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Analysis of the HVI and HVII regions of mitochondrial DNA in 100 individuals from North of Portugal

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Abstract. The analysis of mitochondrial DNA (mtDNA) control region is of great importance in forensic casework and evolution studies. The aim of this study was to create a population database for the HVI and HVII regions of mtDNA in the population of North of Portugal. The HVI and HVII segments were studied in 100 unrelated and healthy individuals by direct sequencing. Nucleotide substitutions were found using Anderson's reference sequence. Length and position heteroplasmy were observed. The genetic structure of the population was analysed. The match probability and discrimination power values were calculated. The phylogenetic analysis for HVI segment was made following classification into haplogroups and genetic distances were calculated. These analyses place the North of Portugal population among the European Caucasoid populations. © 2003 Published by Elsevier B.V.

Keywords: Mitochondrial DNA; HVI–HVII; North of Portugal

1. Introduction

The control region of the human mitochondrial DNA (mtDNA) is highly polymorphic due to a rapid rate of evolution. The mtDNA does not undergo recombination and is present in high copy number per cell. For this reason, its analysis is an important tool for genetic identification and may provide results where the analysis of nuclear DNA fails.

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Table 1
Diversity and genetic parameters in the population of North of Portugal

	HVI	HVII	HVI + HVII
No. of haplotypes	64	55	83
Polymorphic positions	59	45	104
Transitions	56	40	96
Transversions	5	1	6
Insertions	0	4	4
Deletions	1	0	1
Nucleotide diversity	0.012038 ± 0.006654	0.010187 ± 0.005767	0.011116 ± 0.005772
Gene diversity	0.9596 ± 0.0140	0.9739 ± 0.006	0.9882 ± 0.0054
Mean number of pairwise differences	4.32188 ± 2.156573	3.626465 ± 1.853745	7.948283 ± 3.725813

The haploid maternal inheritance of the mtDNA is also useful for the identification of maternally related individuals. The analysis of mtDNA is usually done by direct sequencing of PCR products. It is of great importance in forensic genetics because it has been applied successfully to different types of biological samples. The simultaneous occurrence of more than one type of mtDNA in a single individual is called heteroplasmy, being the length heteroplasmy the most frequent type observed. For purposes of genetic identification, its occurrence must be carefully evaluated. The great amount of variability accumulated in the control region represents a record of the human evolution history. In this sense, the analysis of mtDNA polymorphisms is particularly useful in human evolutionary studies and phylogenetic analysis between populations. The aim of this study was to create a population database for the HVI and HVII regions of mtDNA in the North of Portugal.

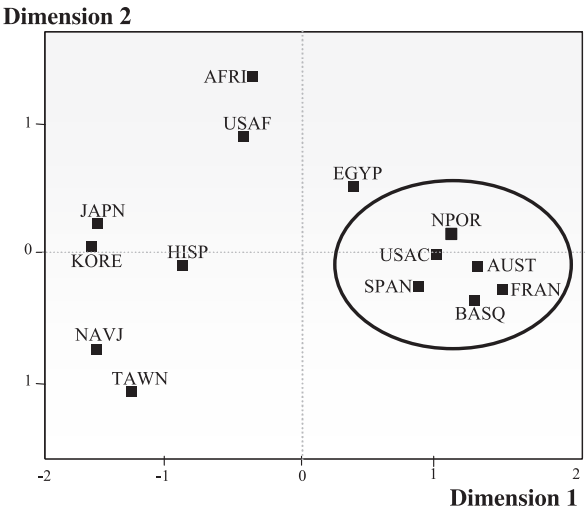

Fig. 1. Nonmetrical multidimensional scaling (MDS) applied on mismatch–intermatch pairwise distances. Total variance for the eigenvectorial reduction = 99.4%, stress = 0.0368.

2. Material and methods

Blood samples were collected from 100 unrelated and healthy individuals from the population of North of Portugal. DNA was extracted using the phenol–chloroform method. The HVI and HVII segments were amplified by PCR using the primers L15 996–H16 401 and L29–H408 [1], respectively, each modified. The two segments were sequenced by capillary electrophoresis (ABI PRISM 310 Genetic Analyser, Applied Biosystems) on both strands using the primers M13 and the "dRhodamine Terminator Cycle Sequencing Ready Reaction kit" (Applied Biosystems). Nucleotide substitutions were found by comparison with Anderson's reference sequence [2]. The genetic structure of the population was analysed [3]. The match probability (PM) and discrimination power (PD) values were calculated. The phylogenetic analysis for HVI segment was made following classification into haplogroups [4] and genetics distances were calculated using the mismatch–intermatch means of pairwise differences [5,6]. The analysis of both segments taken together was made using only their sequences. A nonmetrical multidimensional scaling (MDS) diagram was debugged [7,8].

3. Results and discussion

The sequences of mtDNA HVI and HVII segments were studied from positions 16 033 to 16 391 and 57 to 408, respectively. Population parameters are shown in Table 1.

Taken together both segments, PD is 0.9882 and PM is 0.0118. Length heteroplasmy was observed in 16 individuals. Position heteroplasmy was observed in 2 individuals (16 038R and 16 295Y). The MDS diagram for mtDNA haplogroups in 13 populations (USA Africans, Africans, Egypt, USA Caucasians, Spain, Basques, Austria, France, USA Hispanics, Japan, Korea, Taiwan and Navajo) [9] and North of Portugal is shown in Fig. 1. The phylogenetic analysis between the population of North of Portugal and other 13 populations demonstrates that this population is included in the European population cluster. In conclusion, the population sample here studied represents a mitochondrial database that can be used in forensic casework in the North of Portugal.

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