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Polymorphism of D-loop mitochondrial DNA: study of HV1 and HV2 regions in unrelated individuals living in the East of France

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Abstract

The sequence of two hypervariable regions (HV1 and HV2) of mitochondrial DNA (mtDNA) was obtained for 100 unrelated individuals living in the East of France. A total of 91 different haplotypes was observed for the two hypervariable regions resulting from 72 polymorphic positions in HV1 and 49 in HV2. Analysis of the data showed that the polymorphism of the two hypervariable regions were due to base transitions (75.5%) rather than transversions (1.5%). The genetic diversity was found to be 0.9836 and the random match probability 2.62%.

Keywords: Database; Caucasian population; mtDNA; HV1/HV2

1. Introduction

Owing to its high copy number per cell and its important polymorphism in the D-loop region (HV1 and HV2), automatic sequencing of human mtDNA has proved to be very useful in the analysis of a great number of forensic cases. We established a database by analysing the mitotype of 100 Caucasian individuals living in the East of France in order to assess their frequencies and to estimate random match probability. The goal of this

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study was also to expand work previously performed on 50 French Caucasian individuals [1].

2. Material and methods

DNA was isolated from bloodstrains of 100 individuals using an organic extraction (standard procedure). HV1 and HV2 regions were amplified separately under standard conditions using conventional primers. (HV1: L15977/H16429; HV2: L29/H408). Two sequencing methods were used according to manufacturer's instructions. Reactions were done with the Dye Terminator Cycle Sequencing Ready Reaction Kit (AB) and analysed on an ABI Prism 373A DNA automatic sequencer or with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (AB) and analysed on an ABI Prism 3100 (16 capillary sequencer). Sequencing primers were those used for DNA amplification except for the sequencing of HV1 C-stretch region where an additional sequencing primer was used on one of the strands (H16167). Data were analysed by Sequencing Analysis 3.3 Software (AB) and aligned with the Anderson sequence [2] using Sequence Navigator Software 1.0.1 (AB).

3. Results and discussion

3.1. Population data

In this study, we have determined the nucleotide sequence of the mitochondrial control region from 100 persons unrelated in their maternal lineage (Table 1). Sequence comparison with the Anderson sequence led to the identification of 91 mtDNA types with 72 polymorphic positions in HV1 and 49 in HV2. Of these 91 mitotypes, 86 were observed only in one individual, whereas 3 mitotypes were observed in 2, 1 mitotype in 5 and 1 mitotype in 3 other individuals. Sequence comparison showed also one A to G transition at position 263 in 98% of the individuals and a C insertion at position 315.1 in all subjects. Frequent mitotypes were found to be 263G, 309.1C, 315.1C as well as 73G (62%), 263G (98%), 309.1C (45%), 315.1C (100%). These mitotypes are also typical for nearly all individuals in other European populations [1,3–7].

As expected, transitions were predominant (75.5%) with regard to transversions (1.5%), insertions (22.3%) and deletions (0.5%). A transition from T to C at nucleotide position 16189 occurred in 11 cases and was linked to length heteroplasmy in the homopolymeric tract between positions 16184 and 16193. At position 16189, a clear point heteroplasmy C/T (ratio close to 50/50) was detected in one subject, while the other samples clearly showed only one T peak. In the homopolymeric C tract of HV2, at positions 303–309 and 311–315, 59% of samples showed one or more C insertions but the T at position 310 was constantly present and the stretch was 10 cytosines at most. Insertion of an additional T at position 310 was also observed in two subjects.

Table 1
Distribution of mutations for HV1 and HV2 regions in 100 French Caucasians

HV1																
	16020	16038	16051	16069	16092	16093	6094	6104	6111	6117	6124	6126	6129	6145	6162	6163
Y																
del																
A													4	4		
T	1			8				2	1							
C					2	6	1			1	1	21				
G		1	1												2	1

HV1																
	6172	6177	6179	6183	6185	6186	6189	6192	6193	6193	6193	6195	6209	6213	6216	6221
Y							1									
del																
A														2		
T			1		1	1		10	1	1						1
C	2			4			11			6	1	1	1			
G		1	1												1	

HV1																
	6223	6224	6231	6234	6242	6249	6255	6256	6261	6265	6266	6270	6278	6286	6288	6290
Y																
del																
A					1		2	2								
T	15			3					8	1	1	9	8	3		1
C		12	5			2									1	
G										1						

(continued on next page)

Table 1 (*continued*)

HV1																			
	6291	6292	6293	6294	6295	6296	6298	6301	6304	6309	6311	6317	6319	6320	6325	6327			
Y																			
del																			
A													1						
T	3	2		16	1	5		1						1		1			
C							8		9		19				3				
G			1							4		1							

HV1																			
	6354	6356	6357	6362	6390	6398	6399												
Y																			
del																			
A					5	1													
T	1																		
C		2	2	3															
G							2												

HV2																			
	00063	00064	0072	0073	0093	0114	0119	0143	0146	0147	0150	0151	0152	0153	0159	0185	0188	0189	
Y																			
del																			
A								1								4			
T		2				1					14	1		4					
C	1		7				1		12	12			31		3				
G				62	2												1	2	

HV2																	
	0194	0195	0198	0199	0200	0204	0207	0214	0215	0225	0226	0227	0228	0234	0239	0249	0250
Y																	
del																1	
A							4			5			3				
T	1		1														
C		24		3		4					2				1		2
G					1			1	4			1		1			

HV2																	
	0259	0263	0279	0290	0291	0295	0296	0309	0309	0309	0310	0311	0315	0319			
Y																	
del				1	1		1										
A																	
T						8					2	1					
C			1					45	13	1			100	4			
G	1	98															

Calculation methods previously described gave rise to a genetic diversity value [8] of 0.9836 and a random match probability [9] of 2.62%. These values are in agreement with the ones obtained for other European populations.

3.2. mTDNA analysis of forensic samples

We applied automated sequencing of mtDNA to 30 forensic caseworks with evidence and reference material consisting of hair shaft, hair root, bone, muscle, blood and saliva samples. The mtDNA sequence analysis was successful and informative in 25 cases. The reference samples were excluded or included respectively in 62% and 38% of the cases. All exclusions were based on at least two base differences with no evidence of length heteroplasmy. Conclusive results were obtained for 87% of the samples.

This study proved the utility of the mtDNA sequencing in cases where nuclear DNA analysis failed to give results.

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