Post-Mortem Molecular Identification. Biological Kinship Established by DNA Analysis.

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Introduction:

Molecular characterization allowes to identify individuals when potential relatives are available. Conventional DNA typing complemented with PCR-based forensic analyses such as Short Tandem Repeats (STR) and HLA-DQα evaluation broaden the perspectives in forensic field. The possibility of molecular identification of old cadavers was almost impossible just a few years ago. However, current methodologies made possible the molecular characterization of such remains.

The aim of this presentation is to describe the strategies and methodologies employed in three cases in which from a human body biological kinship evaluation was required. For this report we choose three cases in which cadavers were in different conservational conditions: a- A mummified teenager, b- A burned adult, and c- A semi-putrid newborn.

Based on molecular evaluation, in every case it was possible to establ;ish whether or not biological relatedness existed.

Materials and Methods.

Skin, muscle, teeth and bone were used for DNA extraction in the mummified cadaver, while dermis and muscle were used in the cases of the burned body and the putrid newborn.

DNA extraction was carried out by means of a CTAB extraction protocol (Corach, 1991). Briefly, about 500 mg of tissue are sliced with an scalpel and homogenized in a teflon/glass potter Three volumes of homogenization buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris/HCl, 20mM Na_2EDTA and 0.2% β-mercapto ethanol) were added, following incubation at 60°C for 2-3 hrs with gentle shaking. DNA was extracted with Cl_3CH:isoamyl alcohol (24:1), until the proteic interphase diappeared. Finally, samples are precipitated with 2/3 volumes of isopropyl alcohol, centrifuged

13 000 x g in a microcentrifuge (or 10 000 rpm in a SS-34 Sorvall rotor) for 10 min. at 4°C. DNA extracted by this procedure is nearly always suitable for restriction assays and PCR amplification ,some cadaveric material may require other options. DNA to be used for Southern blot and PCR analysis were purified by means of Glass Max (BRL) or GeneClean II (Bio101) systems. Samples to be used for PCR amplification were purified with Chelex P-100 (Walsh et al.1991. Sex determination was achieved by means PCR amplifications of: a) SRY of a 299 bp fragment, b) Y specific heterochromatin (Yq_{13-ter}) fragment of 154 bp.**DNA Typing:** *Loci* D2S44, D1S7 and D4S139 were evaluated by means of Southern hybridization assays involving *Hinf I* digests and YNH-24 (ATCC), MS1 (BRL) and PH-30 (BRL) probing.**Short Tandem Repeats (STR)** evaluation: THO-1, FABP and ARA were performed according with Edwards et al.(1991, 1992) **HLA-DQα Typing:** Samples were PCR amplified and detected by means of DQα-Amplitype System,(Cetus-Perkin Elmer).

Case Report 1: The cadaver of a teenager, naturally mummified, whose death may had occured 20 years ago was claimed by potential parents as belonging to a young adult who disappeared last year. In order to establish biological kinship between the cadaveric material (CM 1) and the potential relatives four blood samples (1,2,3,4) were sent to our lab, including two volunteers. Tubes were codified (blind double assay).

Case Report 2: An adult was intentionally burned (CM 2) and three women (5,6,7) claimed to be sisters of the victim.

Case Report 3: A couple (8,9) denounced that immediatelly after the birth of his son the infant had been substituted by a dead one(CM 3).

Results and Discussion

Case 1: DNA obtained from CM 1 was highly degraded, suitable only for PCR based typing. Results are summarized in TABLE 1. Sex determination allowed us to discriminate between the potential mother and the other three male samples. Systems assayed denoted at least one exclusion between CM 1 and samples 1 to 4.

Case 2: Although the cadaver was completely burned, DNA obtained was suitable for PCR and Southern blot typing. Results are summarized in TABLE 1. CM 2 and 7 displayed the same genotype for the PCR-based systems. On the other hand, CM 2 and 6 showed the same YNH-24 genotype. The CM 2 ARA (X chromosmome marker) allele was present in 7. Therefore, biological kinship between CM 2 and 5,6,7 was suggested.

Case 3: TABLE 1 depicts the results obtained. PCR and Southern blot typing systems used showed a clear inclusion of CM 3 as son of 8 and 9.

TABLE 1

System	Case 1					Case 2				Case 3		
	CM1	1	2	3	4	CM2	5	6	7	8	CM3	9
SRY / Y	M	F	M	M	M	M	F	F	F	M	M	F
THO-1	9-10	6-7	9-9	7-9	6-7	6-10	7-10	7-10	6-10	7-9	7-10	10-10
FABP	10-10	10-10	8-13	10-10	10-10	10-11	10-11	10-10	10-11	10-13	13-13	11-13
ARA	-	-	ND	ND	ND	+	-	-	+	ND	+	+
DQ-A	1.3-4	3-4	4-4	1.1-3	1.3-4	1.1-4	1.1-3	1.1-4	1.1-4	2-3	1.2-2	1.2-2
YNH-24/Hinf	ND	ND	ND	ND	ND	2.4-4.4	3.7-4.1	2.4-4.4	3.7-4.1	2.6-3.1	2.6-4.4	2.9-4.4
MS1/Hinf I	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.3-4.4	3.3-3.3	3.3-5.5
PH-30/Hinf	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.5-12.6	7.3-12.6	7.3-7.4

Notes: SRY/Y, F=female M=male. THO-1 and FBAP: numbers indicate the number of repetitions for each allele. ARA: + matching allele, -= no matching allele. YNH-24, MS1 and PH-30 numbers indicate the molecular weight of each allele, expressed in kilobases.

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