A	C	T	A	A	C	No. of Individual
H1*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	157
H2	.	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	1
H3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	1
H4	.	.	.	.	T	T	.	.	.	.	.	.	.	.	.	.	1
H5	G	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	3
H6	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	1
H7	.	G	.	A	.		.	.	.	.	.	.	.	.	.	.	1
H8	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	1
H9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	1
H10	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	6
H11	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	1
H12	.	.	.	.	.	.	.	C	.	.	.	.	.	.	G	.	1
H13	.	.	.	.	.	.	.		C	.	.	.	.	.	.	.	3
H14	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
H15	.	.	.	.	T	.	.	.	.	C	.	.	.	.	.	.	1
H16	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	1
H17	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	1
H18	.	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.	3
H19	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	1
H20	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	1
H21	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	2
H22	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	3
H23	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	1
H24	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	1
H25	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	1
H26	G	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	1
H27	.	.	.	.	.	.	.	.	.	.	G	.	.	G	.	.	1
H28	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	1
H29	.	.	.	.	.	.	.	.	C	.	.	.	.	.	G	.	1
H30	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	.	1
H31	G	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	1
H32	.	.	.	.	.	.	T	C	.	.	.	.	.	.	.	.	4
H33	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	1
H34	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
H35	.	.	.	.	.	.	.	.	.	.	.	.	C	.	G	.	1
H36	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	1
H37	.	.	.	.	.	.	.	.	.	C	.	T	.	.	.	.	1
H38	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	1
H39	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	1
H40	.	T	.	.	.	.	.	.	.	.	G	.	.	.	.	T	1
H41	.	.	.	.	T	.	.	.	.	.	G	.	.	.	.	.	1
H42	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	1
H43	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	T	1
H44	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	3
H45	.	T	.	.	.	.	.	.	C	.	.	.	.	.	.	.	1

Table 3. ***Continued.***

	444	447	449	453	456	458	469	471	482	485	493	494	504	508	533	534	
Anderson	A	C	T	T	C	C	C	T	T	T	A	C	T	A	A	C	No. of Individual
H46	G	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	1
H47	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	T	1
H48	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	1
H49	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	1
H50	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	1
H51	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	1
H52	.	.	C	.	.	.	G	.	.	.	.	.	.	.	.	.	1
H53	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	1
H54	.	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.	4
H55	.	T	.	.	.	.	.	C	.	.	.	.	.	.	G	.	1
H56	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	1
H57	.	.	.	.	.	.	T	.	.	.	G	.	.	.	.	.	1
H58	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	1
H59	.	.	C	.	.	.	.	.	.	.	.	.	.	.	G	.	1
H60	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	T	1
H61	.	.	.	.	.	.	.	.	.	.	G	.	C	.	.	.	1
H62	.	T	.	.	.	.	.	.	.	.	.	.	.	.	G	.	1
H63	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	1
H64	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	1
H65	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	T	1
H66	G	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	1
H67	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	1
H68	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
H69	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	1
H70	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	1
H71	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	2
H72	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	1
H73	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	1
H74	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	1
H75	.	.	.	.	.	T	.	.	.	.	.	.	C	.	.	.	1
H76	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	1
H77	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	1
H78	.	.	.	.	.	.	.	.	.	.	.	.	C	G	.	.	1
H79	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	1
H80	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	1
H81	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	1
H82	.	.	.	.	.	.	.	C	.	.	.	.	.	G	.	.	1
H83	.	.	.	.	.	.	.	.	C	.	.	.	.	.	G	.	1
H84	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
H85	.	.	.	.	.	.	T	.	.	.	G	.	.	.	.	.	1
H86	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	1
H87	.	.	.	.	T	.	.	.	.	.	.	.	C	.	.	.	1
Total	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	275

H : Haplotype ; G: Guanine ; T: Thiamine ; C: Cytosine ; A: Adenine.*

Table 4. Types of mutations in variable positions for HVIII.

Positions	Mutation	Type of mutation	Presence in Mitomap	Frequency	Frequency %
444	Transition	A-G	Presence	0.034	34%
447	Transversion	C-G	Presence		
447	Transition	C-T	Presence	0.066	6.6%
449	Transition	T-C	Presence	0.055	5.5%
453	Transversion	T-A	Presence	0.036	3.6%
453	Transition	T-C	Presence		
456	Transition	C-T	Presence	0.04	4%
458	Transition	C-T	Presence	0.04	4%
469	Transition	C-T	Presence	0.04	4%
469	Transversion	C-G	New*		
471	Transition	T-C	Presence	0.05	5%
482	Transition	T-C	New	0.042	4.2%
485	Transition	T-C	Presence	0.052	5.2%
493	Transition	A-G	Presence	0.048	4.8%
494	Transition	C-T	Presence	0.04	4%
504	Transition	T-C	Presence	0.045	4.5%
508	Transition	A-G	Presence	0.028	2.8%
533	Transition	A-G	Presence	0.052	5.2%
534	Transition	C-T	Presence	0.048	4.8%
Genetic diversity* $D= 1-\sum p^2 = 0.965 = 96.5\ \%$					

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 5. 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	280	98	195	111	200	604
No. of variant sites	44	83	149	97	153	233
A→G	64	233	473	323	330	1112
G→A	169	66	81	78	55	219
T→C	119	145	461	382	308	1007
C→T	51	117	321	486	199	688
% transition	96.9%	94.85	92.16	95.77	95.61	97.61
A→T	1	1	2	0	4	2
A→C	0	23	81	15	5	47
G→T	0	0	0	18	0	1
G→C	2	0	3	0	1	6
C→A	0	0	30	17	11	12
C→G	4	4	1	6	19	6
T→A	2	7	5	0	1	0
T→G	0	0	3	0	0	0
% transversion	3%	5.15	7.84	4.23	4.39	2.39
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 substitution times.

Conclusion

Sequence databases are the best source of information regarding the power of mtDNA for identity testing. Sequence analysis of the noncoding region of mtDNA (HVII) and HVIII conducted on a population of 270 unrelated individuals enabled identification of 34 different haplotypes in HVII and 86 different haplotypes in HVIII. New polymorphic positions 57,63,101,469 and 482 are described and that may in future be suitable sources for genetic identification purposes. The ABI Prism Big Dye Terminator Cycle Sequencing kit used for sequencing of the amplified HVII and HVIII region had provided good quality of sequence for the purpose of this study.

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