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MtDNA control region polymorphism: sequence database and forensic applications

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Abstract

Since 1998, the Spanish Scientific Police has been analyzing both hypervariable segments (HVR1 and HVR2) of the mitochondrial control region by sequencing analysis. During this period, different sample types (e.g., blood, saliva, telogen hairs, hair shafts, nails, tissues, bone and dental remains) related to murders, sexual assaults, robberies and identification of human remains have been studied. Some problematic findings detected in routine forensic casework involving sequence heteroplasmy and one single nucleotide mismatch between forensic specimens and reference samples are described in this paper. In specimens which, in principle, due to apparent features should not give any problems when being analysed, a lack of amplification was detected within some of the subregions studied. Subsequently, it was proven that this was caused by point mutations in the primer binding sites. A substitution of the primers by non-conventional ones overcame the absence of reactions. Given that a proper statistical evaluation of the results using a large database is needed, samples from 120 individuals living in Spain have been analysed. The most frequent sequence found was 263-G; 315.1-C (differences compared to the reference sequence), being consistent with other Caucasian population studies.

Keywords: mtDNA database; Primer binding site; Point mutation; Forensic casework; Annealing

1. Introduction

The aim of the present work was firstly to describe the problems that have been found during 4 years of routine mitochondrial DNA forensic casework and the measures adopted

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in order to solve them, and secondly the publication on the internet of the mitochondrial haplotypes of 120 individuals living in Spain.

2. Materials and methods

HV1 and HV2 regions for 120 Caucasian Spanish inhabitants have been studied. Both regions were amplified independently with the primers A1(L15997)/B1(H16395) and C1(L0048)/D1(H0408) as described by Wilson et al. [1].

In addition, 218 casework specimens (5 blood stains, 2 saliva stains, 192 hairs, 3 muscle samples, 2 fingernails, 10 bones, 1 decayed brain sample and 3 teeth) were amplified in four subregions (HV1-A, HV1-B, HV2-A and HV2-B) in cases where they did not present nuclear DNA after quantitation, by using the primers A1(L15997)/B2(H16236), A2(L16159)/B1(H16395), C1(L0048)/D2(H285) and C2(L172)/D1(H0408) as described previously [1].

In all cases, sequencing was performed with the amplification primers and the ABI Prism Big-Dye Terminator Cycle Sequencing Kit (Applied Biosystems) and electrophoresis in an ABI Prism 377 DNA Sequencer (Applied Biosystems).

3. Results

3.1. Population database

The most frequent haplotypes found were "263-G; 315.1-C" (5 out of 120) and "263-G; 309.1-C; 315.1-C" (5 out of 120), being consistent with other European population studies followed by "16.298-C; 72-C; 263-G; 309.1-C; 315.1-C" (http://www.mir.es/policia/cnp/pocien.htm). Length heteroplasmy in poly-C stretches were found in 13 HV1 sequences; while in HV2, the number enlarges to 29. Values obtained for match probability and genetic diversity were 0.01156 and 0.9967, respectively.

3.2. Casework

In two of the cases studied, no amplification in subregion HV1-B was observed while being totally effective in the rest. Sequences analysis of both HV1-A strands showed a mutation in the annealing zone of the primer A2(L16159): in one of them the mutation appeared at position 16150 (C → T) and in the other in position 16153 (G → A). Further amplification with an alternative primer (L16209) allowed a result for the complete HV1 region to be obtained (Fig. 1).

In a third case (hair specimen), in order to obtain more robust results for sequencing analysis of HV2A region, since a first study yielded noise to a certain extent in the L-strand, led to an analysis with a second internal primer (L0066), being unable to produce a clean electropherogram. Enlarging the region of comparison with CRS to positions beyond 73, a mutation was found in position 64 (C → T), which was why the primer could not undergo the appropriate annealing.

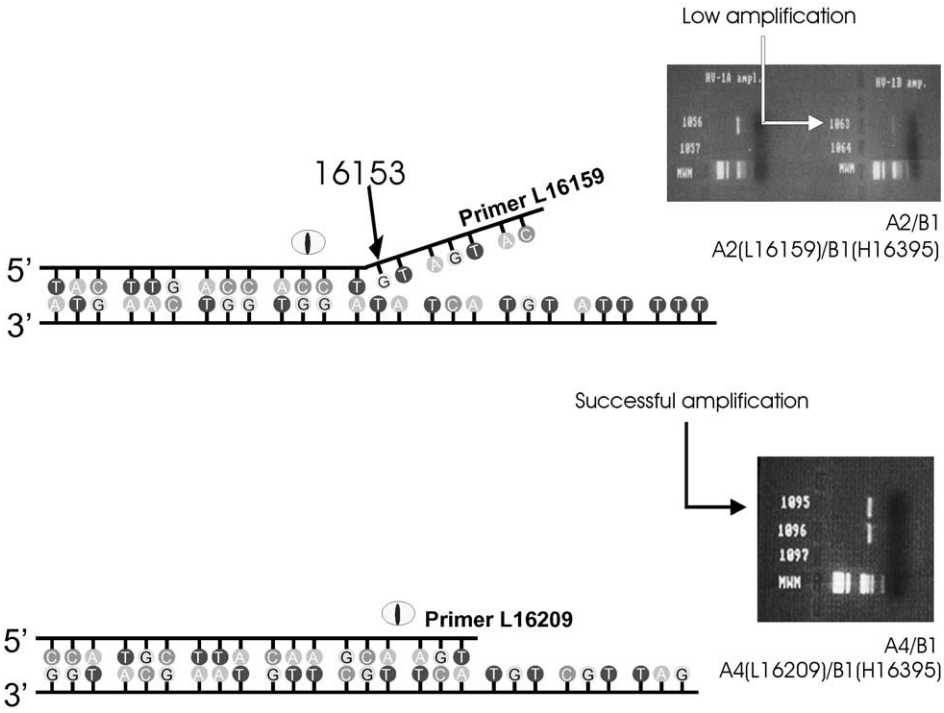

Fig. 1. Mutation in primer binding site.

Sequence heteroplasmy was found in four cases: 16162-G/A in a reference hair sample, 16.292-C/T in a reference hair sample, 16234-T/C in a reference blood sample (this sample belonged to an individual whose brother did not show this polymorphism), and 152-T/C in two reference saliva samples belonging to a mother/child pair (the questioned sample together with that from one of his brothers did not show this heteroplasmy).

Evaluation of evidence with respect to the reference samples was problematic in three cases: in two of them, there was only one difference present between the sequences of both types of specimen, and in a third case, as well as one different position, the evidence sample showed a heteroplasmy absent in the reference sample. In one of these cases, the problem was solved by studying other regions within the D-loop (HV3) and polymorphisms located in positions not habitually edited.

4. Discussion

Negative results in amplification do not imply that the sample has necessarily to be rejected, as mitochondrial primer annealing regions may undergo mutations even though such regions are known to be less polymorphic than the rest. The use of alternative primers in adjacent regions can solve it.

On some occasions, despite the general admission of Wilson's proposals [2] it can be very useful to broaden the regions to edit when analysing sequences, particularly in those cases in which an only difference is observed between reference and evidence samples. Moreover, the study may be completed with the analysis of other regions included into the D-loop, such as HV3 (438–574) [3].

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References

[1] M.R. Wilson, J.A. DiZinno, D. Polanskey, J. Replogle, B. Budowle, Validation of mitochondrial DNA sequencing for forensic casework analysis, Int. J. Legal Med. 108 (1995) 68–74.
[2] M.R. Wilson, M.M. Holland, M. Stoneking, J.A. DiZinno, B. Budowle, Guidelines for the use of mitochondrial DNA sequencing in Forensic Science, Crime Lab. Dig. 20 (4) (1993) 68–77.
[3] S. Lutz, H.J. Weisser, J. Heizmann, S. Pollak, Location and frequency of polymorphic positions in the mtDNA control region of individuals from Germany, Int. J. Legal Med. 111 (2) (1998) 67–77.