CASE REPORT

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Maternal Identification from Skeletal Remains of an Infant Kept by the Alleged Mother for 16 Years with DNA Typing

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ABSTRACT: This is a case study concerning maternal identification by DNA typing at various loci. An infant skeleton was found in the alleged mother's apartment after it was kept for 16 years. We obtained the skeletal remains as well as saliva stains from the alleged mother. DNA typing was conducted for three loci in the HLA class II region (HLA-DQA1, -DPB1, and DRB1), five loci with the AmpliType PM kit (LDLR, GYPA, HBGG, D7S8, and GC), five STR loci (LPL, vWA, F13B, TH01, and TPOX) and D-loop region in mtDNA for maternal identification. Sex determination was accomplished using fluorescent DNA capillary electrophoresis typing. Approximately 5 ng of human DNA was recovered from 1 g of femur bone retrieved from the infant skeletal remains. The probability of two unrelated Japanese sharing the same genotypes was estimated as 7.2×10^{-11}. The combined probability of exclusion that an individual is not the mother was also calculated at 0.998. We therefore conclude that the skeleton is from a female infant, and that there is no inconsistency in the claim that the infant was a daughter of the alleged mother.

KEYWORDS: forensic science, DNA typing, HLA-DQA1, HLA-DPB1, HLA-DRB1, LDLR, GYPA, HBGG, D7S8, GC, short tandem repeat, mitochondrial DNA, sex determination, maternal identification, skeletal remains

Examination of skeletal remains is sometimes required in forensic practice. Adult skulls may be identified by morphological examination, while the identification of other bones or infant skulls is often very difficult. The development of PCR-based DNA typing has made it possible to analyze DNA from compact bone (1–3) and spongy bone (4) for personal identification. STR (short tandem repeat) typing systems and direct sequencing of mitochondrial DNA (mtDNA) have been widely utilized. We now report an infanticide case in which the dead body was kept for 16 years by the alleged mother of the victim. Genetic typing of the skeletal remains and saliva stains of the alleged mother was performed in order to confirm their relationship.

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Case History

According to a woman's confession, police found the skeletal remains of an infant in the woman's apartment in April, 1995. The infant girl, 1.5 years old at that time, was said to have been strangled to death 16 years before by the woman's lover. She confessed to the crime only after the period of time within which a case of murder may be considered in Japan (15 years). The remains were wrapped in clothes belonging to the infant, put into a plastic bag with several deodorants, packed into a hard plastic box, and then kept in a closet. During a period of 16 years, the woman changed her apartment twice, transferring the box with her. It seems that she had opened the box to remove several small pieces of remains and put them in front of a household Buddhist alter to perform services for the memorial of the infant.

Forensic morphological examination only suggested that the remains are derived from a skeleton of an infant aged $1^1/_2$ to 2 years and that at least ten years have passed since the death of the infant. It failed to give information about the sex from the bone.

Materials and Methods

Samples

The right femur of the infant skeleton was used for DNA analysis (Fig. 1). Dust from the femur's surface was washed off by brushing. A segment of compact bone was cut into small pieces, approximately 1 mm^3 in size, using a small grinder. Saliva stains were donated by the alleged mother.

DNA Extraction

DNA was extracted from one gram of small pieces of the compact bone as described by Hochmeister et al. (1) followed by a purification method using cetyltrimethyl ammonium bromide (CTAB) (5) with a minor modification. DNA was extracted from the saliva stain (10 cm^2 on filter paper in size) according to routine organic extraction and ethanol precipitation method.

Quantitative and Qualitative Analysis of DNA Extracted from Bone

The amount of extracted DNA was determined fluorometrically using the TKO 100 Mini Fluorometer (Hoefer Scientific Instruments, CA), and Human DNA was quantified using the QuantiBlot™ kit (Perkin Elmer, NJ) as per manufacturer. The quality

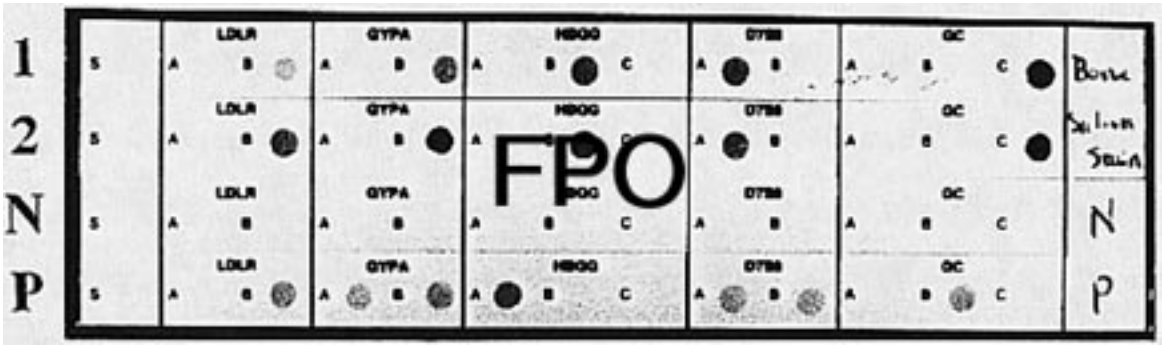

FIG. 3—*Genotyping results of five loci with AmpliType™ PM kit from the infant compact bone and the alleged mother's saliva stain. 1: Infant compact bone. 2: Alleged mother's saliva stain. N: Negative control. P: Positive control (LDLR: B, GYPA: AB, HBGG: A, D7S8: AB and GC: B). Standard dots (marked ''S'') may seem very faint in this photograph although they are positive.*

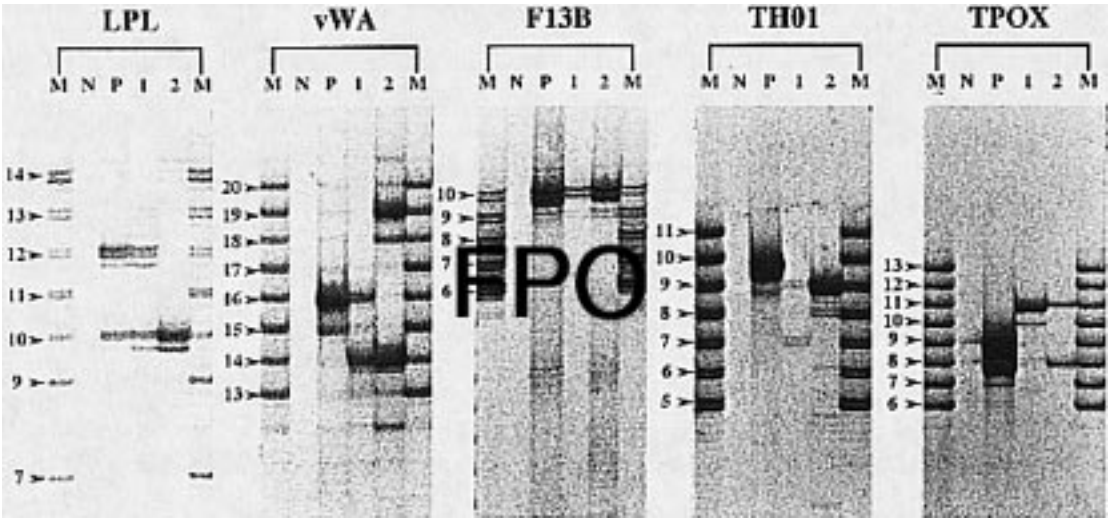

FIG. 4—*Genotyping results of five STR loci by silver staining from the infant compact bone and the alleged mother's saliva stain. 1: Infant compact bone. 2: Alleged mother's saliva stain. N: Negative control. P: Positive control (LPL: 12,10, vWA: 16,16, F13B: 10,10, TH01: 9.3,9.3, TPOX: 9,8). M: Allelic ladder marker. Two bands in the negative control lane at TPOX were contaminated by the PCR product next to the lane in loading.*

DQA1*0103-DRB1*1502-DPB1*0901. It is well known that some haplotypes of DQA1 and DRB1 are so linked that their independent haplotype frequencies cannot be used for calculating the combined probability (9). In this study, we therefore calculated the frequency of the appearance of these types of alleles in the Japanese population based on observed DRB1-DPB1 haplotype frequencies (9). For the alleged mother the frequency was 2.8×10^{-5}, and for the infant 6.3×10^{-3}.

Analysis using the AmpliType™ PM kit (Fig. 3) demonstrated the woman and the infant had identical genotypes at all five loci (LDLR: B, GYPA: B, HBGG: B, D7S8: A and GC: C). The frequency of the appearance of an individual who has the same genotype in the Japanese population was calculated as 1.2×10^{-3} from the allele frequencies (10).

With respect to five STR loci (Fig. 4), the infant bone was typed as 12,10 at LPL, 16,14 at vWA, 10,10 at F13B, 9,7 at TH01 and 11,11 at TPOX as shown in Table 1, and the alleged mother as 10,10 at LPL, 19,14 at vWA, 10,10 at F13B, 9,9 at TH01 and 11,8 at TPOX. According to the allele frequencies reported by Nagai et al. (11), the frequencies of the genotypes of the alleged mother and the infant were estimated as 2.1×10^{-5}, 3.3×10^{-5}, respectively.

Sequence analysis of each twelve clones from the bone and the

TABLE 1—*Results of DNA typing of the infant and the chance of exclusion that a Japanese individual is not the parent of the infant.*

Locus	Genotype		Exclusion Rate (%)
HLA			
DRB1-DPB1	*0901-*0201/*1502-*0901		78.1
PM test			79.7
LDLR	BB	3.5	
GYPA	BB	32.0	
HBGG	BB	8.4	
D7S8	AA	14.5	
GC	CC	60.4	
STRs			71.7
LPL	12,10	1.9	
vWA	16,14	39.2	
F13B	10,10	7.6	
TH01	9,7	11.0	
TPOX	11,11	42.4	
mtDNA			
D-loop (283 bp)	most common type*		85.0†

*The most common type in the Japanese population (15.0%) (13) has two transitions (C to T at 16223rd and T to C at 16362nd) from the Anderson's sequence (12).

†The value is only applied to alleged mothers.

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