APPLICATION OF PCR TECHNIQUE FOR THE CHARACTERIZATION OF HUMAN BONES

C. Previdere', G. Peloso
Institute of Legal Medicine, University of Pavia, Italy

Introduction

One of the sources of more stable DNA and, at times, the only physical evidence of human remains is bone tissue. DNA from bone tissue, even if extensively degraded, can be typed by PCR analysis of loci with a low range of alleles (< 300 bp) such as microsatellites. The aim of this preliminar work is to evaluate the feasibility of genetic identification of human skeletal remains. Moreover we tried to establish a correlation between DNA degradation and storage conditions of bones.
Genetic characterization of bone tissue could be very important for forensic individual identification, expecially if relatives are available for testing.

Materials and methods

Storage study: bone samples were collected in the course of autopsy from 7 cadavers, 24 hours after death. From each one we took a fragment of about 2 grams of spongy (sternum) and compact (femur) bone. These specimens were subjected to different storage conditions: immersed in water or buried in soil for a period of 6 months. Blood samples drawn during autopsy were used as controls for this study.
Old bones: bone samples from unknown skeletal remains were analyzed. In particular the specimens were: 1) fragment of compact bone (temporal) and one tooth (molar) from a partially burnt body recovered in 1986 and stored at room temperature until analyzed (7 years); 2) fragments of compact bone (occipital) from a body that had been recovered in a river and stored at room temperature for 15 years; 3) fragments of compact bone (parietal and femur) recovered in 1964 and stored at room temperature until analysis (30 years); 4) fragments of compact bone (femur,occipital,vertebra,teeth) from different bodies, recovered from a grave, about 50 years old (end of the 2nd World War-1945).
DNA extraction: DNA from bone samples was extracted according to the method described by Hochmeister et al. (1991).
Slot blot analysis: the quantification of human DNA was achieved according to the method of Waye et al. (1989) utilizing an Alu probe.
PCR analysis: the genetic loci analyzed were: HLA-DQ alpha (Helmuth et al.,1990) and microsatellite loci TC11 (Edwards et al.,1992), SE 33 (Polymeropoulos et al.,1992), vWA (Kimpton et al.,1992) and Apo C2 (Weber et al.,1989). The HLA-DQ alpha typing was performed according to the Perkin Elmer Cetus protocol. Microsatellite amplification was carried out including ^{35}S dATP during the reaction. PCR products were fractioned on a 6 % denaturing polyacrilamide gel. Allele sizes were measured relative to DNA sequence ladder derived from a known sequence. In order to prevent PCR inhibition during genetic typing of old bone samples, bovine serum albumin (200 μg/ml) was included in the PCR mix. Finally, positive and negative (no DNA) reactions were routinely included.

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Results

The results of the storage study are reported in Table I. Human DNA was extracted from all bone samples and , as previously reported by Lee et al. (1991), the yield of DNA /gram of bone was higher in spongy bone than in compact one; in addition DNA from samples buried in soil seemed to be more quickly degraded than that from samples immersed in water. All the samples typed by PCR matched the corresponding blood control sample.

Table I :

	bones immersed in water		bones buried in soil	
	spongy	compact	spongy	compact
Samples	7	7	7	7
Human DNA/gram bone tissue (Averange)	4.5 μg	700 ng	2 μg	75 ng
PCR typing (bone typing matches the corresponding blood control sample)	7/7	7/7	5/7[a]	4/7[a]

[a] PCR analysis failed in five samples , probably because of the presence of uncharacterized PCR inhibitors not removed even in presence of bovine serum albumin.

The results of the characterization of the old bone samples are summarized in table II. No correlation was found between PCR typing, quantity of DNA recovered and the age of the samples. In fact, PCR gave good results in the analysis of a 50 years old femur bone sample from which the quantity of human DNA recovered (30 ng/gram) was higher than in a 15 years old occipital bone sample (20 ng/gram) that could not be typed by PCR. The ability of typing the bone samples by PCR was however in part influenced by the presence of uncharacterized PCR inhibitors coextracted with the DNA. Moreover the good stability of the DNA extracted from teeth was confirmed; the quantity of the DNA recovered from a 50 years old tooth was higher than that recovered from bones with similar age and the sample was successfully typed by PCR for all the genetic markers analyzed.

Table II:

Samples	Human DNA/gram bone	PCR analysis
Temporal bone (7years)	120 ng	+
Tooth (7 years)	80 ng	+
Occipital (15 years)	20 ng	−
Parietal (30 years)	30 ng	−
Femur (30 years)	50 ng	+
Occipital (50 years)	10 ng	−
Vertebra (50 years)	20 ng	−
Femur (50 years)	30 ng	+
Tooth (50 years)	40 ng	+

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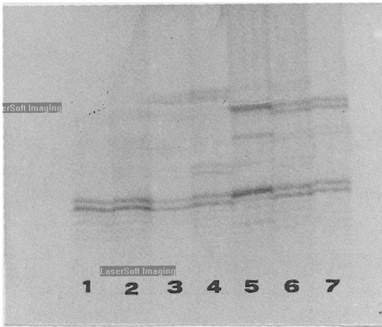

Fig. 1: autoradiogram of the PCR products of the microsatellite locus TC11. In lane 1, 3 and 5 blood control samples typed 183-183, 183-198 and 183-195 respectively In lane 2 DNA from a sample immersed in water (6 months); in lane 4 DNA from a sample buried in soil (6 months); in lane 6 and 7 DNA from the same sample immersed in water and buried in soil respectively. Allele sizes are reported as base pairs (bp)

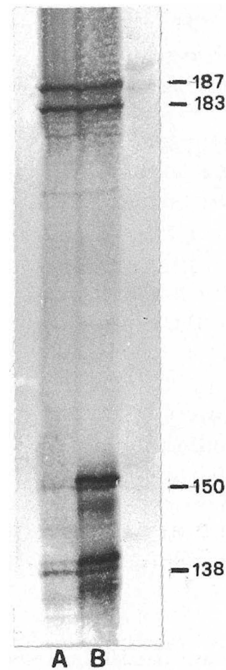

Fig. 2: autoradiogram of the co-electrophoresis of the microsatellite loci TC11 and vWA. DNA from two 50 years old samples: in lane A femur bone sample and in lane B tooth. Allele sizes are in base pairs (bp)

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