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TECHNICAL NOTE

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Usefulness of Dura Mater in Providing DNA Samples for Identifying Cadavers

ABSTRACT: We examined the usefulness of the dura mater in identifying human remains. Dura mater was collected from 50 cadavers, including drowned, charred, and mummified remains. The STR genotype using the AmpFℓSTR® Identifiler™ Kit could be typed at 15 STR and amelogenin loci in 30 samples of 33 cases. Furthermore, the ABO genotype and amelogenin using gel-based methods could be typed in 44 samples of 50 cases. In cases with successful typing of STR, ABO-DNA, and amelogenin, the longest time after death was from 12 to 26 days in a drowned body. The minimum quantity of dura mater required for DNA extraction was about 2.5 mg, dried and fixed by ethanol, in a cadaver 15 h after death. The state of the DNA from the dura mater from the calvaria may be better than that from the basis cranii interna. We found that DNA from dura mater is one of the most useful samples for forensic identification.

KEYWORDS: forensic science, forensic identification, dura mater, AmpFℓSTR® Identifiler™ PCR Amplification Kit, ABO-DNA genotyping, amelogenin typing

Recently, disasters such as hurricane, earthquakes, jetliner crashes, or terrorism have occurred in various countries. It is difficult to identify cadavers that have been charred, drowned, decomposed, or dismembered. Personal identification of these cadavers, particularly the STR genotyping including the CODIS 13 core loci used in various forensic laboratories of the world, is needed (1,2). In recent years, STR genotyping has mainly been carried out using the AmpFℓSTR® Identifiler™ PCR Amplification Kit (3,4). The gender determination of cadavers is often important. Also, the ABO blood group has been regarded as one of the important markers in Japan. Four blood types of A, B, O, and AB are separated by the serological typing method (5), and the distribution of the ABO blood group is A:O:B:AB = 4:3:2:1 in the Japanese population (6). Most Japanese know the ABO blood group of their own. Therefore, the determination of the ABO blood group is thought to be useful in excluding a potential identification based on a differing ABO blood group in Japan.

Because dura mater is protected within the ossa cranii from the outside world, it can often be found intact, unlike the muscles, organs, or other tissues, in drowned, decomposed, or charred bodies, and it likewise may be intact in the heads of dismembered bodies. Therefore, we attempted DNA extraction from dura mater. No previous study examining DNA typing using dura mater have been conducted, although a considerable number of studies have been conducted on DNA typing using hairs, bones, muscles, organs, and other body parts (7–10).

The purpose of this study was to examine the usefulness of dura mater as a DNA sample for forensic identification. We attempted to extract DNA from dura mater and examined the quantity of dura needed for DNA extraction. Furthermore, we experimented with the typing of 15 STR including the CODIS 13 core loci and amelogenin gene using AmpFℓSTR® Identifiler™ PCR Amplification Kit, the DNA typing of the ABO blood group (11,12), and the sex determination by the amelogenin gene (13,14) using gel-based methods.

Materials and Methods

Preparation of Samples

Dura mater was removed from 50 autopsy cases at the Department of Legal Medicine, Graduate School of Medicine, Chiba University. In 12 of these cases, we collected dura mater adhering to the calvaria and basis cranii interna. The samples were stored in 99.5% ethanol before DNA extraction.

DNA Extraction From the Dura Mater

First, the dura mater was minced and dried at 37°C. The DNA was extracted by the phenol/chloroform method from about 50 mg of the dried sample (15,16).

STR Genotyping Using the AmpFℓSTR® Identifiler™ PCR Amplification Kit

To analyze STR typing, we arranged 33 samples as presented in Table 1. To amplify D8S1179, D21S11, D7S820, CSF1PO,

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TABLE 1—*Description of 50 cases.*

Purpose of Typing Number of Samples (Male/Female)	STR 33 (23/10)	ABO-DNA and Amelogenin 50 (37/13)
Cause of death		
Drowning	7 (5/2)	11 (9/2)
Burning	3 (2/1)	5 (4/1)
Exsanguination	4 (2/2)	5 (3/2)
Sickness	4 (3/1)	5 (4/1)
Cerebral injury	3 (1/2)	4 (1/3)
Mummy	1 (0/1)	1 (0/1)
Other	11 (10/1)	19 (16/3)
Age range	5 months in viviparity–93 years	
Time after death	15 h–6 months?	

STR, short tandem repeats.

D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA loci, and the amelogenin gene, we performed multiplex PCR using 2–20 ng target DNA with the AmpFℓSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems, Foster City, CA), according to the manufacturer's recommendation (17). The PCR amplification was carried out using a GeneAmp® PCR System 9700 Thermal Cycler (Applied Biosystems).

The amplified products were electrophoresed on an ABI PRISM® 3130XL Genetic Analyzer (Applied Biosystems). The analysis of the amplified products and allele designation were performed automatically using GeneMapper™ ID software (version 3.1, Applied Biosystems).

ABO Genotyping by PCR–RFLP

Fifty samples for ABO genotyping are presented in Table 1. The ABO genotype was typed by PCR–RFLP analysis based on Lee and Chang's method (11). We analyzed the two polymorphic nucleotide positions (nps) 261 and 703 of the cDNA from A transferase. The sequences of two pairs of primers have been reported (11). Amplification was accomplished in 20 μL of reaction mixture, which contained human genomic DNA 10 ng, 10 pmol of each primer, reaction buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 0.1% Triton X-100), 0.2 mM dNTP, and 1.0 U of Taq DNA polymerase (Ampli Taq Gold®: Applied Biosystems). The cycling conditions were as follows: 10 min at 95°C for a hot start, 30–35 cycles of 94°C for 2 min, 58°C for 2 min, and 72°C for 3 min, followed by 72°C for 7 min in a TAKARA thermal cycler. The amplified 199 or 200 bp products at np 261 were treated with the restriction enzymes *Kpn*I and *Bst*EII. The amplified 128 bp products at np 703 were treated with the restriction enzymes *Alu* and *Nci*I. We newly used *Bst*EII (12) and *Nci*I. *Bst*EII does not digest the O-allele-specific fragment at np 261, but it does digest A- and B-allele common fragments. *Nci*I always digests amplified products at np 703, and 71 and 57 bp fragments are generated. If a B-allele-specific sequence exists in a 57 bp fragment, *Nci*I does not digest the fragment. However, it digests A- and O-allele common fragments. The interpretation of the ABO genotype is shown in Table 2. The digestion patterns were separated on 12.5% polyacrylamide gels (e-PAGEL®: ATTO) by electrophoresis and ethidium bromide staining, and the bands were visualized by UV light.

The ABO phenotype was analyzed using the routine serological blood typing method, namely by forward and reverse grouping (5) in 39 cases. However, the blood was hemolytic in five samples, and it was detected by forward grouping. In six cases without blood, the ABO phenotype could not be detected, but we were able to detect the ABO genotype using bone's DNA.

TABLE 2—*ABO genotype interpretation by RE digestion of PCR products.*

Possible Genotype Predicted by	PCR Products at np 261		PCR Products at np 703	
	*Kpn*I	*Bst*EII	*Alu*I	*Nci*I (57 bp)
Complete digestion	OO	AA, AB, BB	BB	AA, AO, OO
Half digestion	AO, BO	AO, BO	AB, BO	AB, BO
No digestion	AA, AB, BB	OO	AA, AO, OO	BB

Amelogenin Typing Using Gel-Based Methods

Sex determination by the amelogenin gene was carried out in 50 samples, as shown in Table 1. Based on the method of Mannucci et al. (14), we used a single pair of primers spanning part of the first intron from the X–Y homologous gene amelogenin. The sequences of the primers have been reported (14). As 106 and 112 bp PCR products were generated from the X and Y homologs, respectively, the results were expected to be a single band (106 bp) in females and a double band (106/112 bp) in males. The PCR conditions were as follows: human genomic DNA 10 ng, 10 pmol of each primer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.1% Triton X-100, and 1.0 U of Taq DNA polymerase (Ampli Taq Gold®: Applied Biosystems) in a total volume of 20 μL. The cycling conditions were as follows: 10 min at 95°C for a hot start, 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, followed by 72°C for 7 min in a TAKARA thermal cycler. Then, these PCR products were loaded in 12.5% polyacrylamide gels (e-PAGEL®:ATTO, Japan) by electrophoresis and ethidium bromide staining with UV transillumination.

Results

DNA extraction from about 50 mg of dura mater was attempted in 50 cadavers. Furthermore, DNA extraction of nine different quantities of dura was attempted in the body of a person who had died from exsanguination, and who had been dead about 15 h. The final yields of the dura mater are shown in Table 3. Figure 1 indicates the quality of the DNA obtained from the dura. The DNA yield decreased in proportion to the decrease in the dura's weight. Thus, we found that the minimum quantity required for DNA extraction is approximately 2.5 mg from dura mater that had been dried and fixed by ethanol.

In 12 cases, we examined DNA extraction from the dura mater from different regions in the calvaria and basis cranii interna. The DNA yield from the dura obtained from the calvaria was better than that from the basis cranii interna in six cases (data not

TABLE 3—*DNA final yield of dura mater.*

Weight of Dura Mater (mg)	DNA Final Yield (μg)
100.0	101.42
50.0	11.50
20.0	3.70
10.0	3.37
5.0	1.75
2.5	1.00
1.0	0.36
0.5	0.20
0.1	0.12

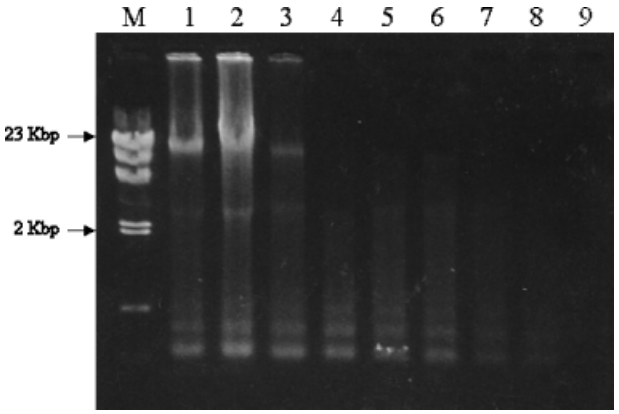

FIG. 1—*Quality of the DNA extracted from dura mater in a cadaver 15 h after death. Lanes 1–9 are from approximately 100, 50, 20, 10, 5, 2.5, 1.0, 0.5, and 0.1 mg of the dura mater. M, Lambda DNA/HindIII markers.*

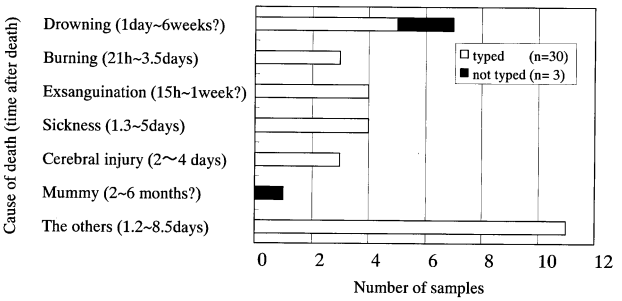

FIG. 2—*Results of STR genotyping using dura mater.*

shown). The final DNA yields were almost the same in dura mater adhering to the calvaria and basis cranii interna.

The result of STR typing and the causes of death in 33 cases are shown in Fig. 2. First, STR typing was performed using DNA samples from blood, muscle, and dura mater in the body of a person who had died from exsanguination, and who had been dead about 15 h. The same type of allele at 15 STR loci and amelogenin gene was detected in three kinds of DNA. The electropherogram using DNA from dura mater is shown in Fig. 3. In 30 samples, STR typing of all loci was successful; it was unsuccessful in three samples of two drowned bodies and one mummy. In a drowned body whose time after death was 4–11 days, STR typing from dura mater was unsuccessful, but that from bone was successful.

In three of the 30 samples, STR typing using the dura's DNA obtained from the calvaria and basis cranii interna was analyzed. In two cases, the result of STR typing using the dura's DNA from the calvaria coincided with that from the basis cranii interna. However, STR analysis using the dura's DNA from the calvaria

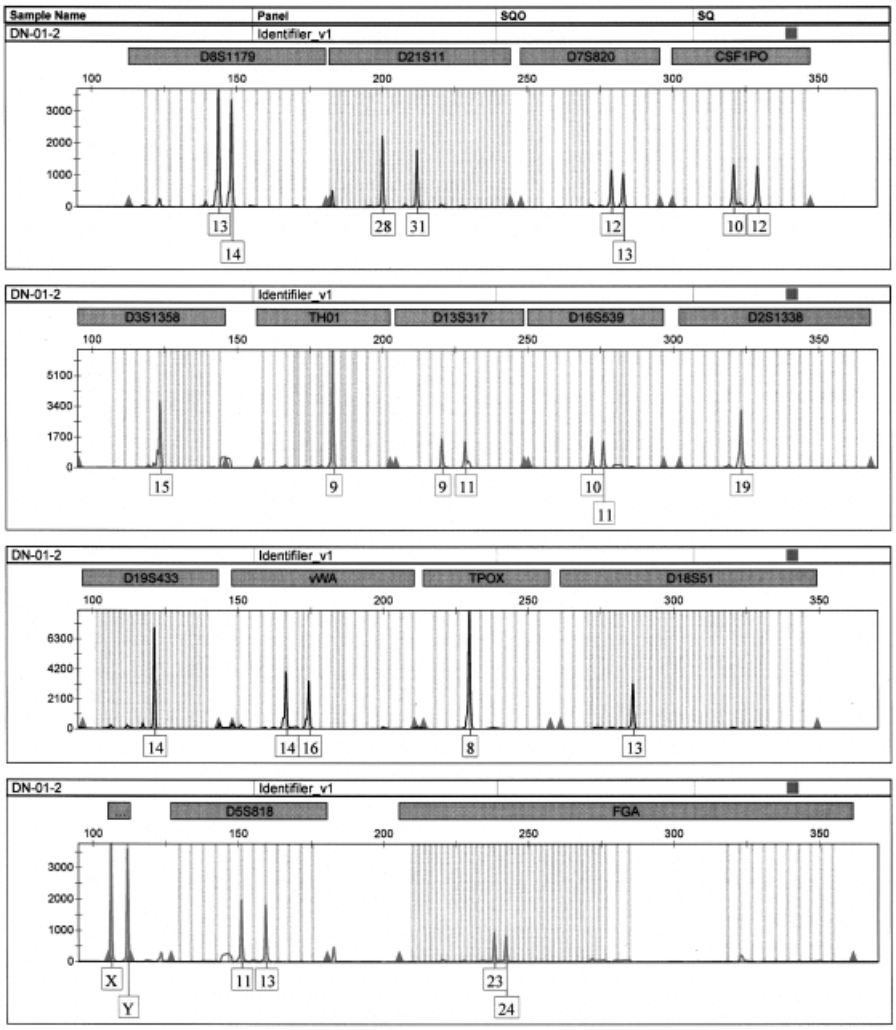

FIG. 3—*Electropherogram of the STR results obtained from the dura mater using the AmpFℓSTR® Identifiler™ PCR Amplification Kit.*

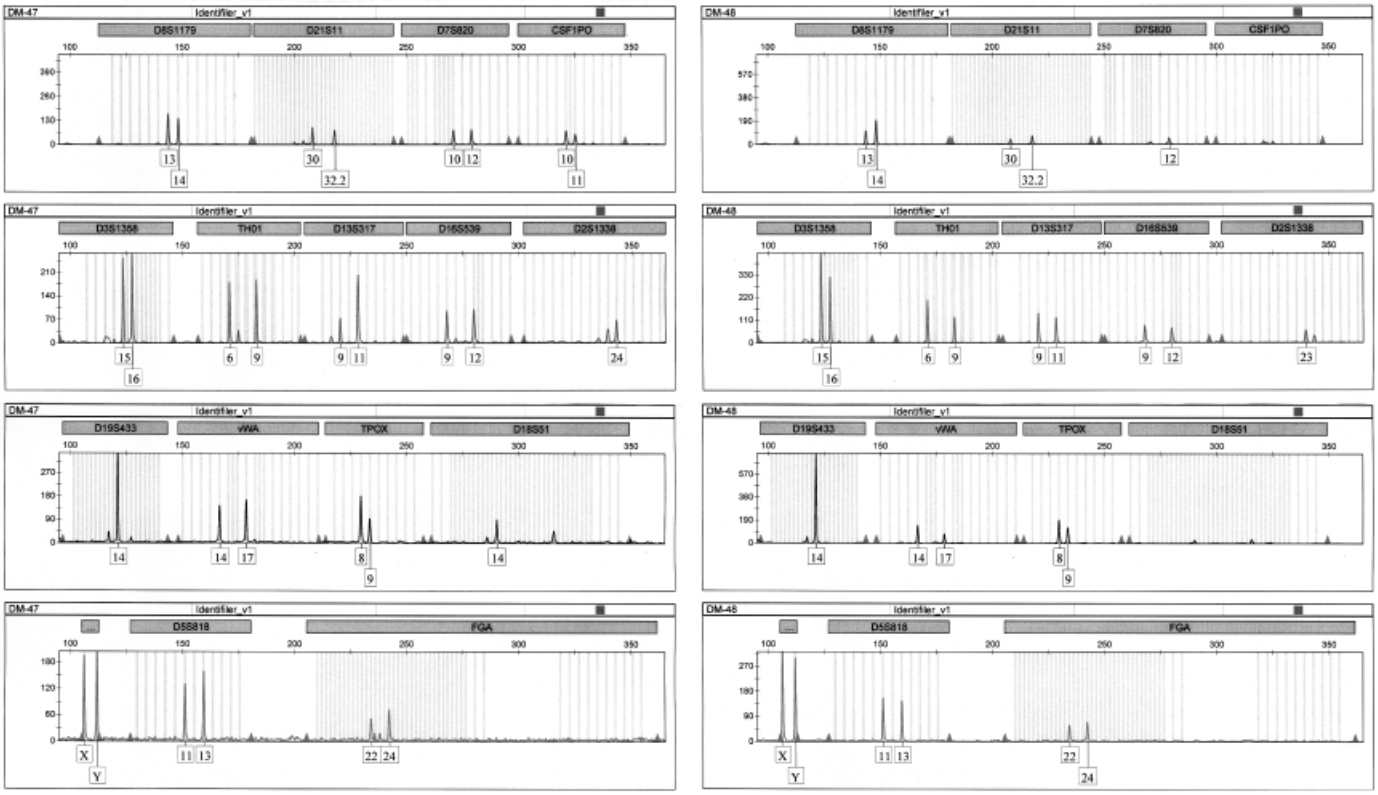

FIG. 4—*Electropherograms affected by regions of the dura mater in a drowned body 12–15 days after death. (a) Calvaria, (b) basis cranii interna.*

and that from the basis cranii interna coincided with 12 STR loci and the ameologenin gene, but not with three STR loci because of the weak signal in the drowned body of a person who had been dead about 12–15 days, as shown in Fig. 4.

In 44 cases, the ABO genotype using the dura's DNA coincided with the phenotype obtained by serological tests. However, the dura's DNA for ABO genotyping could not be amplified from four drowned bodies, a cadaver of cerebral injury, and one mummy. Figure 5 indicates the results of ABO genotyping and the causes of death. Although ABO genotyping using the dura's DNA was not possible in these cases, it was detected using the DNA from the bones in all six cases.

In 44 cases, the sex determination using the dura's DNA coincided with the external appearance, for example of the genital organs. However, as with ABO genotyping, PCR could not be conducted in six cases, as shown in Fig. 5.

The results of STR typing and that of ABO genotyping and amelogenin typing were compared in 33 cases. In 30 samples with successful STR typing, ABO genotyping, and amelogenin typing could be typed in all samples. The longest time after death was from 12 to 26 days in a drowned body. In three samples with unsuccessful STR typing, ABO genotyping, and amelogenin typing could not be accomplished.

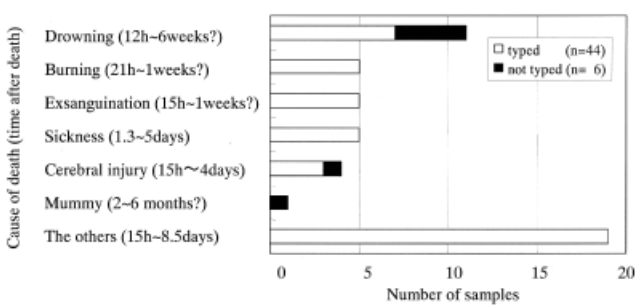

FIG. 5—*Results of ABO genotyping and amelogenin typing using dura mater.*

Discussion

In decomposed bodies, the dura mater is apt to remain intact. The decomposition process of dura mater may be slow, as it is with nerves and blood vessels like the aorta. The dura covering the brain consists of dense connective tissue containing fibroblasts (18). The outer layer of the dura is rich in cells and contains many blood vessels. However, the inner layer contains thin fibers. For the reasons mentioned above, DNA can be extracted from the dura mater.

Dura mater remains in the heads of dismembered bodies that are found at the scenes of natural disasters, terrorist attacks, bombing, jetliner crashes, and the like. Furthermore, dura mater frequently remains in charred bodies in which the truncus has been highly burned and the head slightly burned. In serious traffic accidents, it is not always possible to obtain blood from the hearts and aorta of persons who have died. Because the dura is protected in the ossa cranii, it is usually not affected by the outside environment.

There are, however, limitations to extracting DNA using the dura mater. For example, the procedure cannot be used in skeletons, in charred bodies whose skulls are highly burned, in the bodies of persons who drowned more than 2 weeks before testing, or in other situations where the body is too badly decomposed or damaged.

Although the DNA yields from the calvaria and the basis cranii interna were almost the same in 12 cases, the dura's DNA from the basis cranii interna was fragmented compared with the dura's DNA from calvaria in the case of STR typing in a drowned body 12–15 days after death. This result suggests that the PCR products of long size at some loci are not detected because of DNA fragmentation by putrefaction. Therefore, the sampling of dura mater may be more desirable from the calvaria than that from the basis cranii interna.

When the putrefaction is excessive, DNA must be extracted from hard tissues such as hairs, teeth, and bones. However, DNA extraction from hard tissues is complicated and requires a great deal of time because of delipidation and decalcification, compared with dura mater and other tissues. Therefore, dura mater may be helpful when it remains in the dead body.

This study provides evidence that dura mater can be one of the most useful DNA samples for forensic identification. Therefore, we suggest that DNA extraction be attempted from dura mater, before attempting it from the hard tissues, if dura mater is found to still adhere to either the calvaria or basis cranii interna. If possible, the dura mater from the calvaria should be obtained.

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