International Congress Series 1239 (2003) 535–539

mtDNA analysis in Portuguese populations (Central Portugal and Azores Islands): polymorphic sites in control region sequences

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Abstract

Background: The polymorphism of the two hypervariable segments (HVI and HVII) of the control region of mtDNA was analyzed in a population of 81 unrelated individuals from Central Portugal and 48 from the Azores Islands, using a fluorescent-based electrophoresis sequencing method. *Methods*: Sequences have been obtained with ABI PRISM0 Big Dye Terminator and dRhodamine Terminator Cycle Sequencing Ready Reaction Kits, with Amplitaq DNA Polymerase FS, and have been detected with ABI PRISM 377 DNA sequencer. *Results*: In the Central Portugal population ($n=81$), we observed 69 polymorphic sites of sequence in HVI region and 44 in HVII region. In the Azores population ($n=48$), we observed 48 polymorphic sites of sequence in HVI region and 24 in HVII region. *Conclusions*: Nucleotide substitution rather than insertion/deletion (1 or 2 bp) was the majority of variation. The distribution showed a large bias towards transitional changes than transversional changes. Our sequencing results are similar to other Caucasian population data.

Keywords: mtDNA; Central Portugal; Azores Islands; Population genetics

1. Introduction

The control region of mtDNA has become a useful tool in forensic genetics because we can analyse hair shafts, bones and samples with very little or degraded DNA. The mtDNA

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PII: S0531-5131(02)00580-0

is highly polymorphic and exists in multicopies per cell. The small molecules present a double stranded circular structure of 16569 bp [1].

The aim of this study was to determine the polymorphism of the HVRI and HVRII in two Portuguese populations: Central Portugal and Azores Islands. The results were compared with other Caucasian populations.

2. Material and methods

DNA was extracted from bloodstains (81 unrelated individuals from Central Portugal and 48 from the Azores Islands), using the Chelex 100 extraction method [2].

Primers and amplification conditions were as described by Wilson et al. [3].

PCR products were purified with MicroSpin Sephadex G-50. Purified DNA was estimated using the Phastsystem to determine the optimal PCR product quantity for the sequencing analysis.

Sequencing reactions were performed in both directions (forward and reverse) using the ABI Prism dRhodamine or Big Dye Terminator Cycle Sequencing Ready Reaction Kits (Applied Biosystems) and the same primers as the amplification reactions. After sequencing, samples were purified by $MgCl_2$/ETOH precipitation and analysed on ABI Prism 377 DNA Sequencer.

The nucleotide and sequence diversity was estimated as described by Nei and Tajima [4].

3. Results

In the Central Portugal population ($n = 81$), we observed 58 different sequences for HVRI and 49 for HVRII. HVRI showed 69 polymorphic sites and HVRII showed 44 polymorphic sites (Table 1).

In this population, we found homology with the CRS only in HVRI (14 individuals). The HVRI showed the four polymorphic sites with a high frequency at positions 16126 (T–C 17%), 16189 (T–C 22%), 16294 (C–T 11%) and 16311 (T–C 12%). In HVRII, all individuals revealed a transition A–G at position 263. At position 303–310, the majority of sequences carried 8/9 C nucleotides instead of the 7 reported by Anderson. At position 311–315, the majority of sequences carried 6/7 C nucleotides instead of the 5 in the CRS.

In the Azores population ($n = 48$), we observed 35 different sequences for HVRI and 31 for HVRII. HVRI showed 48 polymorphic sites and HVRII showed 24 polymorphic sites (Table 1).

In this population, we found homology with CRS only in HVRI (seven individuals). The HVRI showed the four polymorphic sites with a high frequency at positions 16126 (T–C 25%), 16189 (T–C 12.5%), 16294 (C–T 16.6%) and 16311 (T–C 14.6%). The HVRII revealed the same polymorphic sites as Central Portugal at positions 263 and 303–310; however, at position 311–315, almost all sequences carried 6 C nucleotides, instead of the 5 in the CRS.

The main variation detected in our populations is due to nucleotide substitutions, rather than insertion/deletion mutational events. The distribution showed a large bias towards

Table 1
The comparison between all performed sequences and the Cambridge Reference Sequence (CRS)

	Central Portugal				Azores Islands			
HVI	58 different sequences				35 different sequences			
	4	Different sequences	2	Times	4	Different sequences	2	Times
	3	were found	3			were found		
	51	Unique sequences			31	Unique sequences		
	14	Sequences homologues with CRS			10	Sequences homologues with CRS		
HVII	49 different sequences				31 different sequences			
	8	Different sequences	2	Times	2	Different sequences	2	Times
	2	were found	3		2	were found	3	
	1	Different sequence	4	Times	1	Different sequence	6	Times
	1	were found	9		1	were found	7	
	1		10					
	36	Unique sequences			25	Unique sequences		

transitional changes rather than transversional changes. The number of pyrimidine transitions is greater than the purine transitions in HVRI, in both populations analysed. However, in HVRII, the opposite occurs: no transversion could be observed in HVRII from Azores Islands data (Table 2).

A polycytosine stretch occurred in both hypervariable segments. These regions are often heteroplasmic, having populations of mtDNA molecules differing in the number of Cs within the C stretch (poly-C).

Length polymorphism is often observed in HVRI, when a homopolymeric tract of Cs is generated by the occurrence of a T–C transition at position 16189 (Bendall and Sykes

Table 2
Sequence polymorphism within the variable regions of mtDNA of 81/48 unrelated individuals of Central Portugal and Azores Islands

		HVR I Portugal	HVR I Azores	HVRII Portugal	HVR II Azores
Total number of sequence polymorphism		193	115	358	216
Transition: Transversion ratio		176:17	108:7	217:5	136:0
Pyrimidine transitions	T → C	82	43	57	32
	C → T	61	46	23	19
Purine transitions	A → G	24	6	124	81
	G → A	13	13	13	4
Transversions	C → A	3	2	1	-
	A → C	10	2	-	-
	G → C	2	-	3	-
	A → T	1	2	-	-
	C → G	1	1	1	-
Insertions	C	16189 (1 pb)	16189 (1 pb)	309 (1-2 pb)	309 (1-2 pb)
				315 (1-2 pb)	315 (1 pb)
	A			291 (1 pb)	

Table 3
HVR I and HVR II mtDNA polymorphism: a comparison between Azores Islands and Central Portuguese sample

	HVR I					HVR II				
	N	*K*	*A*	π	*J*	*N*	*K*	*A*	π	*J*
Azores Islands	48	35	48	0.0113	0.9557	48	31	24	0.0137	0.9610
Central Portugal	81	58	69	0.0112	0.9392	81	49	44	0.0136	0.9702

N—sample size; *K*—number of different sequences found; *A*—number of variable nucleotide positions; π—nucleotide diversity; *J*—sequence diversity.

[5]). This transition was observed in 20% of samples analysed from Central Portugal and 10% from Azores Islands.

The variation in each population is given by sequence diversity value, *J* (Table 3). In our study, $J = 0.9392$ for HVRI and $J = 0.9702$ for HVRII (Central Portugal), and $J = 0.9557$ for HVRI and $J = 0.9610$ for HVRII (Azores Islands).

The nucleotide diversity values (Table 3) were $\pi = 0.0112$ for HVRI and $\pi = 0.0136$ for HVRII (Central Portugal), and $\pi = 0.0113$ for HVRI and $\pi = 0.0137$ for HVRII (Azores Islands).

4. Discussion

In both populations (Central Portugal and Azores Islands), the most frequent mutational events are transitions T–C and C–T.

Table 4
Sequence diversity observed in HVR I in several populations

Population	*N*	*K*	*A*	π	*J*
Galician	92	53	56	0.0087	0.9295
Basque	106	52	52	0.0081	0.9362
Welsh	92	48	51	0.0094	0.9307
Portugal (Central)	**81**	**58**	**69**	**0.0112**	**0.9392**
Portugal (Azores)	**48**	**35**	**48**	**0.0113**	**0.9557**
Portugal (North)	100	67	71	0.013	–
Portugal (Central)	82	62	66	0.014	–
Portugal (South)	59	41	54	0.013	–
British	100	71	67	0.012	0.9760
Spain	89	70	69	0.014	0.9834
Tuscan	49	40	55	0.014	0.9685
Turks	96	79	82	0.015	0.9879
Middle East	42	38	59	0.020	0.9954

N–sample size; *K*—number of different sequences found; *A*—number of variable nucleotides positions; π—nucleotide diversity; *J*—sequence diversity.
Population sources: Galician [6], Basque [7], Welsh [8], Portuguese (Central and Azores) (present study in bold), Portuguese (North, Central and South) [9], British [7], Spain [7], Tuscan [7], Turks [7], Middle-East [7]. All values in all populations are based on the analysis of a fragment of 360 nucleotides, from 16024 to 16383 [1].

The obtained values for sequence and nucleotide diversity reveal a substantial similarity with the described data for other Caucasian populations, namely other Portuguese populations (Table 4).

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