DNA Extraction and STR Typing of Compact Bones from Decomposed Human Skeletal Remains by Using Decalcification Treatment

Tsun-Ying Huang (黃純英)[1]*, Jui-Ming Li (黎瑞明)[1], I-Fang Lee (李宜芳)[1], Chia-Tung Shun (孫家棟)[2]

[1] Institute of Forensic Medicine, Ministry of Justice, and [2] Department of Forensic Medicine, National Taiwan University School of Medicine, Taipei, Taiwan (ROC)

Abstract

We performed DNA extraction and STR typing of decomposed human skeletal remains by using both undecalcified and decalcified methods . The postmortem periods of the studied remains ranged from two weeks to eighteen years. In some cases, they were buried with exhumation. Decalcification using 0.5 M EDTA-3K at 56℃ overnight and repurification were used prior to the digestion and extraction to overcome inhibition of amplification process. DNA was isolated using standard phenol/chloroform/isoamyl alcohol extraction method. This study detected human DNA in 15 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA) from skeletal remains using the kit purchased from Applied Biosystems. Standard phenol/chloroform/isoamyl alcohol extraction followed by decalcification method has been proved as the most successful method. Decalcification with 0.5 M EDTA-3K was shown to improve the success of DNA typing in this study. A duo case, the Combined Paternity Index (CPI) value of the 15 STR loci from decalcified bone sample was higher than undecalcified bone sample in the paternity testing (99.677 % / 99.997 %). Our study demonstrated that DNA extracted from highly decomposed bony tissues of human remains up to eighteen years old by using decalcification treatment was successfully amplified and greatly increased our ability to positively identify previously unknown skeletal remains by a comparative genetic analysis with presumptive relatives.

Key Words: decalcification, human skeletal remains, EDTA-3K , combined paternity index

Introduction

Multiplex PCR-based STR analyses are suitable in human identification and forensic casework dealing with different tissues, even when the sample is heavily decomposed. The extraction of DNA from forensic skeletal remains can provide quite powerful data for analysis, but is plagued by a unique set of methodological problems. Bone is the most resistant tissue in deceased bodies to time depending degradation and putrefaction, but it is often hard to extract DNA from it due to its highly mineralized structure, which makes DNA extraction and/or purification hard to carry out. Standard phenol/chloroform/isoamyl alcohol extraction followed by decalcification method has been proved as the most successful method. Chelating reagents can be used to calcium isolation. EDTA is popular for decalcification treatment because it is very stable. The solubility of EDTA-3K is soluble in water easily. The goals of this study are to investigate the effects of decalcification treatment using 0.5 M EDTA-3K on DNA typing of compact bones from decomposed human skeletal remains. Nuclear DNA typing was performed both with decalcified and undecalcified bone powder samples. In this report we present a comparison of two extracting DNA methods by using decalcified and undecalcified treatments from highly decomposed human skeletal remains.

Materials and Methods

Sample collection: Decomposed human skeletal remains were collected from the Institute of Forensic Medicine, Ministry of Justice, Taiwan. The postmortem periods of the studied remains ranged from two weeks to eighteen years.

Bone tissues pre-treatment and DNA extraction: Compact bones were chosen as the source of DNA extraction. Two sets same amount in weight of bone powder were prepared for all cases. Decalcification was performed using 0.5 M EDTA-3K which was incubated at room temperature, then shaken overnight and changed the EDTA solution twice in the period of time. After spinning down, all supernatant was carefully removed and washed three times with deionized water. The decalcified and undecalcified samples were separately digested with 1ml digestion buffer (TNE buffer containing 2 % SDS and 10 mg/ml proteinase K) at 56 ℃ for 4 h or overnight. DNA Extraction was performed by standard phenol/chloroform/isoamyl alcohol extraction method.

DNA typing: Fifteen STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA) were typed using the kit purchased from Applied Biosystems. After amplification, 1.5 μl samples were electrophoresed using an ABI PRISM 310 genetic analyzer. The data was analyzed by ABI GeneMapper *ID* Software ver. 3.2. according to the manufacturer's recommendations. The peak detection thresholds were set at 50 RFU (relative fluorescence units).

CPI and probability of paternity: The CPI and probability of paternity were calculated from the formulas described by Katsumata Y. et. al. Allele frequencies for 15 STR loci used in the calculations were obtained from unrelated individuals living in Taiwan. All loci are in Hardy-Weinberg equilibrium.

Table 1 DNA typing results for 15 STR loci of skeletal remains.

Cases #		1		2		3		4	
Postmortem periods (years)		1		2		12		18	
Decalcification with EDTA-3K		+	−	+	−	+	−	+	−
15 STR Loci	D8S1179	+	+	+	+	+	+	+	−
	D21S11	+	−	+	−	−	−	+	−
	D7S820	−	−	+	−	−	−	−	−
	CSF1PO	+	−	+	−	−	−	+	−
	D3S1358	+	−	+	−	+	−	+	−
	THO1	+	−	+	−	+	−	+	−
	D13S317	+	−	−	−	−	−	−	−
	D16S539	+	−	−	−	+	−	+	−
	D2S1388	+	−	−	−	−	−	+	−
	D19S433	+	−	+	−	+	−	+	−
	vWA	+	−	+	−	+	−	+	−
	TPOX	+	−	+	−	+	−	+	−
	D18S51	+	−	+	−	−	−	−	−
	D5S818	+	−	+	−	+	−	−	−
	FGA	−	−	−	−	−	−	−	−

+: typeable ; —: nontypeable

Results

® Table 1 displays the electrophoretic results after the decalcified and undecalcified extraction procedures. "+" and "-" denote a typeable vs. a nontypeable result for the STR in the corresponding column. As depicted in Table 1, the decalcified method improve recovery of nuclear DNA from skeletal remains which postmortem periods ranged from one to eighteen years.

® Table 2 shows the STR profiles and CPI values of the victim and victim's children. The CPI values of the 15 STR loci from decalcified bone sample were higher than undecalcified bone sample in the paternity testing.

Table 2 STR profiles and CPI values of the victim and victim's children.

Specimens / STR Loci	Victim Undecalcified bone powder		Victim Decalcified bone powder		Victim's Child 1 Buccal swab		Victim's Child 2 Buccal swab	
STR D8S1179	14	16	14	16	15	16	15	16
STR D21S11	32.2	—	32.2	—	31	32.2	32.2	33.2
STR D7S820	—	—	9	11	11	12	11	11
STR CSF1PO	—	—	10	—	10	11	10	11
STR D3S1358	15	16	15	16	15	16	16	17
STR TH01	9	9.3	9	9.3	7	9.3	7	9.3
STR D13S317	—	—	10	11	10	11	11	12
STR D16S539	—	—	12	12	12	12	9	12
STR D2S1338	—	—	18	—	18	20	18	20
STR D19S433	—	14.2	14	14.2	14.2	15	13	14
STR VWA	—	16	15	16	16	16	15	16
STR TPOX	8	11	8	11	8	11	11	11
STR D18S51	—	—	14	—	15	16	15	16
STR Sex	X	Y	X	Y	X	Y	X	Y
STR D5S818	—	13	11	13	10	11	10	11
STR FGA	—	—	—	26	22	23	22	26
CPI value	789 (undecalcified) 14,974 (decalcified)							
CPI value	309(undecalcified) 35,000(decalcified)							

*—": not detectable

Discussion

® Decalcification with 0.5 M EDTA-3K was shown to improve the success of DNA typing in four cases (Table 1).

® In order to significantly enhance the CPI value, decalcification treatment was performed in a duo case(Table 2). The postmortem period of victim was 12 years. The CPI values of the 15 STR loci from decalcified bone sample (14,974 and 35,000) were higher than undecalcified bone sample(789 and 309) in the paternity testing. The CPI values (14,974 and 35,000) and paternity probability (0.9993 % and 0.9997 %) suggested that the victim (suspicious father) is the biological father of the children.

® Our results showed that decalcification with 0.5 M EDTA-3K increase the molecular information recovered from decomposed skeletal remains and improve the discriminatory power and CPI values of the genetic data.

Conclusions

® Decalcification treatment with 0.5 M EDTA-3K has improved genomic DNA recovery and results in higher detatability than undecalcification process during DNA typing.

® Our study demonstrated that DNA extracted from highly decomposed bony tissues of human remains up to eighteen years old by using decalcification treatment was successfully amplified and greatly increased our ability to positively identify previously unknown skeletal remains by a comparative genetic analysis with presumptive relatives.

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