PCR GENOTYPING IN DENTAL PULP FROM OLD HUMAN SKELETAL REMAINS AND FIRE VICTIMS.

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Introduction

The first attempts to recover genetic information from ancient skeletal material was undertaken from an anthropological and palaeontological point of view. Soon after, an ongrowing forensic interest in the possibility of DNA typing of human skeletal remains emerged, thus solving the identification of recent or ancient victims, and specially difficult parentage testing problems. The successful identification of family relationships in three famous cases: the Karen Price case (Hagelberg, 1991), the identification of the skeletal remains of Josef Mengele (Jeffreys, 1992) and of the Romanov family (Gill, 1994) have stimulated the common usage of skeletal remains in forensic questions involving more or less recently dead individuals. Among these skeletal remains, dental pulp, housed in the pulp cavity in the center of the tooth is a rich source of DNA. Moreover teeth endure post-mortem degradation and extreme environmental conditions better than most biological tissues (Sweet, 1995; Cerri, 1994). Here we present the result of PCR genotyping of dental pulp samples obtained from dental surgeon and from casework.

Material and Methods

Control teeth, freshly removed and healthy, were obtained for orthodontic reasons.

Casework teeth:

A.- alleged father (60 years) of a paternity case, exhumated two years after death.

B.- alleged father (20 years) of a paternity case, exhumated four years after death.

C.- Unidentified burnt corpse, victim of a domestic fire (45 years).

D.- Unidentified male corpse (above 40 years) found outdoors; from the decomposition features time after death was estimated at about fifteen to thirty days.

Sample preparation and DNA extraction

Teeth were thoroughly washed in boiling water with the addition of dishwasher machine detergent (Calgonit®), rinsed with distilled water, dried, immersed in liquid nitrogen for at least 24 h, and finely crushed to fine powder with a mortar and pestle. Powdered teeth were decalcified with 0.5M pH EDTA 7.5, according to Hochmeister (1991). The decalcification process took from four to five days. After washing three times in Milli Q sterile water, 2 ml of prewarmed (56°C) extraction buffer containing 10 mM Tris, 10 mM EDTA, 100 mM NaCl, 40 mM DTT and 150 μL of proteinase K (16.2 mg/mL) were added to each pellet and incubated at 56°C overnight. The solutions were extracted three times with phenol-chloroform-isoamyl alcohol (25:24:1), aqueous solutions combined, extracted once with n-butanol to eliminate phenol residues and DNA was concentrated using a Centricon-30 microconcentrator

device. Retentates were washed three times with Milli Q water and recovered DNA was stored until use at 4°C or -20°C.

DNA sample analysis

The quantity of human DNA in the samples was determined by slot blot quantification using the QuantiBlot human DNA quantitation Kit (Perkin Elmer®).

The DNA quality was assessed by submarine 0.7% agarose minigel electrophoresis in TBE (0.134M Tris- 75 mM Boric Acid - 2.55 mM Na_2 EDTA, pH 8.8). Lambda Hinf III digested DNA was used as molecular weight ladder and UV visualization carried out after immersion of the gel in an ethidium bromide solution (0.5μg/mL) for 20 min after the electrophoresis run.

Amplifications and Typing

DQA1: Amplitype HLA DQA1 Forensic DNA amplification and Typing Kit (Perkin Elmer).

PM: Amplitype PM PCR Amplification and Typing Kit (Perkin Elmer).

D1S80: AmpliFLP D1S80 PCR amplification reagent set (Perkin Elmer). PCR products were detected by vertical PAGE (Gene Amp Detection Gel Perkin Elmer) followed by silver staining (Plus One Silver Staining Kit. Pharmacia).

STR: Gene Print STR systems (Promega) were used for TH01, CSF1P0, TPOX, F13A01, FES/FPS and vWF loci. Typing was carried out by poliacrylamide denaturing gel electrophoresis followed by silver staining, according to Promega protocols.

Results

Table 1 DNA recovery.

Sample	Powdered Teeth Weight (g)	DNA recovery (μg/g)
Control	0.50	2.4
A	1.96	15.9
B	2.18	25.04
C	3.05	295.08
D	1.22	31.96

Dental pulp from the burnt body (C) yielded far more DNA than exhumed specimens. No high molecular weight DNA was recovered in any sample. DQA1 was successfully typed in all the samples. Additionally two STRs were assayed in control teeth (FES/FPS and vWF).

Successful typing of STRs permitted the processing of casework specimens to continue. A set of PCR loci usually amplified in our laboratory was applied. The following results were obtained:

	A	B	C	D
DQA1	+	+	+	+
PM	+	+	+	+
D1S80	+	Undetected	+	+
TH01	+	Spurious bands	+	+
TPOX	+	Spurious bands	+	+
CSF1PO	Faint bands	Spurious bands	+	+
FES/FPS	+	Spurious bands	+	+
vWF	+	Spurious bands	+	+
F13A01	+	+	+	+

+ Successful typing

Comments

Anomalous results or failure in the amplification reaction were obtained only in the tooth sample from the corpse exhumated four years after burial, under the most unfavourable climatic conditions in our region: very high temperatures in summer and a high level of humidity and mild environmental temperatures in winter; moreover the cemetery is located close to the Guadalquivir river.

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