



## Population data of mitochondrial DNA region HVIII in 150 individuals from Bologna (Italy)

C. Bini\*, S. Ceccardi, C. Colalongo, G. Ferri, M. Falconi,  
S. Pelotti, G. Pappalardo

*Department of Medicine and Public Health, Section of Legal Medicine, University of Bologna,  
via Irnerio 49, Bologna 40126, Italy*

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### Abstract

Mitochondrial DNA (mtDNA) sequence variations at the HVIII region in 150 unrelated Italian individuals from Bologna were analysed. A comparison of our sequence data with the Anderson sequence showed that nucleotide substitutions predominate over insertions and deletions. Among nucleotide substitutions the most frequent was a T–C transition.

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*Keywords:* Mitochondrial DNA; Control region; Sequencing; Polymorphism

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### 1. Introduction

The analysis of mitochondrial DNA is an important resource for forensic identification purposes when the evidentiary samples are not suitable for nuclear DNA typing such as single hair shafts and burial remains. The non-coding control region of mitochondrial DNA is highly polymorphic and the majority of the sequence variation is localized in two segments: hypervariable regions I and II (HVI and HVII). Recently, Lutz et al. [1] described within the control region of mtDNA a third hypervariable region located at positions 438–574 and analyzed the polymorphism in 200 unrelated individuals from Germany. In order to evaluate the variability of this additional region in the Italian population, we have studied 150 maternally unrelated individuals from Bologna.

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\* Corresponding author. Tel.: +39-51-243250; fax: +39-51-248896.

*E-mail address:* labemat@medleg.unibo.it (C. Bini).

## 2. Material and methods

DNA from blood samples of 150 maternally unrelated individuals living in Bologna (Italy) was extracted by the salting out method [2]. The PCR primers used to amplify the HVIII region were:

L182: 5'CGCACCTACGTTCAATATTAC 3'

H619: 5'GGTGATGTGAGCCCGTCTAA 3'

PCR amplification was performed in a 25- $\mu$ l reaction volume using the Biometra Trio-thermo block (Biometra, Germany). Each reaction contained 50 mM KCl, 10 mM Tris/HCl, 1.5  $\mu$ M MgCl<sub>2</sub>, 50  $\mu$ M of each dNTP, 0.25  $\mu$ M of each primer and 1.25 U *Taq* DNA polymerase (PE Applied Biosystem, USA). 30 cycles of 1 min at 94 °C, 30 s at 54 °C, 1 min at 72 °C with a pre-denaturation step of 1 min at 94 °C and 7 min of final extension at 72 °C were performed. PCR products were purified by Centriseq (PE Applied Biosystem, USA) and sequenced using BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE

Table 1a  
Nucleotide substitutions, insertions and deletions in the HVIII region of 150 individuals from Bologna

Mutation type	HVIII	
	Number of positions	Total number of mutations
<i>Substitutions</i>		
<i>Transition</i>		
Py–Py		
C–T	5	30
T–C	3	17
Pu–Pu		
A–G	1	1
G–A	2	3
Total	11	51
<i>Transversion</i>		
G–C	1	1
C–G	1	1
Total	2	2
<i>Insertion</i>		
+CA	1	8
+2CA	1	2
+C	1	1
+3C	1	1
+4C	1	1
Total	5	13
<i>Deletion</i>		
–CA	1	14
Total	1	14

Table 1b  
Statistical comparison between Bologna and German populations

Source of variation	<i>df</i>	Sum of squares	Variance components
Among populations	1	0.196	– 0.00311 Va
Within population	348	253,498	0.72844 Vb
Total	349	253,694	0.72534
Fixation Index	FST:	– 0.00428	
		Germans	Bologna
Haplotype diversity		0.62 ± 0.04	0.61 ± 0.03
Mean number of pairwise differences		1.48 ± 0.90	1.42 ± 0.87
Nucleotide diversity		0.0102 ± 0.0068	0.0097 ± 0.0066

Applied Biosystem, USA) according to the manufacturers manual. Sequences were aligned and compared with the reference sequence using the Sequence Navigator computer program (Applied Biosystem, Sequence Navigator version 1.0.1). Data were converted into a Microsoft Excel file (Microsoft 1998, Microsoft Excel version 2000). The population data were analysed by Arlequin software, ver. 2.000 [3].

### 3. Results

In our study, the region between positions 438 and 574, called HVIII, was completely sequenced and analysed for 150 maternally unrelated individuals living in Bologna. Sequencing of both strands was performed to reduce ambiguities in sequence determination as recommended by the DNA Commission of the International Society for Forensic Genetics [4]. In our population sample, 22 polymorphic sites were found giving 23 different haplotypes. The most frequent haplotype was found in 61.3% of the individuals. Compared to the Anderson reference sequence 80 sites were affected by nucleotide substitutions, insertion or deletions. The majority of the nucleotide substitutions were transitions and among them C → T was prevalent (Table 1a). Screening CA-repeat at positions 514–523 (–CA, +CA, +2CA) we found 14 sequences with (CA)<sub>5–1</sub>, 126 with (CA)<sub>5</sub>, 8 with (CA)<sub>5+1</sub> and 2 with (CA)<sub>5+2</sub> repeats. The comparison with the German Caucasian population is shown in Table 1b.

### 4. Discussion

Our population data confirm that HVIII on its own exhibits a lower variability than HVI and HVII regions calculated on the same sample (data not shown) and no significant differences to the German population. Nevertheless, HVIII analysis may be helpful in forensic investigations as emphasized by Lutz et al. [5] and its inclusion in a database is advisable for statistical evaluation.

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