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Identification by mtDNA of exchanged human body remains

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Abstract. This report presents an homicide case which occurred in an African country, involving five Portuguese citizens whose identification was suspected to have been exchanged. HVI and HVII control regions of mtDNA analysis was performed from bones from each of the five corpses in order to achieve identification by comparison with four alleged mothers. Two bodies shared the same sequence, which suggest a kinship relation. Results have confirmed the suspicion of body remains exchange.

Keywords: mtDNA; HVI/HVII; Human identification; Human remains; Degraded DNA

1. Introduction

Five Portuguese citizens were killed in Africa. Three of them were identified and subsequently removed to Portugal, where they were buried. Two children were reported as missing. One year later, two skeletons were found in a grave, 500 m from the crime scene. Genetic typing of DNA extracted from skeletal remains was performed in order to establish their identities. One of the mothers of the two missing minors was excluded from the maternity of the skeletal remains. The hypothesis was raised of an identity exchange of the three corpses previously removed to Portugal. These were then exhumed and subjected to genetic analysis to achieve biological identification.

In this report, we present the results of mtDNA sequence analysis of human body remains of five individuals and their presumptive mothers.

2. Material and methods

Bones and teeth from the five deceased individuals were powdered in a 6800 freezer mill (Fisher Bioblock) and DNA was extracted by an organic method. DNA extraction from bloodstains of the presumptive mothers was performed by Chelex method.

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Table 1
HVI and HVII mtDNA sequences of the five individuals and four alleged mothers compared to the Anderson reference sequence

	Nucleotide position																		
	HVI																		
Sample origin	16069	16126	16129	16148	16168	16172	16187	16188	16189	16193	16223	16224	16230	16264	16270	16278	16293	16311	16320
Anderson	C	T	G	C	C	T	C	C	T	C	C	T	A	C	C	C	A	T	C
Body 1	.	.	A	T	T	C	T	G	C	.	T	.	G	.	.	T	G	C	T
Body 2	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.
Body 3	.	C	.	.	.	.	T	.	C	.	T	.	.	T	T	T	G	C	.
Body 4	T	C	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.
Body 5	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.
Mother 1	.	.	A	T	T	C	T	G	C	.	T	.	G	.	.	T	G	C	T
Mother 2	.	C	.	.	.	.	T	.	C	.	T	.	.	T	T	T	G	C	.
Mother 3	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.
Mother 4	T	C	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.

	HVII																	
	73	93	95	114	150	152	182	185	189	195	198	236	247	263	295	309.1	315.1	316
Anderson	A	A	A	C	C	T	C	G	A	T	C	T	G	A	C	– –	– –	G
Body 1	.	G	C	.	.	.	.	G	G	.	.	C	A	G	.	C	C	.
Body 2	G	.	.	T	.	.	.	.	.	.	.	.	.	G	.	C	C	A
Body 3	G	.	.	.	.	C	T	T	.	C	T	.	A	G	.	.	C	.
Body 4	G	.	.	.	T	.	.	.	.	.	.	.	.	G	T	.	C	.
Body 5	G	.	.	T	.	.	.	.	.	.	.	.	.	G	.	C	C	A
Mother 1	.	G	C	.	.	.	.	G	G	.	.	C	A	G	.	C	C	.
Mother 2	G	.	.	.	.	C	T	T	.	C	T	.	A	G	.	.	C	.
Mother 3	G	.	.	T	.	.	.	.	.	.	.	.	.	G	.	C	C	A
Mother 4	G	.	.	.	T	.	.	.	.	.	.	.	.	G	T	.	C	.

A letter in each position indicates a nucleotide substitution relative to the reference sequence and the absence means that the sequence is according to the reference sequence. Double dash indicates the positions where there is no nucleotide on Anderson sequence.

For HVI region amplification, primers L15997 (A1), L16159 (A2), H16395 (B1) and H16236 (B2) were used [1]. Primers L048 (C1), L172 (C2), H408 (D1) and H 285 (D2) were applied for HVII region amplification [1]. PCR was carried out using the following thermal cycling conditions: 95 °C 11 min, 36 cycles (95 °C 10 s, 60 °C 30 s, 72 °C 30 s), 72 °C 10 min.

PCR products were sequenced with BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and purified with DYNAPURE™ Dye Terminator Removal ver.2 (Dynal®). Sequencing was carried out on an ABI PRISM™ 377 DNA Sequencer.

3. Results

We have obtained mtDNA sequences in all but one sample (a bone) after some changes of the protocol.

Almost all the samples were successfully amplified with the pairs of primers A1/B1 and C1/D1. Results were not obtained for HVII and for HVI regions from teeth of bodies 2 and 4, respectively. On these cases, shorter overlapping fragments were amplified with the pairs of primers C1/D2, C2/D1 (for body 2), A1/B2, A2/B1 (for body 4) and the reconstruction of HVII and HVI sequences were performed.

MtDNA sequence of each body matched always one of the alleged mothers sequence (Table 1). Nucleotide substitutions were the same in bodies 2, 5 and in presumptive mother 3, suggesting a kinship relation.

Results revealed that the three bodies buried in Portugal had been incorrectly identified.

4. Discussion

It was difficult to obtain results namely from teeth and bones retrieved in Africa. The state of bone samples was poor, probably due to humidity, temperature or other African environmental conditions and soil characteristics that accelerate the degradation process.

In some cases, increasing the amount of DNA helped to overcome the low amount of the DNA, but in some others lead to negative results, which can be explained by an increase of Taq polymerase inhibitors in the mix reaction. Several dilutions DNA extract were tested in order to decrease the hypothetical contaminants. Regarding the high fragmentation of DNA associated with this kind of samples [2], the application of different primers to the HVI and HVII regions allowed the amplification of two shorter overlapping fragments for each region and the reconstruction of HVI and HVII sequences.

MtDNA analysis was a useful tool to solve this case. Although it cannot be used to definitely identify the corpses, it provided evidence of maternal relationships and, consequently in this case, the previously exchange of human body remains.

References

[1] M.R. Wilson, J.A. DiZinno, D. Polanskey, et al., Validation of mitochondrial DNA sequencing for forensic casework analysis, Int. J. Legal Med. 108 (1995) 68–74.

[2] H.N. Poinar, The top 10 list: criteria of authenticity for DNA from ancient and forensic samples, Prog. Forensics Genet. 9 (2003) 575–579.