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Mitochondrial DNA polymorphism in 50 unrelated individuals from North Italy

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Abstract

The hypervariable regions HVI and HVII of mitochondrial DNA (mtDNA) were sequenced in 50 unrelated individuals living in Veneto. A total of 48 different mtDNA haplotypes for HVI region (404 bp) and 49 for HVII region (313 bp) were observed. Nucleotide diversity was 0.0116 and 0.0097, respectively.

Keywords: mtDNA; HVI; HVII; Population study

1. Introduction

The two hypervariable regions HVI and HVII of mitochondrial DNA (mtDNA) are becoming more commonly used in forensic applications to differentiate individuals in a population. This analysis is particularly useful in cases involving evidence such as hair shafts and skeletal remains and in maternity testing [1–4]. Furthermore, mtDNA can often be amplified from samples whose nuclear DNA is too degraded. The statistical interpretation of the results depends on the population frequency of a particular sequence, or haplotype. For this reason, it is essential to determine the haplotype frequency distribution of HVI and HVII in any population of interest. The aim of this study is to analyse the sequence data of the two hypervariable HVI and HVII regions from 50 unrelated individuals living in Veneto.

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2. Material and methods

DNA was extracted from peripheral blood, using the kit "Micromix 660" (Talent). The two hypervariable HVI and HVII regions were amplified separately according to Buscemi et al. [5] with minor modifications., using primers L15971, H16414, L15, H389, respectively. Reaction mixture (final volume of 25 μl): 2.5 μl of 10× PCR buffer (Pharmacia), 1 μM of each primer, 100 μM of each nucleotide, 1 U of Taq polymerase (Pharmacia), 20 μl sample and water. Amplification was performed using a 2400 Geneamp thermal cycler (Perkin Elmer) with the following conditions: 94 °C/20 s, 56 °C/15 s, 72 °C/30 s, for 40 cycles. Each of the PCR products was purified with a Microcon 100 (Amicon) and sequenced on both strands, using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystem) (primers: F15971, R16414, F29, R381). Reaction mixture (final volume of 20 μl): 4 μl of Big Dye Terminator (Applied Biosystem), 3.2 pmol of specific primer, 14 μl sample and water. Amplification conditions: 96 °C/10 s, 50 °C/5 s, 60 °C/4 min, for 25 cycles. Analysis was carried out on an ABI Prism 310 (Applied Biosystem).

3. Results and discussion

Complete HVI and HVII sequences were obtained for 50 individuals from Veneto (Northeastern Italy). The sequences were aligned with Anderson's sequences [6] using Sequence Navigator Software. A total of 48 different mtDNA haplotypes was observed for the HVI region (three individuals shared the same sequence of Anderson). For the HVII region, a total of 49 different mtDNA haplotypes was observed (one sequence repeated two times). The sequenced regions included a total of 717 bp, among which 96 nucleotide positions were polymorphic, 70 in HVI and 30 in HVII, respectively. With HVI, 70 polymorphic sites were identified of which the most frequent were the substitutions: C/T at positions 16,223 and T/C at 16,311.

For HVII, the most frequent polymorphic sites were A/G transitions at 73, 93 and 263 positions. Some HVII haplotypes showed insertions of 1–2 C at 309.1, 309.2 and 315.1 positions, whereas no deletions were found. Our results are summarised in Table 1.

Table 1
Results of HVI and HVII sequence in 50 individuals from North Italy

	HVI	HVII
Sample size	50	50
Number of different sequences	48	49
Genetic diversity	0.9976	0.9992
Sequence length (bp)	404	313
Number of variable positions (%)	67 (16.6)	29 (9.3)
Nucleotide diversity	0.0116	0.0097

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