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Population variation of human mitochondrial DNA (HV1 and HV2) in Spanish unrelated individuals (Northeast Spain)

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Abstract. A population database from 200 unrelated Caucasian individuals living in Spain was generated. Sequence polymorphisms of the mitochondrial DNA (mtDNA) control region, hypervariable regions I and II (HVRI and HVRII) were determined by polymerase chain reaction (PCR) and direct sequencing. A total of 175 different sequences were found as defined by 154 variable positions. The most common sequence occurred 10 times, this sequence is also the most frequent in other European populations such as Austrian, German and British. The mean pairwise difference for the two regions taken together was 8.25. The study revealed that transitions made up the majority of the deviations (88%), whereas we observed a significantly lower frequency of transversions (8%). A statistical estimate of the results for this Caucasian population showed a genetic diversity of 0.9965. The probability of two random individuals showing identical mtDNA haplotypes is 0.84%. In order to use the mtDNA analysis in forensic casework, we consider that it is of crucial importance to know the frequency of the different sequences of mtDNA, and this database study could be a useful tool to evaluate statistically the results. © 2003 Published by Elsevier B.V.

Keywords: Mitochondrial DNA; Database; Northeast Spain

1. Introduction

During the last 8 years in our laboratory we have used mitochondrial DNA (mtDNA) analysis to solve routine forensic casework where conventional DNA typing is unavailable. Mainly to type naturally shed hairs or hairs without roots and to confirm the identification of highly decomposed remains and skeletal remains. The present study shows a mtDNA database for the regions hypervariable regions I and II (HVRI and

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Table 1
Statistical data

	HV1	HV2	HV1 + HV2
Number of different haplotypes	131	100	175
Number of variable sites	99	55	154
Number of unique haplotypes	107	77	162
Frequency	0.0229	0.0459	0.0034
Average number of differences	4.555	3.697	8.252
Genetic diversity	0.97709	0.95497	0.99658
Randon match probability (%)	2.78	4.84	0.84

HVRII), from unrelated Caucasian individuals living in Spain. This database is being used to evaluate the results of mtDNA analysis in our laboratory.

2. Materials and methods

DNA was obtained from casework reference samples (blood, saliva, hair) of 200 unrelated Caucasians who live in the Northeast of Spain. DNA was extracted by standard

Table 2
Distribution of mutation distribution

Mutation type	Number of position	Total number of mutations
Transitions		
Py–Py		
C–T	47	257
T–C	39	314
Pu–Pu		
A–G	33	378
G–A	13	63
Total	132	1012
Transversions		
A–C	3	23
C–G	3	3
G–C	2	3
T–G	2	2
G–T	3	6
A–T	2	2
Total	15	39
Insertions		
+C	3	297
+2C	1	18
+3C	1	1
Total	5	316
Deletions		
−A	3	6
−T	1	1
Total	4	7

Table 3
Most frequent haplotypes observed in Spanish population (northeast Spain)

Haplotypes HV1 + HV2	*n*
263G 315.1C	10
263G 309.1C 315.1C	5
16189C 263G 315.1C	3
16069T 16126C 73G 185A 228A 263G 295T 315.1C	2
16311C 263G 315.1C	2
16093C 16224C 16311C 73G 195C 263G 315.1C	2
16069T 16126C 16278T 73G 185A 188G 228A 263G 295T 309.1C 315.1C	2
16145A 16222T 263G 315.1C	2
16069T 16126C 16193T 73G 150T 152C 263G 295T 315.1C	2
263G 309.1C 309.2C 315.1C	2
16192T 16270T 16319A 73G 150T 263G 315.1C	2
200G 263G 315.1C	2
16224C 16311C 73G 263G 315.1C	2

phenol–chloroform method [1]. The mtDNA regions subjected to analysis were 16024–16365 (HVRI) and 73–340 (HVRII). Amplification reaction was performed as described previously [2] employing a GeneAmp System 2400 thermal cycler (Perkin-Elmer, Norwalk, CT).

The amplified products were purified and separated from unreacted primers and dNTPs by filtration in a Centricon-100 (Amicon Beverly, MA) following the manufacturer's recommendations. Sequencing fragments were generated using Taq DyeDeoxy™ Terminator Cycle Sequencing Ready Reaction kit (Foster City, CA, USA) and primers identical to those employed for amplification, according to the protocol provided by PE/ABD with the kit. Afterwards, DNA products were purified by ethanol precipitation and the fragments were analysed using an automated DNA sequencer ABI PRISM 310 (PE/ABD). Each template was sequenced from both directions and the consensus sequence was aligned and compared with the reference sequence [3] using the software SeqEd™ V. 1.0.3 (PE/ABD).

3. Results and discussion

Table 1 shows the statistical data (different haplotypes, variable sites, unique haplotypes frequency, genetic diversity and random match probability) obtained for each region and two region taken together. Table 2 shows the distribution of nucleotide substitution that we have obtained. Table 3 shows the haplotypes observed at least in two individuals in our population.

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