의학박사 학위논문

조선시대 무덤에서 발굴된 사람 시료를 대상으로 한 고유전자 연구

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2017년 8월

서울대학교 일반대학원
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A thesis of the Degree of Doctor of Philosophy

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지도교수 신 동 훈

이 논문을 의학박사 학위논문으로 제출함

2017년 4월

서울대학교 일반대학원
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오창석의 의학박사 학위논문을 인준함

2017년 7월

위 원 장 (인)

부 위 원 장 (인)

위 원 (인)

위 원 (인)

위 원 (인)

The ancient DNA studies on the human remains excavated from the tombs of Joseon Dynasty

by
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A thesis submitted to the Department of Anatomy in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Medicine at Seoul National University College of Medicine

July, 2017

Approved by Thesis committee:

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ABSTRACT

Introduction: The purpose of ancient DNA (aDNA) study is to provide scientific clues to the solution of various problems that have not been resolved through analysis of DNA extracted from living organisms. However, since the soil of Korea is constituted such that bodies are likely to decay rapidly, aDNA extracted from bodies long-buried in tombs is usually considered to be in a poor preservation state; thus, it is likely that failure will be the result if the aDNA is used for genetic analysis. In this study, therefore, I tried to establish a method of aDNA analysis on human remains buried during the Joseon Dynasty. Also, I applied the established method to archeological samples for confirmation of whether or not aDNA analyses could be useful in the field of archaeological science.

Methods: The samples used for this study were ancient skeletal remains, teeth or mummified brains found in lime-soil mixture barrier (LSMB) tombs constructed during the Joseon Dynasty in Korea. All of the samples were subjected to sex determination using archaeological, anatomical, or genetic methods. aDNA from the archaeological human remain samples was isolated using a phenol extraction method and quantified by spectrophotometry or

the real-time polymerase chain reaction (real-time PCR) method. After PCR, cloning and sequence analyses of mitochondrial DNA (mtDNA) hypervariable region (HVR) or short tandem repeat (STR) analyses of nuclear DNA were performed for determination of the preservation state of the aDNA, sex determination, human identification, and contamination control.

Results: The preservation state of the aDNA was analyzed with the AmpFlSTR® Identifiler® PCR Amplification Kit (Identifiler kit) and AmpFlSTR® MiniFiler™ PCR Amplification Kit (Minifiler kit) using DNA extracted from femur samples from eight sets of Joseon skeletal remains. In the analysis using the Identifiler kit, the extracted aDNA showed such a poor preservation state that the subsequent STR analysis failed in most of the loci. However, in the STR analysis with the Minifiler kit, which was designed to reduce the amplicon size on the eight largest loci in the Identifiler kit, the success rate of the locus analysis was more than twice as high.

Also, I investigated whether morphologically well-preserved mummified brain tissue is more valuable as a sample for aDNA analysis than the femur samples that are commonly used as compact bone in aDNA analysis. The Minifiler kit analysis confirmed that the success rate of STR locus analysis using mummified brain samples was higher than that using femur samples. In addition, I

found that, in the mtDNA analysis, the aDNA extracted from the mummified brain tissue could allow for larger-sized PCR amplicons than could the femur. Given these results, mummified brain tissue could indeed be a valuable material for aDNA analysis.

Next, I examined whether aDNA analysis could be helpful in sex determination of controversial archaeological samples. Using archaeological or anatomical methods for a total of 34 individuals buried during the Joseon Dynasty, the sex of five individuals could not be determined anatomically, while another two individuals showed discordant results between the archaeological and anatomical methods. In the results of amelogenin gene analysis on the samples, four of the five individuals for whom the sex could not be determined were confirmed, as three women and one man. Meanwhile, the sexes of the two individuals for whom the results by the two methods were discordant also were confirmed (i.e., the anatomical sex determination had been correct).

Finally, I conducted an investigation to determine whether teeth found in a pocket were those of the individual found in that coffin or another person's. The aDNA techniques used in my thesis were applied to solve this problem. The Minifiler kit was used for analyzing eight STR loci and sex determination based on mummified brain and femur samples obtained from the mummified human

remain. Then, those STR analysis results were compared with the results for the teeth. In fact, the alleles analyzed in the mummified brain, femur, and tooth samples were quite similar to each other. Additionally, the results of a sequence analysis of mtDNA HVR1 confirmed that all of the nucleotide sequences analyzed from the mummified brain, femur and teeth were 100% identical to each other. Considering these genetic analysis results, it is almost certain that the owner of the teeth found in the pocket was most likely to be the mummified human remain buried in that same coffin.

Conclusions: Through this study, I was able to investigate the preservation state of aDNA extracted from archaeological human remains buried in LSMB tombs constructed during the Joseon Dynasty and to establish a research method for analysis of aDNA. And, I confirmed that morphologically well-preserved mummified brain tissue can be a useful material for aDNA analysis. Next, I showed that sex determination and individual identification on archaeological human remains could be successfully accomplished using aDNA analysis methods. Considering that aDNA analysis is still relatively novel in Korea, I hope that my thesis will be helpful to archaeological scientists who are interested in aDNA studies.

Keyword : ancient DNA, skeletal remain, mummy, Joseon Dynasty,

short tandem repeat, sex determination

Student Number : 2008-30570

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LIST OF ABBREVIATIONS

aDNA : ancient DNA

AMEL : amelogenin

BLAST : basic local alignment search tool

CT : Computed tomography

DNA : dioxyribonucleotide acid.

DVI : disaster victim identification

HVR : hypervariable region

Identifiler kit : AmpflSTR® Identifiler PCR Amplification Kit

LR : likelihood ratio

LSMB : lime-soil mixture barrier

Minifiler kit : AmpflSTR® MiniFiler™ PCR Amplification Kit

mtDNA : mitochondrial DNA

PCR : polymerase chain reaction

rCRS : revised cambridge reference sequence

RFU : relative fluorescence unit

STR : short tandem repeat

TEM : transmission electron microscopy

UV : ultraviolet

w/v : weight/volume

Introduction

The purpose of ancient DNA (aDNA) study is to provide, through analysis of DNA extracted from ancient organisms, scientific clues to the solution of various problems that had proved unsolvable. With the development of molecular biological research techniques, studies on aDNA began to be reported by the pioneers in the field (Higuchi et al., 1984; Pääbo 1985, 1989). From the time of the introduction of the initial relevant research, aDNA has attracted a great deal of interest from researchers and the public. However, when looking back at the history of aDNA studies, it has not at all been a smooth process to the achievement of its current, respectable status in the academic community but rather, a crisis- and controversy-filled one.

Higuchi et al. (1984) and Pääbo (1985, 1989) showed for the first time that it was possible to analyze aDNA extracted from archaeological human or animal remains using modern biological techniques. Actually, in most cases, fragmentation of aDNA had been very serious, with the result that it was very difficult to obtain genetic information from ancient samples. aDNA analysis was made possible, finally, by the development of polymerase chain reaction

(PCR) techniques. Using these techniques, many studies on the aDNA analysis of archaeological samples were successfully completed and reported (Spigelman and Lemma, 1993; Rafi et al., 1994; Salo et al., 1994).

However, as the study results and knowledge of aDNA accumulated, the authenticity of some of the earlier studies has come under suspicion. In fact, it was pointed out that PCR technique can amplify not only aDNA fragments but also any modern DNA originating from contamination sources (Willerslev and Cooper, 2005). Academic disputes concerning the authenticity of aDNA analysis have been among the most central and hottest issues within the field of archaeological science over the past several years (Roberts and Ingham, 2008).

As a consequence, researchers have come to agree that there is a potential for modern-DNA contamination in aDNA data and, thus, that clear scientific criteria need to be formulated to enable researchers to resolve the related disputes. Indeed, many provisions have been made to minimize the likelihood of sample contamination and, thus, to ensure the authenticity of aDNA data obtained from archaeological samples. Nowadays, aDNA researchers agree on a set of aDNA study criteria by which their data can be confirmed as authentic (Willerslev and Cooper, 2005).

DNA-based investigation of ancient human samples (aDNA analysis) has been attracting increasing attention from the academic community (e.g. Marota and Rollo, 2002; Cipollaro et al., 2005). Over the past several decades, aDNA analysis has expanded the related fields of archaeological science considerably (Marota and Rollo, 2002; Cipollaro et al., 2005). For example, DNA profiles derived from archaeological human samples have proved integral to researchers seeking to reconstruct the lives of historical peoples (de Pancorbo et al., 1995; Baca et al., 2012).

aDNA analysis also has been instrumental in solving the great mystery of the fate of the Romanov family. Some skeptics had believed that a member of the family might have escaped execution and found sanctuary in another European country. However, DNA analysis proved that none of the Tsar's family survived after 1918, thus closing the hitherto lengthy debate on the tragedy of the Russian royal family (Gill et al., 1994; Coble et al., 2009).

Di Nunno et al. (2007) identified the discrepancy in STR genotype frequencies between medieval and present-day populations living in the same geographical region of Italy. This finding supported the hypothesis that there might have been strong Germanic and Asian (Goths, Lombards, Avars) gene flows into the region during the Middle Ages. Likewise, various ancient DNA

studies also have been performed in order to reconstruct the family trees of historically important people or rule out possible contamination of ancient samples by modern DNA (Gill et al., 1994; de Pancorbo et al., 1995; Hummel et al., 1999; Clisson et al., 2002; Ricaut et al., 2005a,b; Vanek et al., 2009; Baca et al., 2012).

Recently in Korea, cases of archaeological excavation have grown rapidly in number due to the many cases of rescue archaeology necessitated by the construction boom. With the discovery of ancient human or animal bones at such sites, the need for archaeologists to undertake aDNA analysis has correspondingly, and remarkably, increased. In fact, there have been several recent cases of aDNA analysis utilizing mtDNA or autosomal DNA obtained from archaeological samples in Korea.

Briefly, Lee et al. (2008) performed mtDNA analyses on 35 skeletal samples from museums in Korea, on which basis they determined that the early Korean population shared a common origin with the peoples living in the northern regions of the Altai Mountains and Lake Baikal of southeastern Siberia. Kim et al. (2010), having performed mtDNA analysis on ancient bodies excavated on Neukdo Island, established that their haplogroups were M and D. Later, Kim et al. (2011)'s SNP analysis revealed the mtDNA haplogroups and blood types of mummies dating back 450–

500 years.

A number of paleoanthropological and bioanthropological studies have already been published in Korea. However, aDNA techniques suitable for analysis of samples obtained from domestic archaeological sites have not yet been established. Korean soil tends to be weakly acidic with a low base content, in which environment, the high decomposition activity of microorganisms quickly degrades organic material (Kim et al., 2004, Jee et al., 2008). And because DNA preservation is negatively correlated with tissue decomposition (Michaud and Foran, 2011), the possibility of aDNA damage in human remains excavated at Korean archaeological sites would be considered to be very high.

In the present study, in order to determine the applicability of aDNA analysis techniques to archaeological samples in Korea, I investigated the preservation state of aDNA using skeletal remains and mummified samples obtained from bodies buried during the Joseon Dynasty. To that end, I tried to find the condition for successful analysis in ancient DNA study with nuclear and mitochondrial DNA. Then, I confirmed the usefulness of the established aDNA analysis techniques by employing them in the archaeological field for the purposes of sex determination or identification of ancient human remains found at Korean

archaeological sites.

CHAPTER 1

A study on the preservation state of aDNA extracted from skeletal remains buried during Joseon Dynasty

Introduction

Autosomal short tandem repeat (STR) genotyping enables anthropologists to perform a variety of genetic analyses (e.g. human identification or paternity testing) on ancient human specimens discovered at archaeological sites. Most of these analyses have been performed with conventional multiplex STR genotyping kits such as AmpFlSTR Profiler Plus, AmpFlSTR identifiler, or Powerplex ES (Hummel et al., 1999; von Wurmb-Schwark et al., 2003, 2004; Iwamura et al., 2004; Ricaut et al., 2004, 2005a, 2005b, 2006; Amory et al., 2007; Di Nunno et al., 2007). As these conventional STR kits enable analysis of a large number of autosomal STR loci in one reaction, effective analysis is possible even with a small amount of sample (Collins et al., 2004).

However, obtaining a complete autosomal STR profile on archaeological human remains using conventional autosomal STR kits is actually difficult, because aDNA remaining in ancient samples often becomes fragmented or denatured by various chemical reactions (Pääbo, 1989; Hofreiter et al., 2001; Dabney et al., 2013). Therefore, conventional STR kit analysis on aDNA is expected to have a low success rate, as would be the case with a typical degraded forensic sample.

In this regard, the miniSTR kit (e.g. AmpflSTR® MiniFiler™ PCR Amplification Kit) could be very useful to aDNA analysis. The kit is constructed using a primer designed to reduce the size of the amplification products for the 8 largest loci in the Identifiler kit (D7S820, D13S317, D16S539, D21S11, D2S1338, D18S51, CSF1PO and FGA). Also, the miniSTR kit is known to have a higher success rate of locus analysis than the conventional STR kit (Wiegand et al., 2001; Tsukada et al., 2002; Butler et al., 2003; Drábek et al., 2004; Coble and Butler, 2005; Grubwieser et al., 2006; Hill et al., 2007; Alenizi et al., 2009).

However, endogenous DNA from, for example, ancient archaeological samples is far more degraded than that from forensic samples. It is also uncertain whether the miniSTR kit could be used complementarily to conventional STR kit analysis of aDNA samples. Therefore, in this study, I investigated the preservation state of aDNA extracted from the skeletal remains of individuals buried during the Joseon Dynasty, using the Identifiler kit as a conventional STR kit. Next, miniSTR kit analysis was applied to confirm whether the analysis results from the Identifiler kit could be improved when the same 8 largest loci were analyzed with a smaller amplification product, using the Minifiler kit as the miniSTR kit.

Materials and methods

A total of eight human femurs collected from 16th to 18th century Korean tombs were used in this study. The surfaces of the bones were removed using a sterilized knife, after which they were exposed to UV irradiation for 20 minutes, and subsequently immersed in 5.4 % (w/v) sodium hypochlorite. After the samples were washed with distilled water and absolute ethanol, they were air-dried and pulverized to a fine powder using a SPEX 6750 Freezer/Mill (SPEX SamplePrep, Metuchen, NJ, USA) (O'Rourke et al., 2000; Rohland and Hofreiter, 2007).

The bone powder (0.3-0.5g) obtained from the femurs was incubated in 1 ml of lysis buffer (EDTA 50 mM, pH 8.0; 1mg/ml of proteinase K; SDS 1%; 0.1M DTT) at 56℃ for 24 hr. Total DNA was extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1), and then treated with chloroform/isoamyl alcohol (24:1). DNA isolation, purification and elusion were performed using a QIAmp PCR purification kit (QIAGEN, Hilden, Germany). (Yang et al., 1998; Casas et al., 2006; Calvignac et al., 2008; Blow et al., 2008).

The DNA extracted from the femur samples was amplified with the Quantifiler^{TM} kit (Applied Biosystems, Foster City, CA, USA),

showing the total amount of amplifiable human DNA remaining in each sample. All of the procedures were carried out in accordance with the manufacturer's instructions. The Quantifiler data were analyzed by 7000 system SDS software version 1.2.3 (Applied Biosystems, Foster City, CA, USA).

Autosomal STR analyses were repeated ten times for each sample with AmpFlSTR MiniFiler and Identifiler kits (i.e. five times for each) (Applied Biosystems, Foster City, CA, USA). Briefly, after 10 μl of sample DNA was amplified by MiniFiler or Identifiler kit following manufacturer's instructions, amplified products were analyzed on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems). When allelic profiles were reproducible in at least three of the five replicates, they were regarded authentic consensus profiles (Budowle et al., 2009). Peak height thresholds for the MiniFiler and Identifiler kits were set at 50 and 100 relative fluorescence units (RFUs), respectively.

In the course of sampling and lab works, protection gloves, masks, gowns and head caps were always used. aDNA lab facilities were set up in accordance with the protocol of Hofreiter et al. (2001): the rooms for aDNA extraction or PCR preparation were physically separated from main PCR lab; further, the DNA extraction/PCR preparation rooms were equipped with night UV irradiation, isolated

ventilation, and laminated flow hoods. Other criteria for authentic aDNA analysis, suggested by Willerslev and Cooper (2005), were also followed. To determine whether the specimen caused modern DNA contamination, we compared the autosomal STR profile of the researchers involved in this study with those of the ancient samples (under the permission of Institutional Review Board of Seoul National University, H-0909-049-295).

Results

In the Identifiler kit analysis (total locus number = 15), consensus profiles were obtained in 43 out of 120 autosomal STR loci (35.8 %). Among them, 51.8 % (29 out of 56) were 7 smaller sized loci (D8S1179, D3S1358, TH01, D19S433, vWA, TPOX and D5S818). Meanwhile, 21.9 % (14 out of 64) were 8 larger sized loci (D7S820, D13S317, D16S539, D21S11, D2S1338, D18S51, CSF1PO and FGA). The range of the number of loci in which consensus profiles were acquired by Identifiler kit analysis was 0 – 12 per a sample (average = 5.5 ± 5.3). The range of the number of larger sized loci observing consensus profiles was 0 – 5 per a sample (average = 1.8 ± 2.2).

In MiniFiler kit analysis (total locus number = 8), consensus profile were determined as 75 % (48 out of 64). And the range of the number of locus in which consensus profiles increased to 2 – 8 per sample (average = 6 ± 2) as well.

Significant improvement in the number of loci profiles was not found when the analysis of only MiniFiler kit (48/49 = 97.96%) was compared with combined MiniFiler and Identifiler kit analyses (49/49 = 100%). However, the number of 15-Identiflier locus profiles rose as high as 65.83 % (79/120 loci) in combination with

MiniFiler and Identifiler kit, a remarkable improvement compared with the result obtained with the Identifiler kit alone (36.7 %, 44/120 loci).

When comparing the results of the MiniFiler and Identifiler kit analyses, the full concordance of the profile was observed in 12.24% (6 out of 49) of the STR loci. The number of different profile was 43, accounting for the STR loci in the MiniFiler kit, including D7S820 (n=4), D21S11 (n=4), D18S51 (n=5), and CSF1PO (n=8), D13S317 (n=7), D16S539 (n=4), D2S1338 (n=7), FGA (n=4) (Table 2). In the Identifiler and MiniFiler kit analyses, some larger sized loci showed potential drop-outs (6 in Identifiler; and 10 in MiniFiler). This might be due to the low copy number of starting DNA templates in PCR (Table 1).

None of the STR profiles of the samples was matched those of the researchers who participated in this study. It confirms the authenticity of the results, reassuring that the data was endogenic, but not the outcome of environmental contamination.

Table 1. Autosomal STR analyses repeated 10 times (i.e. 5 for MiniFiler; 5 for Identifiler) for each individual sample. MF, MiniFiler; ID, Identifiler; Cons, Consensus profile obtained from either MiniFiler or Identifiler kit analyses (allele profiles repeated 3 out of 5 repetitions were considered authentic); MF+ID, Consensus profile obtained from combined use of MiniFiler and Identifiler kit analyses. Shaded heterozygous loci showed the drop-outs in small sized alleles, possibly by processing a small number of starting DNA templates during PCR.

SN 1-32																	
Kit	Exp.	D8 S1179	D21 S11	D7 S820	CSF1PO	D3 S1358	TH 01	D13 S317	D16 S539	D2 S1338	D19 S433	vWA	TPOX	D18 S51	D5 S818	FGA	Quantifiler (pg/ul)
MF	1		28,–	–	11,–			12,–	11,–	25,–				–		–	3.39
	2		28,30.2	–	11,–			12,–	11,12	17,25				23,–		24,–	3.39
	3		28,30.2	10,11	11,12			12,–	–	17,25				13,23		19,24	10.4
	4		28,30.2	10,11	11,12			12,–	–	17,25				13,23		19,24	10.4
	5		28,30.2	10,11	11,12			12,–	–	17,–				13,23		19,24	10.4
	Cons		28,30.2	10,11	11,12			12,–	–	17,25				13,23		19,24	
ID	1	–	28,–	–	–	–	–	–	–	–	–	17,–	8,–	–	–	–	3.39
	2	–	28,–	–	–	–	–	–	–	–	–	17,–	8,–	–	–	–	3.39
	3	11,15	28,30.2	10,11	11,–	16,17	9,–	–	11,12	25,–	13,15	17,18	8,–	13,23	12,13	19,24	10.4
	4	11,15	28,30.2	10,11	11,12	16,17	9,–	12,–	11,12	17,25	13,15	17,–	8,–	13,–	12,13	–	10.4
	5	11,–	28,30.2	10,–	11,12	16,17	9,–	12,–	11,12	17,–	13,15	17,18	8,–	13,23	12,–	–	10.4
	Cons	11,–	28,30.2	10,–	11,–	16,17	9,–	–	11,12	–	13,15	17,–	8,–	13,–	12,–	–	
MF+ID		11,–	28,30.2	10,11	11,12	16,17	9,–	12,–	11,12	17,25	13,15	17,–	8,–	13,23	12,–	19,24	

SN 4-18-1																	
Kit	Exp.	D8 S1179	D21 S11	D7 S820	CSF1PO	D3 S1358	TH 01	D13 S317	D16 S539	D2 S1338	D19 S433	vWA	TPOX	D18 S51	D5 S818	FGA	Quantifiler (pg/ul)
MF	1		31,–	11,–	9,11			10,–	–	–				–		–	20.2
	2		31,–	11,–	9,11			10,13	10,11	20,24				15,16		24,28	20.2
	3		31,–	11,–	9,11			10,13	–	24,–				15,16		–	20.2
	4		31,–	–	9,11			10,13	–	20,24				16,–		24,28	23.1
	5		31,–	11,–	11,–			10,13	10,–	20,24				15,16		24,28	23.1
	Cons		31,–	11,–	9,11			10,13	–	20,24				15,16		24,28	
ID	1	14,–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	20.2
	2	13,–	–	–	–	–	9,–	–	10,–	–	–	17,18	–	–	9,–	–	20.2
	3	–	–	–	–	–	–	–	–	–	–	17,18	–	–	–	–	20.2
	4	14,–	31,–	–	–	15,–	9,–	10,–	11,–	–	16.2,–	17,18	8,–	15,–	9,13	24,–	23.1
	5	14,–	31,–	–	–	15,–	9,–	10,–	11,–	–	14,16.2	17,18	9,–	–	13,–	–	23.1
	Cons	14,–	–	–	–	–	9,–	–	–	–	–	17,18	–	–	–	–	
MF+ID		14,–	31,–	11,–	9,11	–	9,–	10,13	–	20,24	–	17,18	–	15,16	–	24,28	

SN 4-18-2																	
Kit	Exp.	D8 S1179	D21 S11	D7 S820	CSF1PO	D3 S1358	TH 01	D13 S317	D16 S539	D2 S1338	D19 S433	vWA	TPOX	D18 S51	D5 S818	FGA	Quantifiler (pg/ul)
MF	1		29,31	10,12	10,12			–	10,11	24,–				–		–	12.8
	2		29,31	10,–	10,–			11,–	10,11	24,–				–		26,–	12.8
	3		29,31	10,–	10,12			11,–	10,11	17,–				–		26,–	12.8
	4		29,31	10,12	10,12			11,–	10,11	17,24				14,15		21,26	12.6
	5		29,31	10,12	10,12			11,–	10,11	17,24				14,15		21,26	12.6
	Cons		29,31	10,12	10,12			11,–	10,11	17,24				–		26,–	
ID	1	10,–	29,–	10,–	–	17,–	6,–	11,–	11,–	–	14,–	14,–	8,–	–	9,11	–	12.8
	2	10,–	29,–	–	OL	17,18	6,–	–	–	–	14,–	14,–	–	14,–	11,–	–	12.8
	3	10,11	31,–	–	12,–	17,18	6,–	–	–	–	14,–	14,–	–	–	–	–	12.8
	4	10,11	29,31	–	–	–	6,–	–	10,11	–	14,–	14,–	8,11	–	9,11	–	12.6
	5	10,11	29,31	–	–	17,18	6,–	11,–	10,11	–	14,–	14,–	8,11	–	9,11	21,–	12.6
	Cons	10,11	29,31	–	–	17,18	6,–	–	11,–	–	14,–	14,–	8,–	–	9,11	–	
MF+ID		10,11	29,31	10,12	10,12	17,18	6,–	11,–	10,11	17,24	14,–	14,–	8,–	–	9,11	26,–	

SN 4-25-2																	
Kit	Exp.	D8 S1179	D21 S11	D7 S820	CSF1PO	D3 S1358	TH 01	D13 S317	D16 S539	D2 S1338	D19 S433	vWA	TPOX	D18 S51	D5 S818	FGA	Quantifiler (pg/ul)
7.	1		–	11,–	11,–			11,–	–	–				–		–	3.47
	2		–	11,–	11,13			11,–	9,–	20,–				13,15		22,–	3.47
	3		29,31	–	11,–			11,–	–	24,–				15,–		22,–	3.47
	4		–	–	–			11,–	8,–	–				–		–	17.2
	5		–	–	–			–	–	–				–		–	17.2
	Cons		–	–	11,–			11,–	–	–				–		–	
ID	1	13,14	–	10,–	–	16,–	–	–	–	–	–	18,–	–	–	–	–	3.47
	2	–	29,OL	–	11,–	–	–	–	–	–	15,–	15,–	–	–	–	–	3.47
	3	–	–	11,–	–	16,–	–	–	–	–	15,–	–	11,–	–	–	–	3.47
	4	–	–	–	–	–	–	–	–	–	–	–	–	15,–	–	–	17.2
	5	–	–	–	–	–	–	–	–	–	–	18,–	–	–	–	–	17.2
	Cons	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
MF+ID		–	–	–	11,–	–	–	11,–	–	–	–	–	–	–	–	–	

SH 2-10-1																	
Kit	Exp.	D8 S1179	D21 S11	D7 S820	CSF1PO	D3 S1358	TH 01	D13 S317	D16 S539	D2 S1338	D19 S433	vWA	TPOX	D18 S51	D5 S818	FGA	Quantifiler (pg/ul)
MF	1		28,31	11,12	11,13			10,12	11,12	17,23				16,–		22,24	12
	2		28,31	11,12	11,13			10,12	–	17,23				16,–		22,24	12
	3		28,31	11,12	11,13			10,12	11,12	17,23				16,–		22,24	12
	4		28,–	11,12	11,13			10,12	11,12	23,–				16,–		22,24	10.2
	5		28,31	11,12	11,13			10,12	11,12	17,23				16,–		22,24	10.2
	Cons		28,31	11,12	11,13			10,12	11,12	17,23				16,–		22,24	
ID	1	11,–	28,31	11,12	–	15,–	7,9	10,12	11,12	–	13,–	14,18	8,11	16,–	12,13	22,24	12
	2	11,–	28,31	11,–	11,13	15,–	7,9	10,12	11,12	–	13,–	14,18	8,11	15,16	12,13	22,24	12
	3	11,–	–	–	–	–	–	–	–	–	–	14,–	–	–	–	–	12
	4	11,–	28,–	–	–	15,–	7,–	12,–	11,12	–	–	14,–	–	–	13,–	–	10.2
	5	11,–	–	11,12	–	15,–	7,9	10,12	11,12	–	13,–	18,–	–	16,–	12,–	–	10.2
	Cons	11,–	28,–	11,–	–	15,–	7,9	10,12	11,12	–	13,–	14,18	–	16,–	12,13	–	
MF+ID		11,–	28,31	11,12	11,13	15,–	7,9	10,12	11,12	17,23	13,–	14,18	–	16,–	12,13	22,24	

EP 1-50-2																	
Kit	Exp.	D8 S1179	D21 S11	D7 S820	CSF1PO	D3 S1358	TH 01	D13 S317	D16 S539	D2 S1338	D19 S433	vWA	TPOX	D18 S51	D5 S818	FGA	Quantifiler (pg/ul)
MF	1		29,30	–	10,–			9,–	9,10	20,–				13,17		–	0.56
	2		29,–	11,–	10,12			9,–	9,–	17,20				17,–		–	0.56
	3		29,–	–	10,12			9,–	–	17,20				13,–		–	0.56
	4		–	–	–			9,–	–	–				17,–		–	0.32
	5		–	–	–			–	9,–	–				–		–	0.32
	Cons		29,–	–	10,–			9,–	9,–	20,–				17,–		–	
ID	1	–	30,–	–	–	16,–	–	–	9,–	17,–	13,15	16,–	–	–	–	–	0.56
	2	–	30,OL	–	–	–	–	–	–	–	–	16,–	–	–	–	–	0.56
	3	–	–	–	–	–	–	–	–	–	13,–	–	–	–	–	–	0.56
	4	–	–	–	–	–	–	–	–	–	14,–	–	–	–	–	–	0.32
	5	13,–	–	–	–	–	6,9	–	–	–	–	18,–	8,–	–	–	–	0.32
	Cons	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
MF+ID		–	29,–	–	10,–	–	–	9,–	9,–	20,–	–	–	–	17,–	–	–	

EP 1-188-1																	
Kit	Exp.	D8 S1179	D21 S11	D7 S820	CSF1PO	D3 S1358	TH 01	D13 S317	D16 S539	D2 S1338	D19 S433	vWA	TPOX	D18 S51	D5 S818	FGA	Quantity (pg/ul)
MF	1		31.2,32	12,–	10,11			10,11	12,–	18,22				15,19		21,23	30.2
	2		31.2,–	12,–	10,11			10,11	12,–	18,22				15,19		23,–	30.2
	3		31.2,32	12,–	10,11			10,11	12,–	18,22				15,19		–	22.1
	4		31.2,32	–	10,11			10,11	–	18,22				15,19		–	22.1
	5		–	–	10,11			10,11	–	18,22				15,19		–	22.1
	Cons		31.2,32	12,–	10,11			10,11	12,–	18,22				15,19		–	
ID	1	11	32,–	12,–	10,–	15,16	7,8	11,–	–	18,–	13,15.2	14,16	8,–	19,–	11,12	21,–	30.2
	2	11,13	–	12,–	10,–	16,–	7,–	–	12,–	–	13,15.2	14,16	8,–	–	12,–	–	30.2
	3	13,–	–	–	–	–	–	–	–	–	–	14,–	–	–	–	–	22.1
	4	11,13	–	–	–	–	–	–	–	22,–	–	14,16	8,–	–	–	–	22.1
	5	11,13	31.2,–	12,–	10,–	15,16	7,8	10,11	12,–	18,–	13,15.2	14,16	8,–	15,19	11,12	21,23	22.1
	Cons	11,13	–	12,–	10,–	16,–	7,–	–	–	–	13,15.2	14,16	8,–	–	12,–	–	
MF+ID		11,13	31.2,32	12,–	10,11	16,–	7,–	10,11	12,–	18,22	13,15.2	14,16	8,–	15,19	12,–	–	

EP 1-188-2																	
Kit	Exp.	D8 S1179	D21 S11	D7 S820	CSF1PO	D3 S1358	TH 01	D13 S317	D16 S539	D2 S1338	D19 S433	vWA	TPOX	D18 S51	D5 S818	FGA	Quantity (pg/ul)
MF	1		30,–	11,–	–			9,12	9,–	22,–				–		19,22	3.09
	2		29,–	11,–	12,–			9,12	9,–	22,–				16,–		22,–	3.09
	3		–	–	12,13			9,12	–	22,–				14,16		–	15.1
	4		–	–	12,13			9,12	–	22,–				14,16		–	15.1
	5		–	–	12,13			9,12	–	22,–				14,16		–	15.1
	Cons		–	–	12,13			9,12	–	22,–				14,16		–	
ID	1	13,–	–	–	–		–	–	–	–	13,–	–	–	16,–	13,–	–	3.09
	2	12,–	–	–	13,–	–	–	–	–	–	15.2,–	–	–	–	13,–	–	3.09
	3	12,13	–	–	–	–	–	–	–	–	–	17,–	8,–	–	–	–	15.1
	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	15.1
	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	15.1
	Cons	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
MF+ID		–	–	–	12,13	–	–	9,12	–	22,–	–	–	–	14,16	–	–	

Table 2. Loci profiles between MiniFiler and Identifiler kit analysis

	Sample	Locus	MiniFiler	Identifiler
1	SN1-32	D7S820	10,11	10,-
2	SN1-32	CSF1PO	11,12	11,-
3	SN1-32	D13S317	12,-	-
4	SN1-32	D16S539	-	11,12
5	SN1-32	D2S1338	17,25	-
6	SN1-32	D18S51	13,23	13,-
7	SN1-32	FGA	19,24	-
8	SN4-18-1	D21S11	31,-	-
9	SN4-18-1	D7S820	11,-	-
10	SN4-18-1	CSF1PO	9,11	-
11	SN4-18-1	D13S317	10,13	-
12	SN4-18-1	D2S1338	20,24	-
13	SN4-18-1	D18S51	15,16	-
14	SN4-18-1	FGA	24,28	-
15	SN4-18-2	D7S820	10,12	-
16	SN4-18-2	CSF1PO	10,12	-
17	SN4-18-2	D13S317	11,-	-
18	SN4-18-2	D16S539	10,11	11,-
19	SN4-18-2	D2S1338	17,24	-
20	SN4-18-2	FGA	26,-	-
21	SN4-25-2	CSF1PO	11,-	-
22	SN4-25-2	D13S317	11,-	-
23	SH2-10-1	D21S11	28,31	28,-
24	SH2-10-1	D7S820	11,12	11,-
25	SH2-10-1	CSF1PO	11,13	-
26	SH2-10-1	D2S1338	17,23	-
27	SH2-10-1	FGA	22,24	-
28	EP1-50-2	D21S11	29,-	-
29	EP1-50-2	CSF1PO	10,-	-
30	EP1-50-2	D13S317	9,-	-
31	EP1-50-2	D16S539	9,-	-
32	EP1-50-2	D2S1338	20,-	-
33	EP1-50-2	D18S51	17,-	-
34	EP1-188-1	D21S11	31.2,32	-
35	EP1-188-1	CSF1PO	10,11	10,-
36	EP1-188-1	D13S317	10,11	-
37	EP1-188-1	D16S539	12,-	-
38	EP1-188-1	D2S1338	18,22	-
39	EP1-188-1	D18S51	15,19	-
40	EP1-188-2	CSF1PO	12,13	-
41	EP1-188-2	D13S317	9,12	-
42	EP1-188-2	D2S1338	22,-	-
43	EP1-188-2	D18S51	14,16	-

Discussion

A report has shown that most DNA extracted from various samples aged between 4,000 and 13,000 years breaks down into fragments of 40–500 bp, and that the reason for fragmentation could be due to damage resulting from oxidative or hydrolytic reaction (Pääbo, 1989). Moreover, some reports have determined that the preservation state of endogenous DNA can be altered by burial-environment conditions such as low temperature rather than sample age (Höss et al., 1996; Poinar et al., 1996; Poinar and Stankiewicz, 1999). These reports suggest, in other words, that the fragmentation and preservation states of aDNA are related to burial environment.

During the Joseon Dynasty, a type of tomb constructed with a lime-soil mixture barrier (LSMB tomb) was adopted by the ruling class and spread widely (Chung, 1994). Considering that most of the well-preserved skeletal or mummified human remains are found in LSMB tombs, it seems that the burial environment does indeed affect the decomposition state of human remains in Korea (Chang et al., 2006a,b). Therefore, based on previous reports (Pääbo, 1989; Höss et al., 1996; Poinar et al., 1996; Poinar and Stankiewicz, 1999), it can be inferred that LSMB tombs might have affected the

preservation states of aDNA.

In this study, I found that most of the aDNA extracted from skeletal remains found in LSMB tombs was not well enough preserved for analysis using conventional STR kits such as the Idenfiler kit. In Identifiler kit analysis, the amplification success rate for smaller-sized loci (51.8%, 29 of 56) was higher than that for larger-sized loci (21.9%, 14 of 64) (Table 1). Considering that the average amplicon size for larger-sized loci is about 280 bp in the Identifiler kit, in order to perform aDNA analysis using skeletal samples dating to the Joseon Dynasty, I inferred that the PCR amplicon size of such samples should be no larger than 280 bp for successful autosomal STR analysis.

This prediction was verified by using a miniSTR kit, specifically the Minifiler kit. The Minifiler kit is an autosomal STR analysis kit designed for an average amplicon size of about 150 bp for the largest 8 loci of the Identifiler kit (Applied Biosystems, 2006; Mulero, 2008). In this study, when the Minifiler kit was used, the success rate of locus analysis was increased to 75% (48 of 64) (Table 1).

Additionally, it was discovered that the combination of miniSTR and the conventional STR kit can enhance STR analysis rate for skeletal remains found in LSMB tombs. The number of consensus

locus profiles was increased to as high as 79 of 120 loci (65.83%) by the combined use of MiniFiler and Identifiler analyses, which is a far better result than could be achieved by single use of the Identifiler kit (36.7%, 43 of 120).

In this study, I discovered that aDNA extracted from skeletal remains found in LSMB tombs constructed during the Joseon Dynasty was not well preserved, but that a reduced amplicon size could improve the locus analysis. Therefore, considering the characteristics of aDNA and the technical benefits of the miniSTR kit, the miniSTR kit is recommended when aDNA researchers perform STR analysis or need to improve conventional STR analysis of aDNA extracted from Joseon samples buried in LSMB tombs.

CHAPTER 2

A comparative study on the preservation state of aDNA extracted from the femur and mummified brain found in the tomb of Joseon Dynasty

Introduction

Dead bodies undergo degradation by microorganisms, eventually disappearing entirely. This process is called decomposition. Decomposition takes place within the soft tissues (i.e. brain) relatively early and subsequently in the mineralized parts such as bone (Thali et al., 2003; Dent et al., 2004; Perper 2006).

From the perspective of archaeological science, it is also well-known that the acidic-pH soil in Korea degrades dead organisms readily, and much more quickly than is the case in many other countries (Jee et al., 2008). However, a remarkable discovery has been mummified brain tissue, which has been found even where mineralized materials have been entirely decomposed.

Actually, there have been many reports on mummified brains found in Joseon Dynasty tombs (Shin et al., 2003, 2010, 2013; Kim et al., 2006, 2008, 2014; Lee et al., 2007, 2009; Lim et al., 2008) (Table 3). Kim et al. (2008) conducted the first scientific research on such tissue. According to their report, even though the overall volume of the mummified brain had been decreased to 14-20% of its original size, morphologically well-preserved brain lobes with sulcus and gyri could still be observed. They also found myelin-sheath-like structures by transmission electron microscopy (TEM).

This finding suggests that if a mummified brain is morphologically well-preserved, biomolecules such as DNA can survive. And if this is actually proven, mummified brain samples can be very useful to aDNA analyses on archaeological human remains.

However, there has as yet been no scientific study on whether mummified brain tissue actually contains endogenous DNA that can be analyzed, and if so, how much there is. Also, it is necessary to determine if it can be more useful than the compact bone (e.g. femur) that typically is used for DNA analysis.

Therefore, in this study, I investigated the morphologic features of a mummified brain found within an intact skeleton and compared the preservation status of endogenous DNA samples extracted from it and femur bone, respectively, in order to confirm whether mummified brain tissue is a suitable material for aDNA analysis.

Materials and methods

Two mummified human brains and femurs (Yongin and SN PK) collected from the LSMB tombs constructed during Joseon Dynasty were examined in this study (Fig. 1).

Yongin mummy

Briefly, in November 2005, archaeologists of Dankook University Museum discovered a Joseon tomb in Yongin City, Gyeonggi province, South Korea. Based on the archaeological evidence, they estimated that it had been constructed in the 15th or 16th century. Like the other Joseon tombs, it was encapsulated by LSMB. After the accompanying Joseon Dynasty cultural artifacts were collected by archaeologists, the human remains were moved to bioanthropology laboratory (Kim et al., 2008).

The sex of the individual was tentatively determined to be female, as suggested by archaeological method (the hairstyle and clothes), which conclusion was confirmed by the anatomical method (non-metric features of the skull and hip bones). According to the method of Lovejoy et al. (1985), the age range of the individual was 18-24 years (Kim et al., 2008).

SN PK mummy

In 2007, the archaeologists of Hangang Institute of Cultural Heritages investigated another Joseon tomb (SN PK) discovered in Sinnae-dong of Seoul City, South Korea. According to carbon dating analysis, the tomb was constructed in the 16th-17th century. The case was a male, judging from the pelvic (Phenice 1969, Kromgan and Iscan 1986) and cranial morphologies (Buikstra and Ubelaker 1994, Ubelaker 1999). His age at death was estimated to be a middle-aged (35-50 years) by the method of Lovejoy et al. (1985).

Morphological analysis

After the clothing was collected by textile historians, the human remains were subject to anatomical examination. When the skull was cut with an electric saw, the mummified brain within the calvaria could be investigated with the naked eye. Transmission electron microscopy (TEM) on the mummified brains was performed in accordance with methods outlined previously (Hayat, 1970; Bozzola and Russell 1992). Ultrathin sections were observed under H-7600 TEM (Hitachi, Japan).

aDNA analysis

In each of the two cases, DNA samples were extracted from the brain and the mid-shaft of the femur, respectively. The surfaces of the samples were scraped by surgical scalpel or hand drill. The femur samples were bleached with 10% commercial bleach solution, washed with distilled water and ethanol, air-dried and subsequently exposed to 20 min ultraviolet (UV) irradiation. The samples, thus decontaminated, were pulverized using a SPEX 6750 Freezer/Mill (Metuchen, NJ, USA) preparatory to aDNA extraction.

Both the brain and bone samples (0.2-0.3g) were incubated at 56℃ for 24 hrs in 1 ml of TE buffer (pH 8.0) including 100 mM of EDTA (Bioneer, Daejeon, Korea), 1% SDS (Bioneer, Daejeon, Korea), 1 mg/ml of proteinase K (Invitrogen, Carlsbad, CA, USA) and 0.1M DTT (Invitrogen, Carlsbad, CA, USA). DNA was then extracted with equal volumes of phenol/chloroform/isoamyl alcohol (25:24:1) solution (Sigma Aldrich, Saint Louis, MO, USA). DNA isolation and purification was carried out with a QIAquick PCR purification kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The purified DNA was then eluted in 50 μl of EB buffer (QIAGEN, Hilden, Germany) (Oh et al., 2010, Kim et al., 2011b).

The DNA amounts in the respective extracted samples were measured using a Nanodrop® ND-1000 spectrophotometer

(Thermo Scientific, Wilmington, DE, USA). Next, the same amounts of brain and femur DNA (40 ng) were amplified with a Quantifiler™ kit (Applied Biosystems, Foster City, CA, USA). The Quantifiler data were analyzed by 7000 system SDS software, version 1.2.3 (Applied Biosystems, Foster City, CA, USA). The Quantifiler-determined amplifiable DNA quantities were statistically analyzed by Student's t-test. p values < 0.05 were considered statistically significant. The final data were presented as means ± standard deviation.

A PCR amplification assay using the AmpFlSTR® MiniFiler™ kit (Applied Biosystems, Foster City, CA, USA) was repeated seven times for each sample, according to the manufacturer's instructions. Subsequently, amplified products were analyzed on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). When allele profiles were reproducible in at least four of the seven replicates, they were regarded as authentic consensus profiles (Budowle et al., 2009). The peak height thresholds for the AmpFlSTR® MiniFiler™ kit were set at 100 relative fluorescence units (RFUs).

Forty ng of total DNA was mixed with PCR premix containing 1 mg/ml of BSA (New England Biolabs, MA), 10 pmol of each primer (Integrated DNA Technology, USA), 0.25 mM of dNTP mixture

(Invitrogen, USA), 1X PCR buffer, 2 mM of $MgSO_4$ and 1 unit of Platinum^{TM} *Taq* Polymerase High Fidelity (Invitrogen, USA). Hypervariable regions 1 (HV1) and 2 (HV2) of the human mitochondrial DNA (mtDNA) control region were amplified with extracted DNA from each brain and femur. The primer sets used in this study were as follows. PS1 (263 bp): F15989 (5' -CCC AAA GCT AAG ATT CTA AT-3') and R16251 (5' -GGA GTT GCA GTT GAT GT-3'); PS2 (221 bp): F16190 (5' -CCC CAT GCT TAC AAG CAA GT-3') and R16410 (5' -GAG GAT GGT GGT CAA GGG AC-3'); PS3 (226 bp): F015 (5' -CAC CCT ATT AAC CAC TCA CG-3') and R240 (5' -TAT TAT TAT GTC CTA CAA GCA-3'); PS4 (227 bp): F155 (5' -TAT TTA TCG CAC CTA CGT TC-3') and R381 (5' -GCT GGT GTT AGG GTT CTT TG-3'); PS5 (821 bp): F15989 and R240 (Holland and Huffine, 2001).

PCR amplification was performed using a PTC-200 DNA Engine (Bio-Rad Laboratories, Hercules, CA), and the PCR conditions were as follows: pre-denaturation at 94℃ for 10 min; 35 cycles of denaturation at 94℃ for 30 sec; annealing at 50℃ for 30 sec; extension at 72℃ for 30 sec; final extension at 72℃ for 10 min. The PCR products were separated on 2.5% agarose gel, and then stained with ethidium bromide. They were photographed using a

Vilber Lourmat ETX-20.M equipped with Biocapt software (Vilber Lourmat, France).

The amplicons were isolated using a Qiagen gel extraction kit (Qiagen, Germany), and cloning was performed with the pGEM-T easy vector system (Promega, Madison, WI, USA) and ECOS-101 competent cells (Yeastern Biotech, Taipei, Taiwan) following the manufacturer's instructions. Plasmid isolation and purification from cultured bacteria were carried out using a QIAprep spin miniprep kit (Qiagen, Germany). Sequencing analyses were performed by using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) and an ABI Prism 3100 automatic sequencer (Applied Biosystems, USA). The obtained DNA sequences were aligned by MEGA5 (Tamura et al., 2011). The consensus sequences were compared with the revised Cambridge Reference Sequence (rCRS; accession number: NC_012920) in order to identify the sequence differences in BLAST (bl2seq) (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Care was taken to minimize possible aDNA contamination by modern DNA, following the method suggested by Hofreiter et al. (2001). In the course of sampling and other lab work, protective gloves, masks, gowns and head caps were worn at all times (Fig. 2). aDNA lab facilities were set up also in accordance with the protocol

of Hofreiter et al. (2001): the rooms for aDNA extraction or PCR preparation were physically separated from the main PCR lab; further, the DNA extraction/PCR preparation rooms were equipped with night UV irradiation, isolated ventilation, and laminated flow hoods (Fig. 3).

To determine if the samples had incurred any modern-DNA contamination, the autosomal short tandem repeat (STR) profiles and mitochondrial haplotypes of the researchers involved were determined and then compared with the ancient-sample STR profiles (under the auspices of the Institutional Review Board of Seoul National University, H-0909-049-295).

Results

In the gross examination on the mummified brains of Yongin and SN PK, though the volume was remarkably reduced, the brain hemispheres and lobes were morphologically well preserved (Fig. 4). The weight of the mummified brain was 220 grams for Yongin and 358.55 grams for SN PK.

Like previous histological studies (Radanov et al., 1992; Hess et al., 1998; Kim et al., 2008), the myelin sheaths in mummified brains were clearly observed in TEM. They were shown as if it were a major component of the mummified brain. Structures that look like axon were also found in myelin sheaths (Fig. 3).

The Quantifiler analysis revealed that amplifiable autosomal DNA was present in the mummified brains. It was also confirmed that the amount of DNA extracted from mummified brain was more than from femur. The difference in amplification was statistically significant ($p < 0.05$; two-tailed) (Table 4).

Next, autosomal STR analysis was performed using a Minifiler kit to confirm whether the extracted DNA was endogenous, and whether gene analysis was possible when using the DNA extracted from mummified brain.

The sex determinations based on the amelogenin gene (X for

Yongin; XY for SN PK) by Minifiler kit was matched well with the archaeological and anatomical evidence when using mummified brain samples. Surprisingly, however, under the same conditions, femur sample failed to analysis the amelogenin gene (Table 5).

These differences were observed in autosomal STR analysis. In the autosomal STR analysis using mummified brain samples, consensus profiles were obtained from 14 out of 16 STR loci (87.5%), whereas in the case of the femur samples from the same subjects, only one locus (D13S317 of SN PK; 1 of 16, 6.25%) showed a consensus profile (Table 5).

Since the number of consensus profiles of autosomal STR loci from the femur samples was too small, the authenticity of the data obtained from the mummified brains could not be confirmed by comparison with the respective profiles obtained with the same number of PCR cycles (i.e. 30). The problem with using the damaged DNA is PCR failure, and it is known that increasing the number of PCR cycles is one of the ways to solve the problem (Gill, 2001; Alaeddini et al., 2010).

Therefore, I increased the number of PCR amplifications (cycles=35) for a better success rate of AmpFlSTR® MiniFiler™ kit analysis with the same femur samples. As a result, the success rate of STR analysis using femur samples increased to 75% (12 out

of 16). Specifically, full concordance between the STR locus profiles of the brain (PCR cycles=30) and femur (PCR cycles=35) was observed for 50% (8 out of 16) of the STR loci (CSF1PO of Yongin; D13S317, D7S820, D2S1338, D16S539, D18S51, CSF1PO and FGA of SN PK). The differences that were observed in the other locus profiles were caused by allelic drop-outs, possibly as a result of processing only low copy number DNA (LCN) during the PCR (Table 6, Fig. 6). The authenticity of the STR profiles obtained from the mummified brains could be proven by the concordance between them and those of the femur samples.

In order to confirm whether the DNA contamination of researchers occurred in this study, STR profile obtained from the brain samples was compared with those of the researchers who participated. However, any identical locus profiles were not found in the comparison; therefore, it can be confirmed that the autosomal STR profiles be endogenous, not that of contamination (Table 7).

Next, I also compared the preservation status of mtDNA between the femur and the mummified brain. For this experiment, I chose hypervariable region 1 (HV1) and 2 (HV2) as amplification target site because the location has a high sequence variation, so that it can be used for human identification and contamination test at the same time by analyzing the sequence of the region.

All of mtDNA fragment (PS1–PS5) for HV1 and HV2 region were successfully amplified with the mummified brain samples (Figs. 7 and 8). However, in case of the femur samples, only the short DNA fragments (PS1–PS4) under 263 bp were amplified, and the 821 bp mtDNA amplicons (PS5) could be obtained only from the mummified brain samples.

The consensus sequence on mtDNA HV1 and HV2 from each amplified PCR fragment were successfully determined by cloning and sequencing (Fig. 9), and the 821 bp mtDNA sequence containing HV1 HV2 from the mummified brain samples (PS5) were completely identical to the corresponding sequences of the PS1–PS4 fragments obtained from the mummified brain and femur samples (Fig. 10).

The possibility of modern DNA contamination was investigated by mtDNA haplotype comparison of the Yongin mummy, SN PK mummy and participating researchers, following the previous reports (Ricaut et al., 2004, 2005, 2006a, 2006b; Haak et al., 2005). I could not find identical sequences among them (Table 7 and 8). This means that the mtDNA results was not the outcome of contamination by modern DNA.

Table 3. Mummified human brains examined since 2006

Name of Tomb	Sex	Excavation Date	Preservation Status of the Human Bodies
Yongin	Female	2005-09-14	Skeletonized
Hadong 1	Female	2006-04-08	Mummified
GJ 1-1	Male	2007-02-13	Skeletonized
SN 3-14	Male	2007-06-23	Skeletonized
SN 2-19-1	Male	2007-07-23	Skeletonized
SN 2-19-2	Female	2007-07-23	Skeletonized
EP B-III-1 1F	Female	2007-08-31	Skeletonized
SN 1-2	Male	2007-10-25	Half-mummified
SN PK	Male	2007-11-16	Half-mummified
EP C-8-1	Male	2008-03-21	Skeletonized
Dangjin	Female	2008-08-08	Mummified
Seocheon	Female	2008-08-22	Skeletonized
Waegwan	Male	2008-10-30	Skeletonized
Hadong 2	Female	2009-06-01	Mummified
Mungyeong	Female	2010-04-18	Mummified
JinJu-Sabong	Male	2010-07-22	Skeletonized
Sapgyo	Male	2011-02-16	Half-mummified
Sacheon	Female	2011-12-12	Skeletonized
Konkuk	Female	?	Mummified
Seoul	Male	?	Mummified
Hwasung	Male	2012-10-26	Mummified
Andong	Male	2013-01-DD	Mummified
Dalsung	Female	2014-05-28	Mummified
Cheongdo	Male	2014-10-15	Mummified

Table 4. aDNA measured by Quantifiler Human DNA Quantification Kit

Case	Sample from	Quantifiler (pg/ul)
SN PK	Femur	6.5 ± 5.6
	Brain	19.1 ± 9.3*
Yongin	Femur	3.3 ± 3.5
	Brain	12.8 ± 3.9*

*$p < 0.05$

Table 5. STR analysis of brain and femur samples in this study

Yongin														
Sample	Labs	Cycle #	DNA (ng)	PCR	AMEL	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
Brain	A	30	124.5	1	X,–	12,–	8,9	18,23	30,–	8,–	22,–	10,12	–
		30		2	X,–	10,12	8,9	18,23	28,30	8,14	15,22	10,12	–
		30		3	X,–	10,12	8,9	18,23	28,30	8,14	15,22	10,12	–
		30	26.8	4	X,–	10,12	8,9	18,23	28,–	8,14	15,22	10,12	24,25
		30		5	X,–	10,12	8,9	18,23	28,–	8,–	15,22	10,12	24,25
	B	30	81.81	1	X,–	10,12	–	18,23	28,30	–	15,–	10,12	–
		30		2	–	10,12	–	18,23	–	–	15,22	10,12	24,–
	Cons				**X,–**	**10,12**	**8,9**	**18,23**	**28,30**	**8,–**	**15,22**	**10,12**	–
Femur	A	30	124.5	1	–	–	–	–	–	–	–	–	–
		30		2	–	–	–	–	–	–	–	–	–
		30		3	–	–	–	–	–	–	–	–	–
		30	26.8	4	–	–	–	–	–	–	–	–	–
		30		5	–	–	–	–	–	–	–	–	–
	B	30	81.81	1	–	–	–	–	30,31	–	–	–	–
		30		2	–	–	–	–	30,31	–	–	–	–
	Cons				–	–	–	–	–	–	–	–	–

Table 5. continued.

SN PK													
Sample	**Labs**	**Cycle #**	**DNA (ng)**	**PCR**	**AMEL**	**D13S317**	**D7S820**	**D2S1338**	**D21S11**	**D16S539**	**D18S51**	**CSF1PO**	**FGA**
Brain	A	30	98.16	1	XY	13,–	–	19,20	29,–	10,–	13,14	12,–	23,24
		30		2	XY	13,–	–	19,20	–	10,–	13,14	12,–	23,–
		30		3	XY	13,–	11,–	19,20	–	10,–	13,14	12,–	24,–
		30	68.69	4	XY	13,–	11,12	19,20	–	10,–	13,14	12,–	24,–
		30		5	XY	13,–	11,12	19,20	29,–	10,–	13,14	12,–	–
	B	30	31.98	1	XY	13,–	11,–	18,19,20	29,–	10,–	13,14	12,–	23,24
		30		2	XY	13,–	11,12	19,20	31,–	10,–	13,14	12,–	23,–
	Cons				**XY**	**13,–**	**11,–**	**19,20**	**–**	**10,–**	**13,14**	**12,–**	**23,24**
Femur	A	30	98.16	1	–	–	–	–	–	–	–	–	–
		30		2	–	13,–	–	–	–	–	13,–	–	–
		30		3	–	–	–	–	–	–	–	–	–
		30	68.69	4	–	13,–	–	–	–	–	–	–	–
		30		5	–	–	–	–	–	–	–	–	–
	B	30	31.98	1	–	13,–	–	20,–	30,–	–	13,–	12,–	–
		30		2	–	13,–	11,12	–	–	–	–	–	–
	Cons				**–**	**13,–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**

Table 6. Loci profiles between Brain and Femur

Sample	Locus	Brain 30 cycle	Femur 30 cycle	Femur 35 cycle
Yongin	D13S317	10,12	–	10,–
	D7S820	8,9	–	–
	D2S1338	18,23	–	–
	D21S11	28,30	–	–
	D16S539	8,–	–	8,14
	D18S51	15,22	–	22,–
	CSF1PO	10,12	–	10,12
	FGA	–	–	–
SN PK	D13S317	13,–	13,–	13,–
	D7S820	11,–	–	11,–
	D2S1338	19,20	–	19,20
	D21S11	–	–	29,32.2
	D16S539	10,–	–	10,–
	D18S51	13,14	–	13,14
	CSF1PO	12,–	–	12,–
	FGA	23,24	–	23,24

Table 7. Autosomal STR profiles from brain and femur (30 or 35 PCR cycles). The profiles from researchers (1, 2, and 3) joining in this study were also presented.

Site	Sample	Cycle #	DNA (ng)	PCR	AMEL	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
Yongin	Brain	30		Cons	X,–	10,12	8,9	18,23	28,30	8,–	15,22	10,12	–
	Femur	30		Cons	–	–	–	–	–	–	–	–	–
	Femur	35	124.5	1	X,–	10,–	–	–	28,30	8,14	15,22	10,12	–
		35		2	–	10,–	9,–	–	–	14,–	22,–	10,–	24,–
		35		3	X,–	10,–	–	18,–	–	8,14	22,–	10,12	–
		35		Cons	X,–	10,–	–	–	–	8,14	22,–	10,12	–
SN PK	Brain	30		Cons	X,Y	13,–	11,–	19,20	–	10,–	13,14	12,–	23,24
	Femur	30		Cons	–	13,–	–	–	–	–	–	–	–
	Femur	35	98.16	1	X,Y	13,–	–	19,20	–	–	10,11	12,–	24,–
		35		2	X,Y	13,–	11,12	19,20	29,32.2	10,–	13,14	12,–	23,24
		35		3	X,Y	13,–	11,–	19,20	29,32.2	10,–	13,14	12,–	23,24
		35		Cons	X,Y	13,–	11,–	19,20	29,32.2	10,–	13,14	12,–	23,24
Researchers	1	30			X,Y	8,12	8,12	22,23	29,32.2	12,–	17,21	10,12	19,22
	2	30			X,Y	8,11	11,–	20,25	29,31	9,12	15,–	10,–	22,23
	3	30			X,Y	8,–	11,12	20,24	29,32	10,–	14,27	10,12	18,22

Table 8. mtDNA haplotype comparison of the Yongin, SN PK mummies and participating researchers

Subject	Hypervariable region	
	HVI (16026-16365)	HVII (73-340)
SN PK	16129A 16182C 16183C 16189C 16222A 16249C 16304C 16311C 16344T	73G 152C 249D 263G 315.1C
Yongin	16189C 16193.1C 16223T 16362C	73G 150T 263G 315.1C
*RS1	16183C 16189C 16220C 16254G 16298C 16362C	73G 248d 263G 315.1C
*RS2	16172C 16174T 16223T 16362C	73G 263G 309.1C 315.1C
*RS3	16189C 16223T 16265C 16274A 16362C	73G 143A 152C 315.1C

*RS1-3, researchers participated in this study

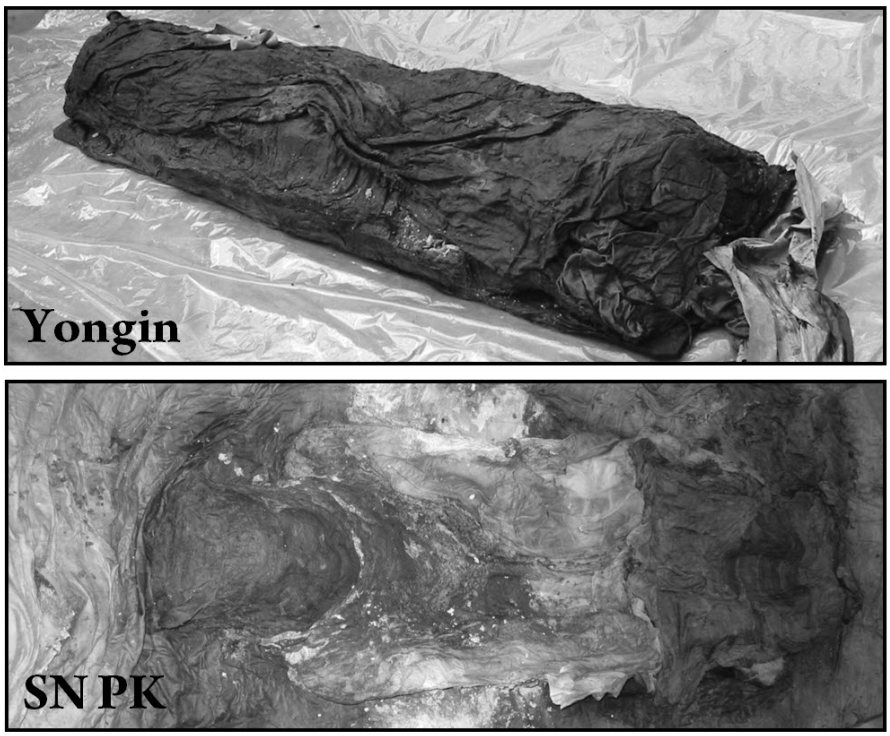


Figure 1. Yongin and SN PK cases examined in this study. The human remains discovered in the coffins were wrapped by clothing. The removal of clothing was performed under well-appointed lab condition, following the method suggested by Hofreiter et al. (2001).

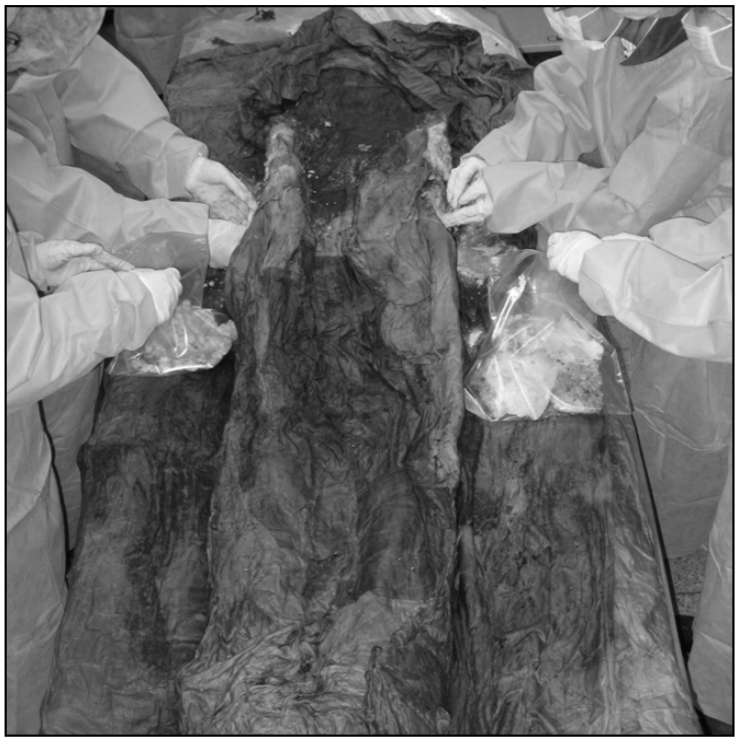


Figure 2. Removal of clothes from the dead body. Every participant wore sterilized gown, gloves, masks and head caps.

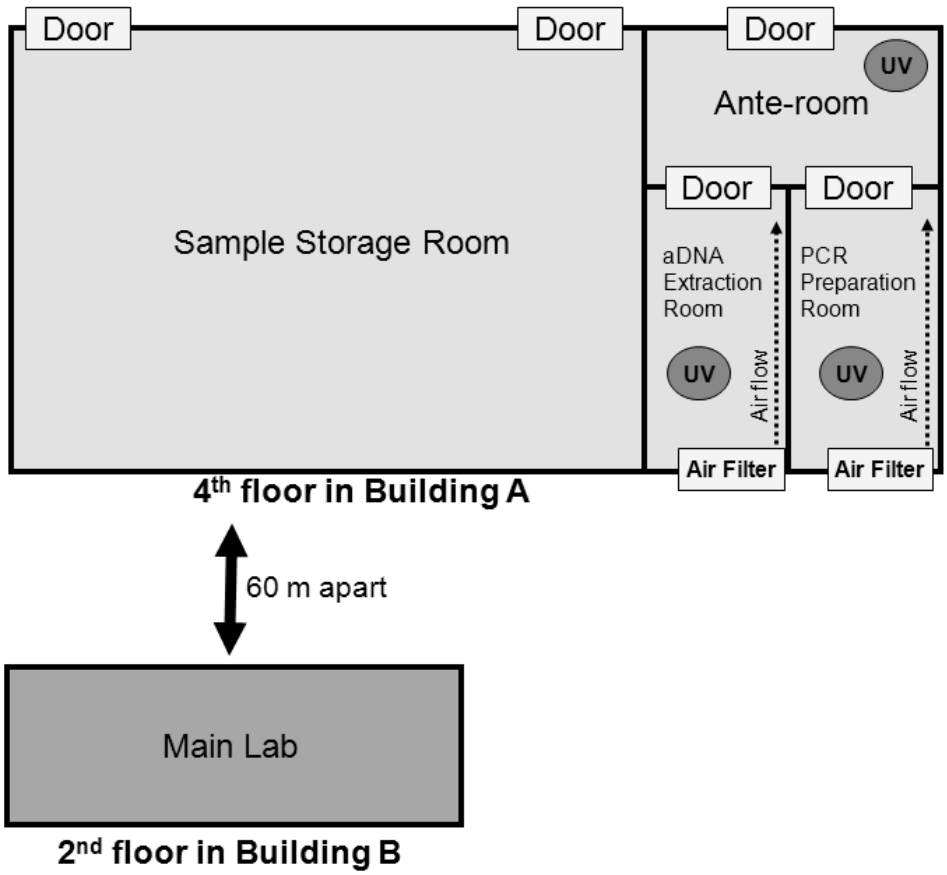


Figure 3. Laboratory exclusively dedicated to aDNA work. The distance between aDNA extraction or PCR preparation rooms of Building A and main lab of Building B is about 60 meters. There is no lab performing PCR amplification of modern DNA on 4th floor in Building A. None could enter into aDNA extraction or PCR preparation rooms without permission.

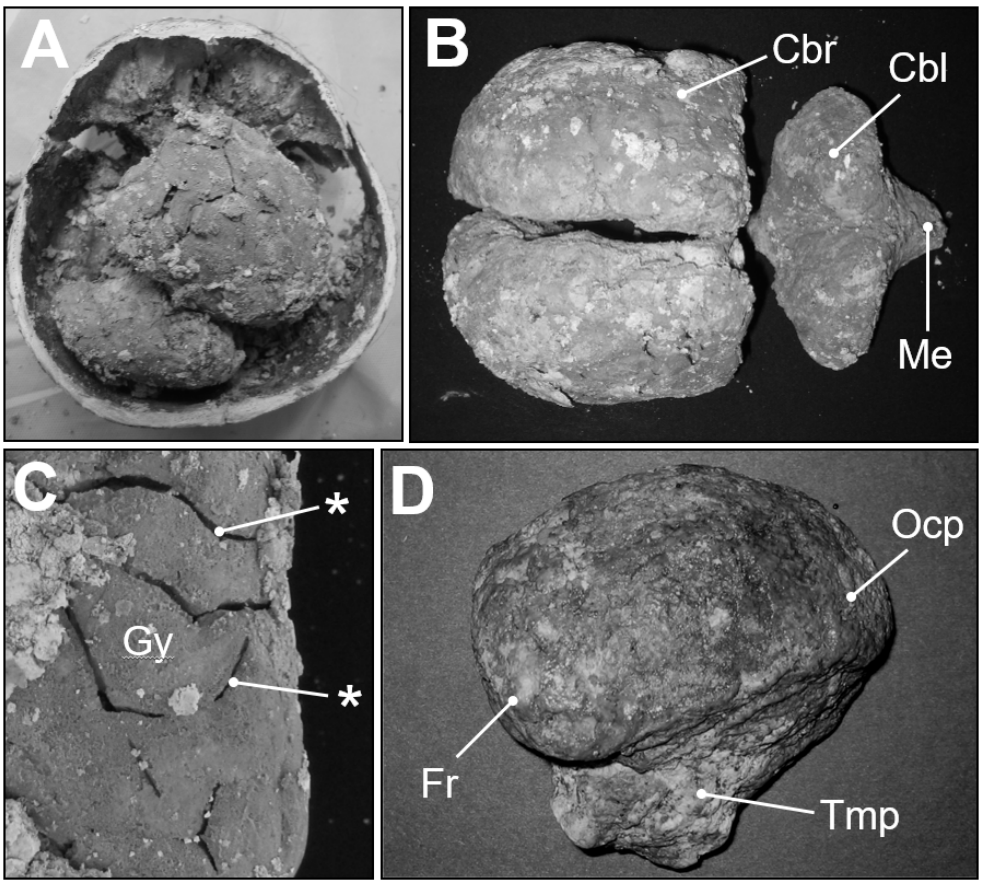


Figure 4. Examples of mummified brain dissection. (A) to (C) are for SN PK. (A) When the skull was opened with electric-saw, the mummified brain was observed very well preserved within the cranial cavity. (B) The cerebral (Cbr) and cerebellar (Cbl) hemispheres are recognized in SN PK case. Me, medulla oblongata. (C) Gyri (Gy) and sulci (asterisks) could be clearly identified in some part of mummified brain surface. (D) is the brain from Yongin case. Fr, frontal lobe; Ocp, occipital lobe; Tmp, temporal lobe.

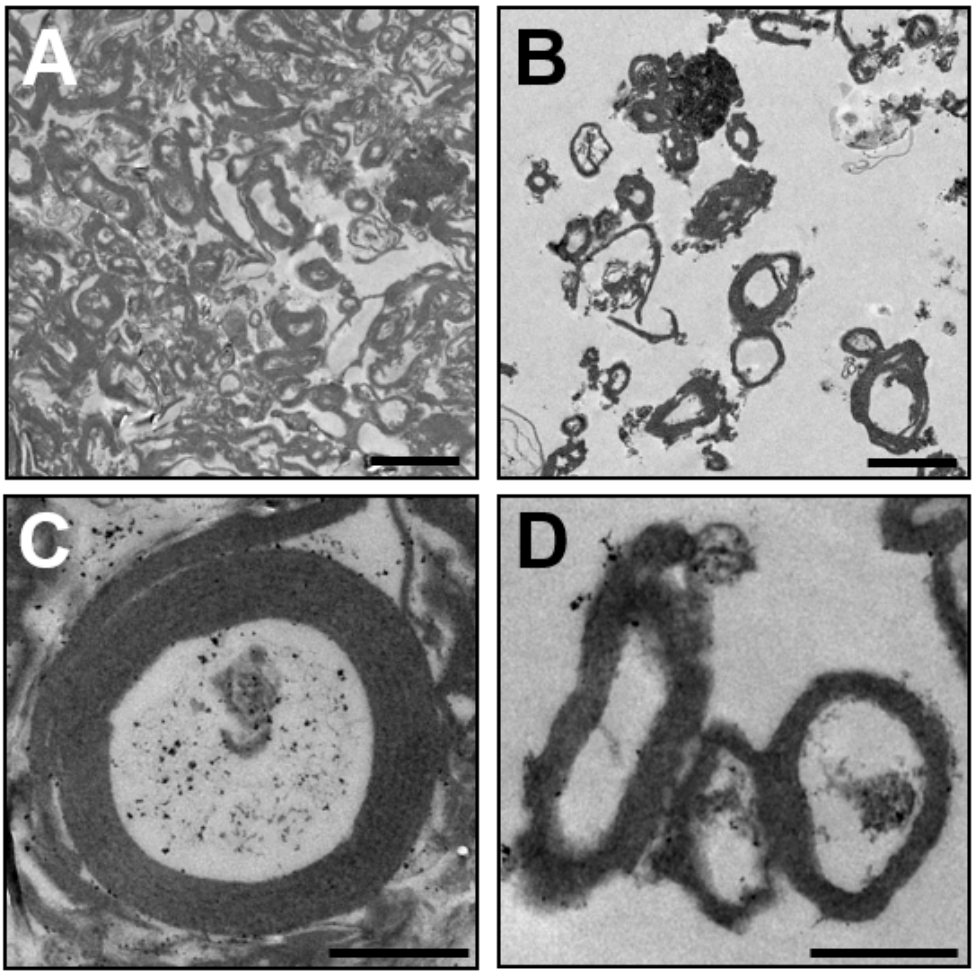


Figure 5. Transmission electron microscopy on mummified brains. Concentric patterns of myelin sheath could be preserved well. (A) and (C) for Yongin case; (B) and (D) for SN PK cases. (C) and (D) are magnified images of (A) and (C) respectively. Scale bars, 2 μm for (A) and (B); 500 nm for (C) and (D).

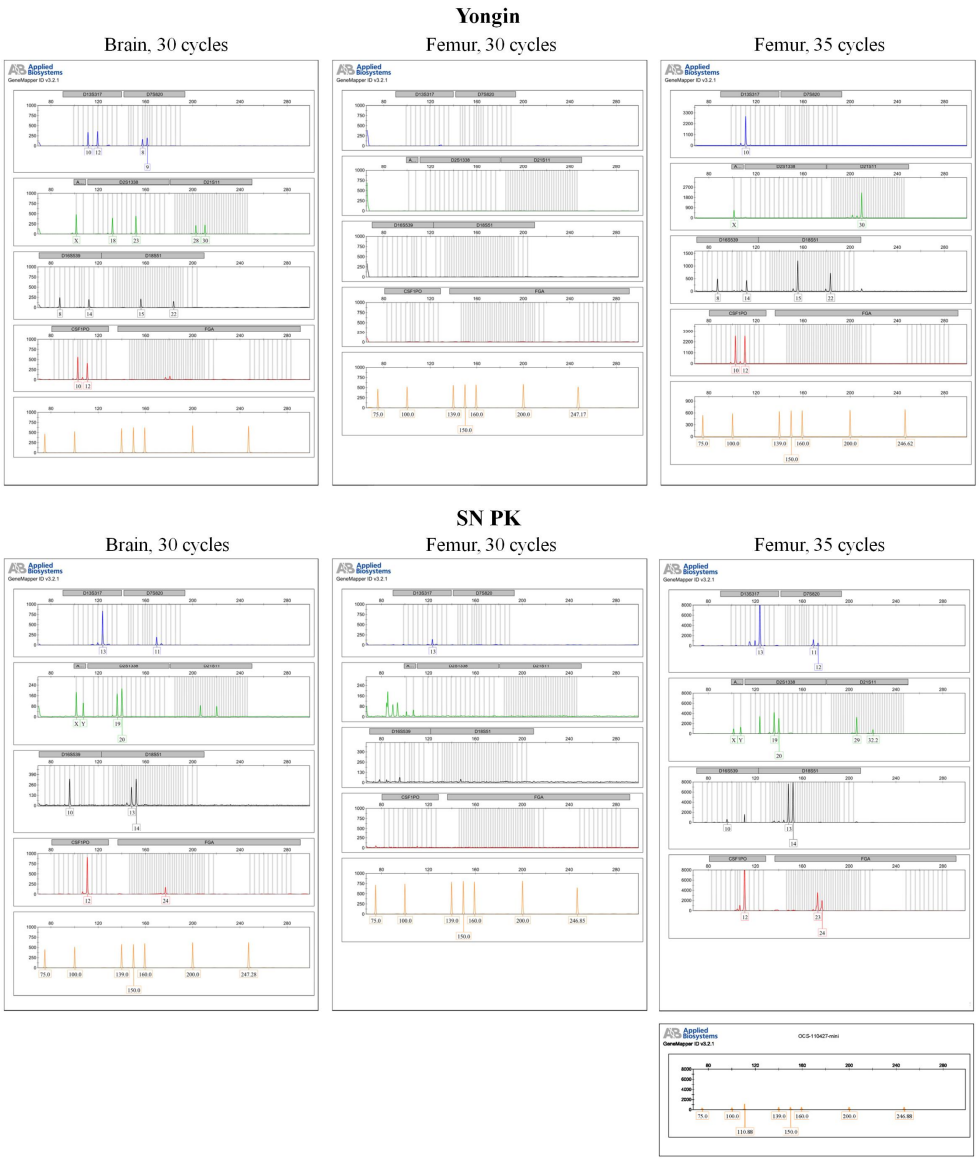


Figure 6. Autosomal STR results. In both cases of Yongin and SN PK, the autosomal STR profiles were much better obtained from the brain (30 cycles) than from the femur samples (30 cycles). In MiniFiler kit analysis on the same skeletons, with an increased number of amplifications (PCR number=35), a number of

Figure 6. continued

the STR locus profiles could be newly determined, showing a significant PCR amplification improvement. Since most of the differences observed in the locus profiles were caused by allelic drop-outs, the authenticity of the STR profiles obtained from the mummified brains could be proven.

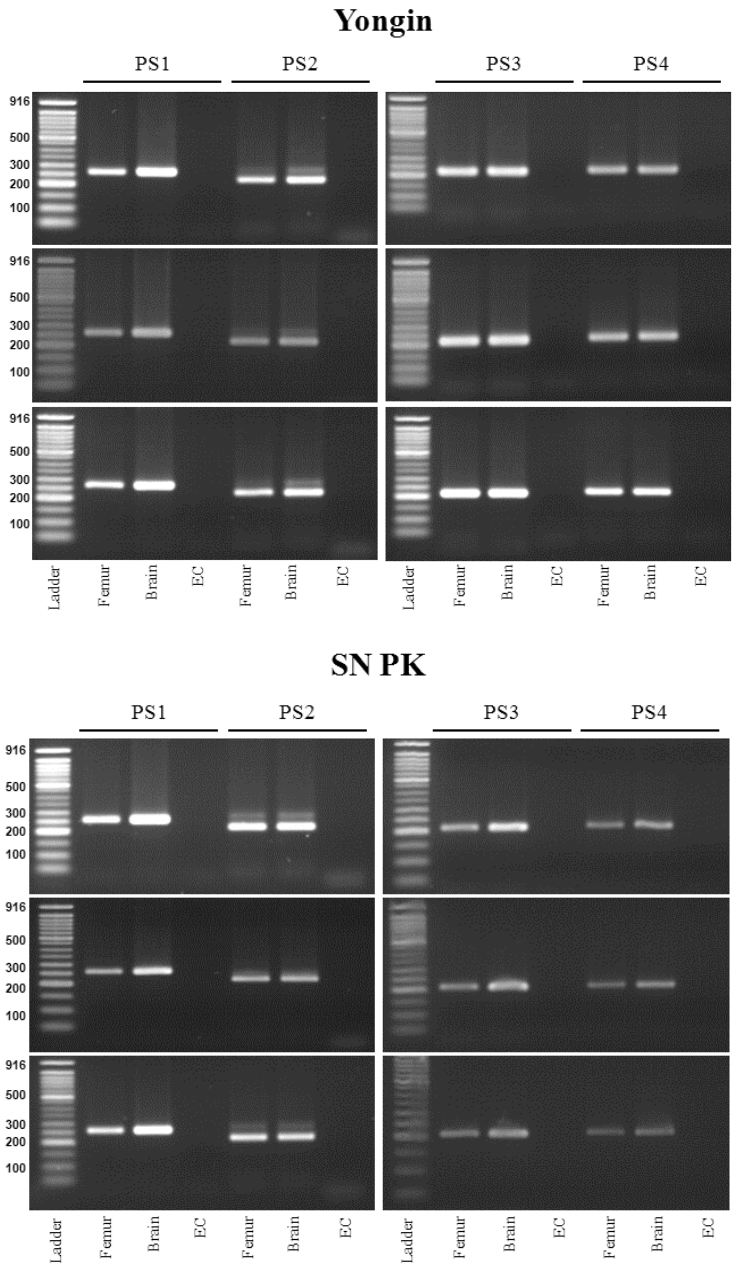


Figure 7. PCR result of Hypervariable region. 221–263 bp mtDNA fragments (PS1–PS4) were successfully amplified with femur and brain samples from Yongin and SN PK mummies. EC, extraction control.

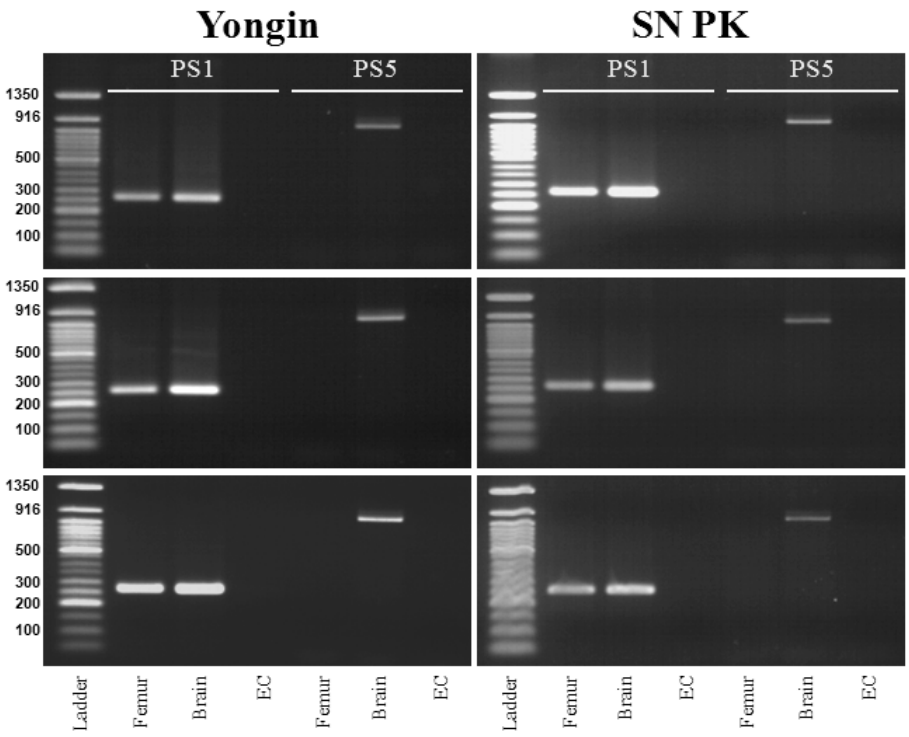


Figure 8. Amplified bands of PS1 (15989–16251, 263 bp) and PS5 (15989–240, 821 bp) fragments of mitochondrial DNA from Yongin and SN PK mummies on agarose gel electrophoresis. Experiments were repeated three times. In cases of femur and brain samples, PS1 DNA fragments could be successfully amplified. On the other hand, 821-bp PS5 amplicons were only obtained from brain samples. EC, extraction control.

Yongin Femur

PS1(16009-16234)

```
Consensus   1    TTAAACTATTCTCTGTTCTTTCATGGGGAAGCAGATTTGGGTACCACCCAAGTATTGACTCACCCATCAACAACCGCTATGTATTTCGTACATTACTGCCAGCCACCATGAATATTGTAC  120
YIF1-1      1    ....................................................................................................................  120
YIF1-2      1    ....................................................................................................................  120
YIF1-3      1    ....................................................................................................................  120
YIF2-1      1    ..................................................C.................................................................  120
YIF2-2      1    ....................................................................................................................  120
YIF2-3      1    ....................................................................................................................  120
YIF3-1      1    ....................................................................................................................  120
YIF3-2      1    ....................................................................................................................  120
YIF3-3      1    ....................................................................................................................  120

Consensus   121  GGTACCATAAATACTTGACCACCTGTAGTACATAAAAACCCAATCCACATCAAAACCCCCCCCCCCATGCTTACAAGCAAGTACAGCAATCAACCTTCAACTATCAC  227
YIF1-1      121  ....................................................................................................................  227
YIF1-2      121  ....................................................................................................................  227
YIF1-3      121  ....................................................................................................................  227
YIF2-1      121  ....................................................................................................................  227
YIF2-2      121  ....................................................................................................................  227
YIF2-3      121  ..........................A.........................................-.....................................  226
YIF3-1      121  ....................................................................................................................  227
YIF3-2      121  ....................................................................................................................  227
YIF3-3      121  ....................................................................................................................  227
```

PS2(16210-16390)

```
Consensus   1    ACAGCAATCAACCTTCAACTATCACACATCAACTGCAACTCCAAAGCCACCCCTCACCCACTAGGATACCAACAAACCTACCCACCCTTAACAGTACATAGTACATAAAGCCATTTACCG  120
YIF1-1      1    ....................................................................................................................  120
YIF1-2      1    ....................................................................................................................  120
YIF1-3      1    ....................................................................................................................  120
YIF2-1      1    ....................................................................................................................  120
YIF2-2      1    ....................................................................................................................  120
YIF2-3      1    ....................................................................................................................  120
YIF3-1      1    ..................................................................................................T.................  120
YIF3-2      1    ..................................................................................................G.................  120
YIF3-3      1    ....................................................................................................................  120

Consensus   121  TACATAGCACATTACAGTCAAATCCCTTCTCGCCCCCATGGATGACCCCCCTCAGATAGGG  181
YIF1-1      121  .............................................................  181
YIF1-2      121  .............................................................  181
YIF1-3      121  .............................................................  181
YIF2-1      121  .............................................................  181
YIF2-2      121  .............................................................  181
YIF2-3      121  .............................................................  181
YIF3-1      121  .............................................................  181
YIF3-2      121  .............................................................  181
YIF3-3      121  .............................................................  181
```

PS3(35-219)

```
Consensus   1    GGAGCTCTCCATGCATTTGGTATTTTCGTCTGGGGGGTGTGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCTATGTCGCAGTATCTGTCTTTGATTCCTGCCTCATTCTAT  120
YIF1-1      1    ....................................................................................................................  120
YIF1-2      1    ............................-.........A.....................................................................  119
YIF1-3      1    ....................................................................................................................  120
YIF2-1      1    ....................................................................................................................  120
YIF2-2      1    ....................................................................................................................  120
YIF2-3      1    ....................................................................................................................  120
YIF3-1      1    ....................................................................................................................  120
YIF3-2      1    ....................................................................................................................  120
YIF3-3      1    ....................................................................................................................  120

Consensus   121  TATTTATCGCACCTACGTTCAATATTACAGGCGAACATACTTACTAAAGTGTGTTAATTAATTAA  185
YIF1-1      121  ...........................................................  185
YIF1-2      120  ...........................................................  184
YIF1-3      121  ...........................................................  185
YIF2-1      121  ...........................................................  185
YIF2-2      121  ...........................................................  185
YIF2-3      121  ...........................................................  185
YIF3-1      121  ...........................................................  185
YIF3-2      121  ...........................................................  185
YIF3-3      121  ...........................................................  185
```

PS4(175-361)

```
Consensus   1    AATATTACAGGCGAACATACTTACTAAAGTGTGTTAATTAATTAATGCTTGTAGGACATAATAATAACAATTGAATGTCTGCACAGCCGCTTTCCACACAGACATCATAACAAAAAATTT  120
YIF1-1      1    ....................................................................................................................  120
YIF1-2      1    ....................................................................................................................  120
YIF1-3      1    ....................................................................................................................  120
YIF2-1      1    ....................................................................................................................  120
YIF2-2      1    ....................................................................................................................  120
YIF2-3      1    ....................................................................................................................  120
YIF3-1      1    ....................................................................................................................  120
YIF3-2      1    ....................................................................................................................  120
YIF3-3      1    ....................................................................................................................  120

Consensus   121  CCACCAAACCCCCCCTCCCCCGCTTCTGGCCACAGCACTTAAACACATCTCTGCCAAACCCCAAAAA  188
YIF1-1      121  .....................................................................  188
YIF1-2      121  .....................................................................  188
YIF1-3      121  .....................................................................  188
YIF2-1      121  .....................................................................  188
YIF2-2      121  .....................................................................  188
YIF2-3      121  .....................................................................  188
YIF3-1      121  .....................................................................  188
YIF3-2      121  .....................................................................  188
YIF3-3      121  .....................................................................  188
```

Yongin Brain

PS1(16009-16234)

```
Consensus   1   TTAAACTATTCTCTGTTCTTTCATGGGGAAGCAGATTTGGGTACCACCCAAGTATTGACTCACCCATCAACAACCGCTATGTATTTCGTACATTACTGCCAGCCACCATGAATATTGTAC  120
B1-1        1   ....................................................................................................................  120
  2         1   ....................................................................................................................  120
  3         1   ....................................................................................................................  120
B2-1        1   ....................................................................................................................  120
  2         1   ....................................................................................................................  120
  3         1   ....................................................................................................................  120
B3-1        1   ....................................................................................................................  120
  2         1   ....................................................................................................................  120
  3         1   ....................................................................................................................  120

Consensus   121 GGTACCATAAATACTTGACCACCTGTAGTACATAAAAACCCAATCCACATCAAAACCCCCCCCCCCATGCTTACAAGCAAGTACAGCAATCAACCTTCAACTATCAC  227
B1-1        121 ....................................................................................................................  227
  2         121 .....................................G.....................T.........-.....................................  226
  3         121 ....................................................................................................................  227
B2-1        121 ....................................................................................................................  227
  2         121 ..........................................................-.....................................  226
  3         121 ....................................................................................................................  227
B3-1        121 ....................................................................................................................  227
  2         121 ....................................................................................................................  227
  3         121 ....................................................................................................................  227
```

PS2(16210-16390)

```
Consensus   1   ACAGCAATCAACCTTCAACTATCACACATCAACTGCAACTCCAAAGCCACCCCTCACCCACTAGGATACCAACAAACCTACCCACCCTTAACAGTACATAGTACATAAAGCCATTTACCG  120
B1-1        1   ....................................................................................................................  120
  2         1   ....................................................................................................................  120
  3         1   ....................................................................................................................  120
B2-1        1   ....................................................................................................................  120
  2         1   ....................................................................................................................  120
  3         1   ..........................................................G.....................T.....................  120
B3-1        1   ....................................................................................................................  120
  2         1   ....................................................................................................................  120
  3         1   ....................................................................................................................  120

Consensus   121 TACATAGCACATTACAGTCAAATCCCTTCTCGCCCCCATGGATGACCCCCCTCAGATAGGG  181
B1-1        121 .........................................................  181
  2         121 .........................................................  181
  3         121 .........................................................  181
B2-1        121 .........................................................  181
  2         121 .........................................................  181
  3         121 .........................................................  181
B3-1        121 .........................................................  181
  2         121 .........................................................  181
  3         121 .........................................................  181
```

PS3(35-219)

```
Consensus   1   GGAGCTCTCCATGCATTTGGTATTTTCGTCTGGGGGGTGTGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCTATGTCGCAGTATCTGTCTTTGATTCCTGCCTCATTCTAT  120
B1-1        1   ....................................................................................................................  120
  2         1   ....................................................................................................................  120
  3         1   ........................................-...........................................................................  119
B2-1        1   ....................................................................................................................  120
  2         1   ....................................................................................................................  120
  3         1   ....................................................................................................................  120
B3-1        1   ....................................................................................................................  120
  2         1   ....................................................................................................................  120
  3         1   ....................................................................................................................  120

Consensus   121 TATTTATCGCACCTACGTTCAATATTACAGGCGAACATACTTACTAAAGTGTGTTAATTAATTAA  185
B1-1        121 .........................................................  185
  2         121 .........................................................  184
  3         120 .........................................................  185
B2-1        121 .........................................................  185
  2         121 .........................................................  185
  3         121 .........................................................  185
B3-1        121 .........................................................  185
  2         121 .........................................................  185
  3         120 .........................................................  185
```

PS4(175-361)

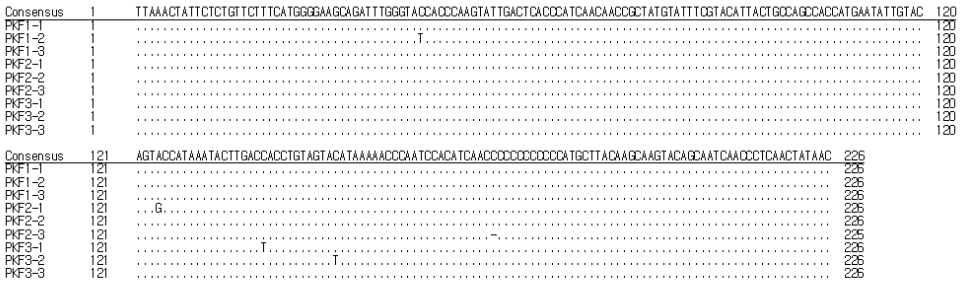
```
Consensus   1   AATATTACAGGCGAACATACTTACTAAAGTGTGTTAATTAATTAATGCTTGTAGGACATAATAATAACAATTGAATGTCTGCACAGCCGCTTTCCACACAGACATCATAACAAAAAATTT  120
B1-1        1   ....................................................................................................................  120
  2         1   ....................................................................................................................  120
  3         1   ..............G.....................................................................................................  120
B2-1        1   ....................................................................................................................  120
  2         1   ....................................................................................................................  120
  3         1   ....................................................................................................................  120
B3-1        1   ....................................................................................................................  120
  2         1   ....................................................................................................................  120
  3         1   ....................................................................................................................  120

Consensus   121 CCACCAAACCCCCCCTCCCCCGCTTCTGGCCACAGCACTTAAACACATCTCTGCCAAACCCCAAAAA  188
B1-1        121 .........................................................  188
  2         121 .........................................................  188
  3         121 .........................................................  188
B2-1        121 .........................................................  188
  2         121 .........................................................  188
  3         121 .........................................................  188
B3-1        121 .........................................................  188
  2         121 .........................................................  188
  3         121 .........................................................  188
```

PS5(16009-219)

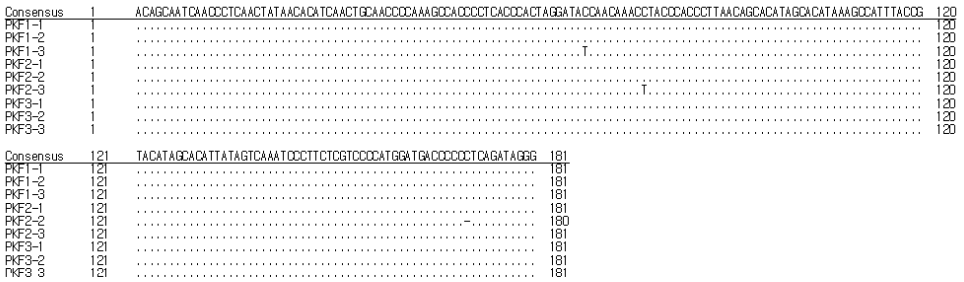
```
Consensus   1   TTAAACTATTCTCTGTTCTTTCATGGGGAAGCAGATTTGGGTACCACCCAAGTATTGACTCACCCATCAACAACCGCTATGTATTTCGTACATTACTGCCAGCCACCATGAATATTGTAC  120
B1-1        1   ....................................................................................................................  120
    2       1   ....................................................................................................................  120
    3       1   ....................................................................................................................  120
B2-1        1   ....................................................................................................................  120
    2       1   ....................................................................................................................  120
    3       1   ....................................................................................................................  120
B3-1        1   ....................................................................................................................  120
    2       1   ....................................................................................................................  120
    3       1   ....................................................................................................................  120

Consensus   121 GGTACCATAAATACTTGACCACCTGTAGTACATAAAAACCCAATCCACATCAAAACCCCCCCCCCCATGCTTACAAGCAAGTACAGCAATCAACCTTCAACTATCACACATCAACTGCAA  240
B1-1        121 ....................................................................................................................  240
    2       121 ....................................................................................................................  240
    3       121 ...................................................................-................................................  239
B2-1        121 ....................................................................................................................  240
    2       121 ....................................................................................................................  240
    3       121 ....................................................................................................................  240
B3-1        121 ....................................................................................................................  240
    2       121 ...................................................................-................................................  239
    3       121 ....................................................................................................................  240

Consensus   241 CTCCAAAGCCACCCCTCACCCACTAGGATACCAACAAACCTACCCACCCTTAACAGTACATAGTACATAAAGCCATTTACCGTACATAGCACATTACAGTCAAATCCCTTCTCGCCCCCA  360
B1-1        241 ....................................................................................................................  360
    2       241 ....................................................................................................................  360
    3       240 ....................................................................................................................  359
B2-1        241 ....................................................................................................................  360
    2       241 ..........T.....................................................................................................  360
    3       241 ....................................................................................................................  360
B3-1        241 ....................................................................................................................  360
    2       240 ....................................................................................................................  359
    3       241 ....................................................................................................................  360

Consensus   361 TGGATGACCCCCCTCAGATAGGGGTCCCTTGACCACCATCCTCCGTGAAATCAATATCCCGCACAAGAGTGCTACTCTCCTCGCTCCGGGCCCATAACACTTGGGGGTAGCTAAAGTGAA  480
B1-1        361 ....................................................................................................................  480
    2       361 ....................................................................................................................  480
    3       360 ....................................................................................................................  479
B2-1        361 ....................................................................................................................  480
    2       361 ....................................................................................................................  480
    3       361 ............C.......................................................................................................  480
B3-1        361 ....................................................................................................................  480
    2       360 ....................................................................................................................  479
    3       361 ....................................................................................................................  480

Consensus   481 CTGTATCCGACATCTGGTTCCTACTTCAGGGCCATAAAGCCTAAATAGCCCACACGTTCCCCTTAAATAAGACATCACGATGGATCACAGGTCTATCACCCTATTAACCACTCACGGGAG  600
B1-1        481 ....................................................................................................................  600
    2       481 ....................................................................................................................  600
    3       480 ....................................................................................................................  599
B2-1        481 ....................................................................................................................  600
    2       481 ....................................................................................................................  600
    3       481 ....................................................................................................................  600
B3-1        481 ....................................................................................................................  600
    2       480 ....................................................................................................................  599
    3       481 ....................................................................................................................  600

Consensus   601 CTCTCCATGCATTTGGTATTTTCGTCTGGGGGGTGTGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCTATGTCGCAGTATCTGTCTTTGATTCCTGCCTCATTCTATTATT  720
B1-1        601 ....................................................................................................................  720
    2       601 ....................................................................................................................  720
    3       600 ....................................................................................................................  719
B2-1        601 ....................................................................................................................  720
    2       601 ....................................................................................................................  720
    3       601 ..................................-.................................................................................  719
B3-1        601 ....................................................................................................................  720
    2       600 ....................................................................................................................  719
    3       601 ..................................-.................................................................................  719

Consensus   721 TATCGCACCTACGTTCAATATTACAGGCGAACATACTTACTAAAGTGTGTTAATTAATTAA  781
B1-1        721 ..........................................................  781
    2       721 ..........................................................  781
    3       720 ..........................................................  780
B2-1        721 ..........................................................  781
    2       721 ..........................................................  781
    3       720 ..........................................................  780
B3-1        721 ..........................................................  781
    2       720 ..........................................................  780
    3       720 ..........................................................  780
```

SN PK Femur

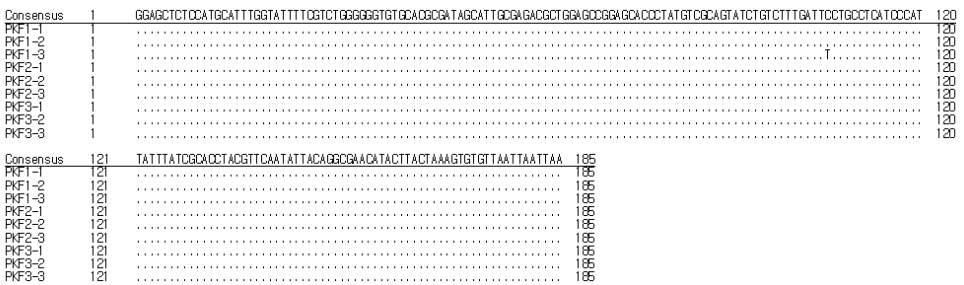
PS1(16009-16234)



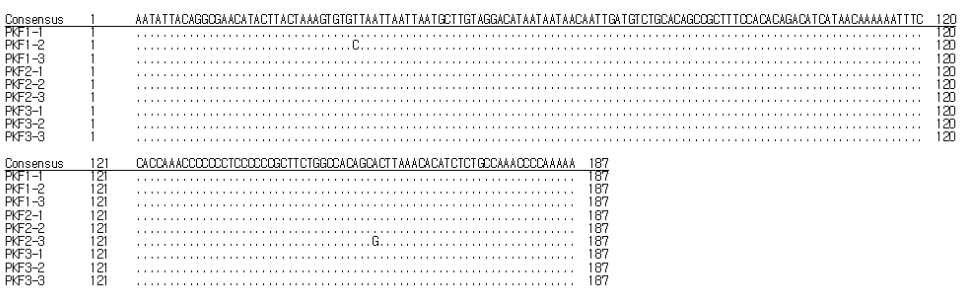
PS2(16210-16390)



PS3(35-219)



PS4(175-361)



SN PK Brain

PS1(16009-16234)

```
Consensus   1    TTAAACTATTCTCTGTTCTTTCATGGGGAAGCAGATTTGGGTACCACCCAAGTATTGACTCACCCATCAACAACCGCTATGTATTTCGTACATTACTGCCAGCCACCATGAATATTGTAC  120
B1-1        1    ....................................................................................................................  120
   2        1    ....................................................................................................................  120
   3        1    ....................................................................................................................  120
B2-1        1    ....................................................................................................................  120
   2        1    ....................................................................................................................  120
   3        1    ....................................................................................................................  120
B3-1        1    ....................................................................................................................  120
   2        1    ..............C.....................................................T.............................................  120
   3        1    ....................................................................................................................  120

Consensus   121  AGTACCATAAATACTTGACCACCTGTAGTACATAAAAACCCAATCCACATCAACCCCCCCCCCCC-ATGCTTACAAGCAAGTACAGCAATCAACCCTCAACTATAAC  225
B1-1        121  ....................................................................-...............................G....  226
   2        121  ....................................................................-...............................  226
   3        121  ....................................................................-...............................  226
B2-1        121  ....................................................................-..............................T....  226
   2        121  ....................................................................-...............................  226
   3        121  G...................................................................C...............................  227
B3-1        121  ....................................................................-...............................  226
   2        121  ....................................................................-...............................  226
   3        121  ....................................................................-...............................  226
```

PS2(16210-16390)

```
Consensus   1    ACAGCAATCAACCCTCAACTATAACACATCAACTGCAACCCCAAAGCCACCCCTCACCCACTAGGATACCAACAAACCTACCCACCCTTAACAGCACATAGCACATAAAGCCATTTACCG  120
B1-1        1    ....................................................................................................................  120
   2        1    ....................................................................................................................  120
   3        1    ....................................................................................................................  120
B2-1        1    ....................................................................................................................  120
   2        1    ....................................................................................................................  120
   3        1    ....................................................................................................................  120
B3-1        1    ....................................................................................................................  120
   2        1    ....................................................................................................................  120
   3        1    ....................................................................................................................  120

Consensus   121  TACATAGCACATTATAGTCAAATCCCTTCTCGTCCCCATGGATGACCCCCCTCAGATAGGG  181
B1-1        121  ............................................................  181
   2        121  ............................................................  181
   3        121  ............................................................  181
B2-1        121  ............................................................  181
   2        121  ............................................................  181
   3        121  ............................................................  181
B3-1        121  ............................................................  181
   2        121  ............................................................  181
   3        121  ............................................................  181
```

PS3(35-219)

```
Consensus   1    GGAGCTCTCCATGCATTTGGTATTTTCGTCTGGGGGGTGTGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCTATGTCGCAGTATCTGTCTTTGATTCCTGCCTCATCCCAT  120
B1-1        1    ....................................................................................................................  120
   2        1    ....................................................................................................................  120
   3        1    ....................................................................................................................  120
B2-1        1    ....................................................................................................................  120
   2        1    ....................................................................................................................  120
   3        1    ....................................................................................................................  120
B3-1        1    ....................................................................................................................  120
   2        1    ..............C.....................................................................................................  120
   3        1    ....................................................................................................................  120

Consensus   121  TATTTATCGCACCTACGTTCAATATTACAGGCGAACATACTTACTAAAGTGTGTTAATTAATTAA  185
B1-1        121  ............................................................  185
   2        121  ............................................................  185
   3        121  ............................................................  185
B2-1        121  ............................................................  185
   2        121  ............................................................  185
   3        121  ............................................................  185
B3-1        121  ............................................................  185
   2        121  ..........................................................G.  185
   3        121  ............................................................  185
```

PS4(175-361)

```
Consensus   1    AATATTACAGGCGAACATACTTACTAAAGTGTGTTAATTAATTAATGCTTGTAGGACATAATAATAACAATTGATGTCTGCACAGCCGCTTTCCACACAGACATCATAACAAAAAATTTC  120
B1-1        1    ....................................................................................................................  120
   2        1    ....................................................................................................................  120
   3        1    ....................................................................................................................  120
B2-1        1    ....................................................................................................................  120
   2        1    ....................................................................................................................  120
   3        1    ....................................................................................................................  120
B3-1        1    ....................................................................................................................  120
   2        1    ....................................................................................................................  120
   3        1    ....................................................................................................................  120

Consensus   121  CACCAAACCCCCCCTCCCCCCGCTTCTGGCCACAGCACTTAAACACATCTCTGCCAAACCCCAAAAA  187
B1-1        121  ............................................................  187
   2        121  ..........T.................................................  187
   3        121  ..........T.................................................  187
B2-1        121  ..........T.................................................  187
   2        121  ..........T.................................................  187
   3        121  ............................................................  187
B3-1        121  ............................................................  187
   2        121  ..........................................................T.  187
   3        121  ............................................................  187
```

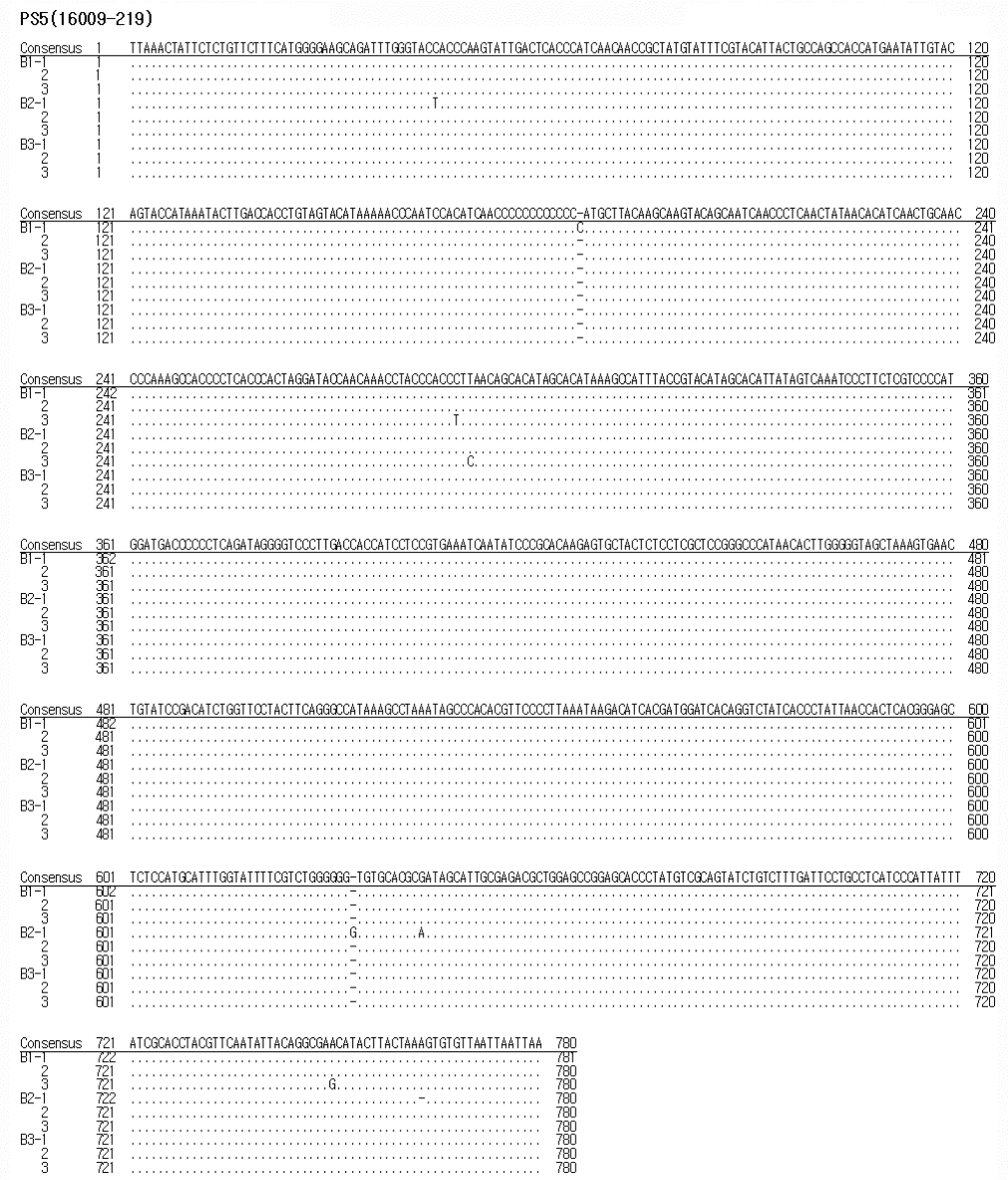
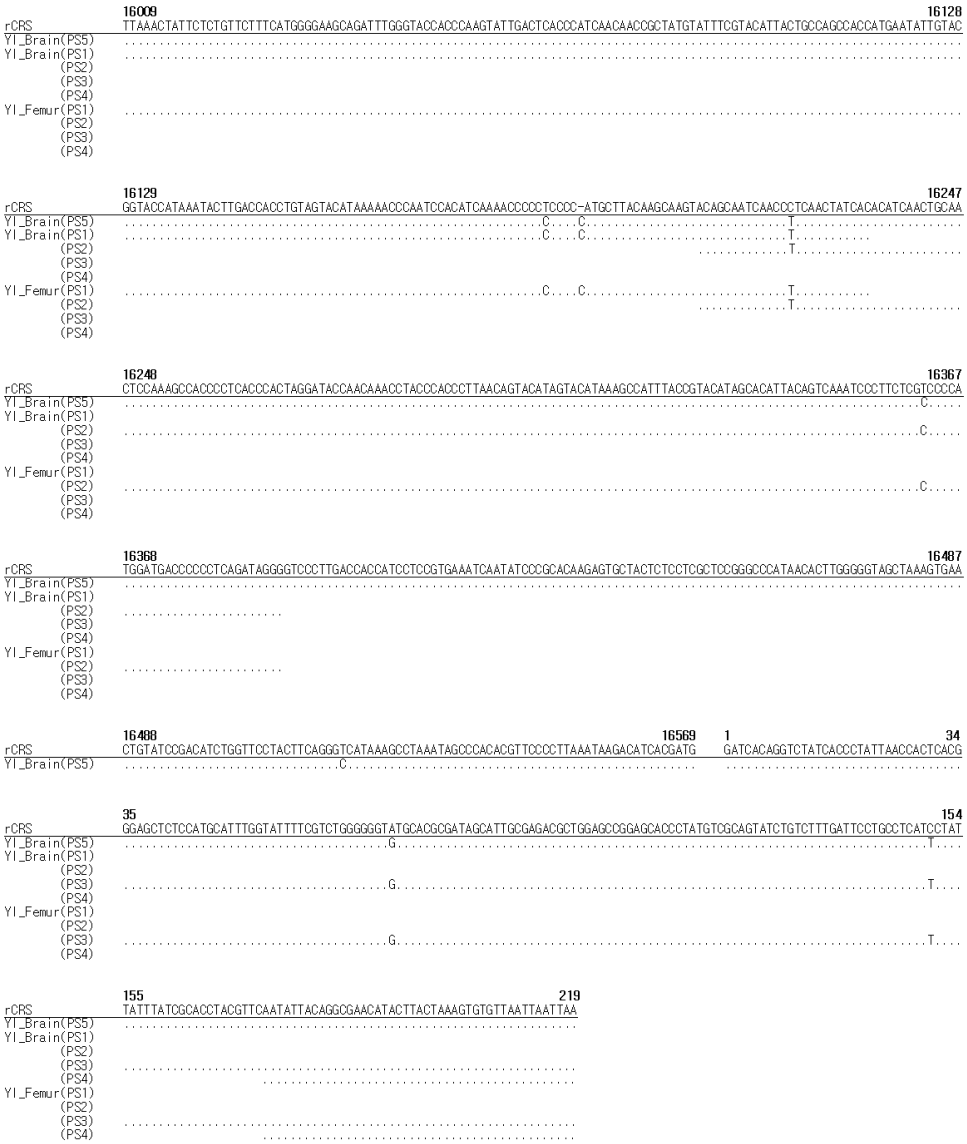


Figure 9. Sequence analysis results. PCR amplicons of PS1 (15989-16251, 263 bp), PS2 (16190-16410, 221 bp), PS3 (15-240, 226 bp), PS4 (155-381, 227bp), and PS5 (15989-240, 821 bp) mtDNA were cloned and sequenced. Consensus sequence could be determined by alignment of 9 individual clone sequences.

Figure 9. continued

PCR was repeated three times on the femur and brain of Yongin or SN PK mummy. rCRS, revised Cambridge Reference Sequence (accession number NC_012920).

Yongin mummy



SN PK mummy

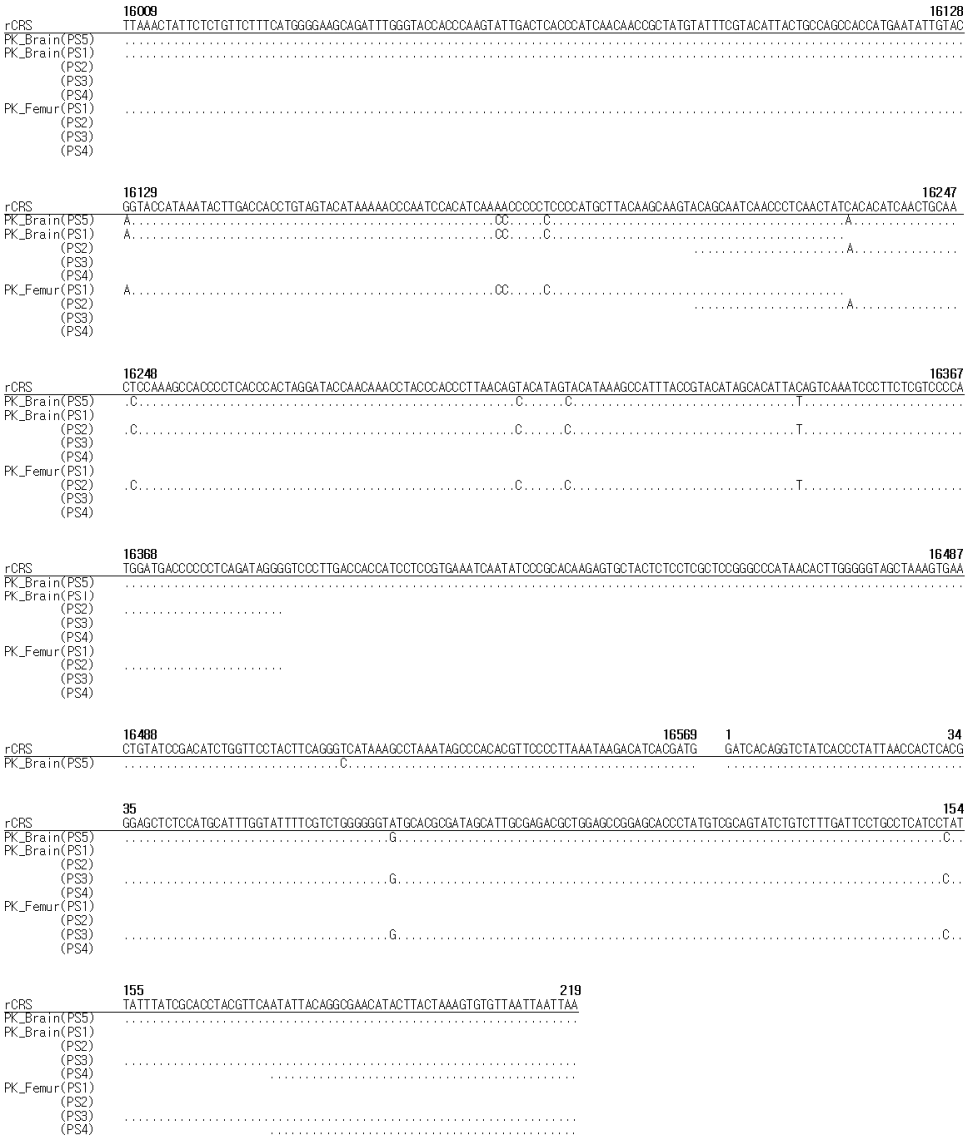


Figure 10. Sequence comparison result. The 821-bp mtDNA sequence of PS5 from brain samples were 100% identical to the correspondent sequences of PS1 (15989-16251, 263 bp), PS2 (16190-16410, 221 bp), PS3 (15-240, 226 bp), and PS4 (155-381, 227bp) fragments from brain and femur samples.

Discussion

A number of mummified brains have been discovered at archaeological sites in Korea, and various studies on them have shown that their typical morphological preservation state is good even after hundreds of years (Shin et al., 2003; Lee et al., 2007, 2009; Kim et al., 2008; Lim et al., 2008). However, there have been no studies showing the preservation status of aDNA in morphologically well preserved mummified brains.

This is probably due to skepticism that human brain tissue can be suitable for aDNA experiments. It is supposed that, as postmortem degradation occurs in the brain much earlier than in other human organs (Aufderheide and Rodriguez-Martin, 1998), biomolecules such as aDNA will be very poorly preserved therein. Generally, in Korea as in other countries, long bone such as femur have been the preferred materials for aDNA analysis. Actually, the femur has a more compact structure than any other skeletal components in the human body (Andelinović et al., 2005).

Even so, in this study, the preservation state of aDNA extracted from femur bone was not much better than that from mummified brain tissues. In a Quantifiler analysis, hTERT gene fragments taken from aDNA removed from brain tissue were amplified very

successfully, indicating that endogenous DNA in the mummified brain was less damaged than was that in the femurs from the same subject.

The preservation status of the aDNA in the mummified brain also was revealed in an amelogenin gene and autosomal STR analysis using the Minifiler kit. When I compared the outcomes of the autosomal STR analysis of the mummified brain with those of the femur from the same subjects, the number of consensus STR locus profiles from the mummified brain was far higher than in the case of the femur.

Like the Minifiler analysis, an mtDNA analysis also showed that aDNA from the mummified brain had a better preservation status than did that from the femur of the same individual. Briefly, in the case of the aDNA from the brain samples, the longer mtDNA fragments (PS5, 821 bp) were successfully amplified, whereas only the shorter DNA amplicons (PS1–PS4, 221–263 bp) were observed in the femur results.

In particular, in this experiment, the size of the fragmented mtDNA template remaining in the two mummified brains was at least 800 bp. In the autosomal STR analysis using the Minifiler kit, moreover, the size of the largest locus was up to 268 bp (Applied Biosystems, 2006). Therefore, further studies on the preservation

state of nuclear DNA extracted from mummified brain tissue are needed.

In this study, using aDNA extracted from mummified brain and femur samples representing the same individual, I investigated whether endogenous and authentic DNA was present in the mummified brains and whether genetic analysis could be performed successfully on aDNA. Through this series of studies, I confirmed that aDNA extracted from mummified brains was more valuable to the genetic analysis than that taken from the femur.

In the future, whenever a well-preserved mummified brain is found, it might be a good idea to utilize it for successful genetic analysis.

CHAPTER 3

A study on the usefulness of aDNA analysis in sex determination of ancient human remains

Introduction

In anthropological studies on archaeological human remains, sex determination is an essential step in the identification process. In archaeological studies, in most cases, sex determination has been based on cultural information (Vaňharová & Drozdová, 2008; Reinhold, 2003).

However, cultural and anatomical information sometimes cannot provide sufficient data for authentic sex determination, or in other cases, the cultural and anatomical evidence is discordant. In those cases, aDNA analysis can, as previous studies have proved, play a decisive role. For example, many PCR markers designed for amplification of X- or Y-chromosomes have been integral to successful sex determination (Nakahori et al., 1991; Akane et al., 1992; Bailey et al., 1992; Sullivan et al., 1993; Faerman et al., 1995; Butler, 2005). Among them, amelogenin gene analysis has been widely used in determining the sex corresponding to skeletons (Stone et al., 1996; Faerman et al., 1995; Cipollaro et al., 1998).

The sex determination of pre-modern Korean skeletons also has been performed with reference to cultural remains (e.g. clothing). Other information, for example archaeological indications, such as the reciprocal locations of coffins, has provided particularly strong

clues, given that a husband's coffin generally was on the right side of his wife's during the Joseon Dynasty period (1392–1910) (Fig. 11). Anatomical features also have been utilized by Korean physical anthropologists seeking to determine the sex of archaeological human remains.

However, when using the employed technique, the possibility of misdiagnosis cannot be completely excluded. For example, in cases of sex determination based on the relative husband and wife coffin locations in two-person tombs, coincidence between historical-documentary evidence and real objects is not always found.

Indeed, because the seriousness or consistency of that particular Joseon funeral rite has not yet been established, the possibility of exceptional cases in which the husband was not actually buried on the right side of his wife cannot be ruled out. However, what should be emphasized here is the fact that there are few reports on the degree of coincidence among the sex determination techniques commonly used in Korea.

In this study, therefore, I performed sex determination on archaeological human remains using cultural, anatomical and molecular biological techniques, and I evaluated the concordance between them. Subsequently, I tried to identify the most useful method for sex determination of human remains buried during the

Joseon Dynasty.

Materials and methods

A total of 34 skeletons discovered at eight archaeological sites were examined in this study. Fourteen skeletons were obtained from Sinnae, eight from Eunpyung, six from Shiheung, and one or two each at Seochen, Hadong, Yongin, Waegwan, and Gongju (Fig. 12).

Where a wife and husband were buried in the same tomb, cultural sex determination, based on the reciprocal locations of the coffins (i.e. the husband positioned to the right of his wife), was conducted. I also considered any cultural artifacts (e.g. clothes) as clues (Table 9, Fig. 12).

Sex determinations were made also on the basis of morphological differences manifest in the pelvic bone. To determine the pelvic dimorphism, for example, I examined the greater sciatic notch, the pre-auricular sulcus, the ischiopubic ramus, the subpubic angle, the subpubic concavity, and the ventral arc (Phenice, 1969; Kromgan and Iscan, 1986). When any of these sex indicators failed to show clear characteristics of either sex, I deemed the case borderline. Ancillary indicators considered were skull structures, specifically the nuchal crest, the matoid process, the supraorbital margin, the glabella, and the mental eminence (Buikstra and Ubelaker, 1994;

Ubelaker, 1999).

Bone samples were removed from the mid-shaft of the femur, as is consistent with the previous methods (Kolman and Tuross, 2000; Wurmb-Scwark et al., 2003; Gilbert et al., 2005). Long bone (e.g. femur) normally is used in aDNA analysis, owing to the fact that the bony structure is much more compact than anywhere else in skeletons (Andelinović et al., 2005).

To minimize modern DNA contamination, I followed the Criteria of Authentication suggested by Hofreiter et al. (2001) and Willerslev and Cooper (2005). Accordingly, the surfaces of the bones were scraped off using a sterilized blade. The bones were then decontaminated with 10% commercial bleach solution, and subsequently exposed to UV irradiation for 20 min. Pulverization by a SPEX 6750 Freezer/Mill (Metuchen, NJ, USA) reduced the bones to powder, some of which (0.3-0.5 g) was incubated in 1 ml of lysis buffer (50 mM of EDTA; 1mg/ml of proteinase K; 1% SDS; 0.1M of DTT) at 56℃ for 24 hr. Total DNA was extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1), followed by treatment with chloroform/isoamyl alcohol (24:1). DNA isolation and purification were performed using a QIAmp PCR purification kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. DNA extraction was repeated twice for each sample.

A Quantifiler® human DNA quantification kit assay (Applied Biosystems, Foster, CA, USA) which measure the total amount of amplifiable human nuclear DNA by amplifying of a 62-bp-sized human telomerase reverse transcriptase gene (hTERT) and internal PCR control DNA (IPC), was performed on the extracted DNA.

Amelogenin gene amplification was performed with an AmpFlSTR MiniFiler PCR Amplification Kit (Applied Biosystems, Foster, CA, USA). Briefly, after sample DNA (10 μl) was mixed with AmpFlSTR MiniFiler Master Mix (10 μl) and AmpFlSTR MiniFiler Primer set (5 μl), the PCR amplification was driven by the PTC-200 DNA Engine (Bio-Rad Laboratories, Hercules, CA, USA). The thermal cycling conditions were as follows: the initial incubation step, at 95℃ for 11 min; denaturation at 94℃ for 20 sec; annealing at 59℃ for 2 min, and extension at 72℃ for 1 min. After 35 cycles, the final extension proceeded at 60℃ for 45 min. The PCR product was analyzed with the ABI PRISM 3130 Genetic Analyzer and Gene Mapper Software (Applied Biosystems, Foster, CA, USA).

Results

The sex determinations based on the cultural, anatomical and aDNA data are summarized in Figure 13 and 14, and Tables 10, 11 and 12.

Of the 34 skeletons, 11 (32.3%) showed perfect matches among the three methods (Table 10). Seven cases (20.6%) exhibited discordances. The remaining 16 samples (47.1%) could not be resolved by amelogenin-gene analysis in this study, even in cases where both the cultural and anatomical approaches showed positive results individually as well as perfect matches between them (Table 13).

As in other cases of two-person tombs, two samples (SN 2-15-2 and SN 2-15-1) were considered to be a wife and husband. According to prevailing archaeological opinion, the individual on the right side should have been the husband. However, suspicion arose when, on anatomical examination, a hip bone from SN 2-15-2 exhibited male characteristics and, furthermore, a typical female specimen was found in the other, SN 2-15-1 coffin. The contradiction was solved by an amelogenin assay, which showed that SN 2-15-2 was in fact XY, and SN 2-15-1 XX. Thus, aDNA analysis had corroborated the anatomical determination while disproving the cultural one (Table 11, Fig. 15).

Another type of disputed case also was discovered among samples (SN 2-19-2, SH 2-10-2, EP C-10-1, EP 188-2 and SN 1-2): the anatomical sexes were borderline cases, whereas the cultural evidence had been definitive for both sexes. In all of them

except for one instance (EP C-10-1), the amelogenin gene could be successfully amplified, the sexes determined by the amelogenin assay being 100% identical to those based on the cultural evidence (Fig. 15).

Table 9. Sex determination by cultural evidences

Name	Reciprocal Location in burial pit	Cloths	Funeral banner	Final Evaluation
SN 1-1-1	M	-	M	M
SN 1-1-2	F	-	F	F
SN 2-15-1	M	-	-	M
SN 2-15-2	F	-	-	F
SN 2-19-1	M	-	M	M
SN 2-19-2	F	-	-	F
SN 3-7-1	M	-	-	M
SN 3-7-2	F	-	-	F
SN 4-18-1	M	-	-	M
SN 4-18-2	F	-	-	F
SN 4-25-1	M	-	-	M
SN 4-25-2	F	-	-	F
EP C-10-1	M	-	-	M
EP C-10-2	F	-	-	F
EP 2-43-1	M	-	M	M
EP 2-43-2	F	-	F	F
EP 188-1	M	-	-	M
EP 188-2	F	-	-	F
EP 379-1	M	-	-	M
EP 379-2	F	-	-	F
SH 2-3-1	M	-	-	M
SH 2-3-2	F	-	-	F
SH 2-10-1	M	-	-	M
SH 2-10-2	F	-	-	F
SH 2-14-1	M	-	-	M
SH 2-14-2	F	-	-	F
GJ 1-1-1	M	-	-	M
GJ 1-1-2	F	-	-	F
SN 1-2	-	M	M	M
SN PK	-	M	-	M
Yongin	-	F	F	F
Hadong	-	F	F	F
Seocheon	-	F	F	F
Waegwan	-	M	M	M

Table 10. Cases showing perfect matches among sex determinations by cultural, anatomical and molecular biological methods

Samples	Sex Estimation Methods			Quantifiler (pg/ul)
	Cultural	Anatomical	Amelogenin (aDNA)	
SN 2-19-1	M	M	XY	15.1
			X-	16.9
SN 4-18-1	M	M	XY	20.2
			XY	23.1
SN 4-18-2	F	F	X-	12.8
			X-	12.6
SN 4-25-2	F	F	X-	17.9
			X-	17.2
SH 2-10-1	M	M	XY	12.0
			XY	10.2
EP 188-1	M	M	XY	30.2
			XY	22.1
SN PK	M	M	XY	8.2
			XY	15.4
Yongin	F	F	X-	9.2
			X-	10.4
Hadong	F	F	X-	26.1
			X-	21.5
Seocheon	F	F	X-	13.3
			X-	28.7
Waegwan	M	M	XY	18.4
			XY	11.3

Table 11. Results of sex estimation for disputed cases

Samples	Sex Estimation Methods			Quantifiler (pg/ul)
	Cultural	Anatomical	Amelogenin (aDNA)	
SN 2-15-1	M	F	X-	5.69
			X-	
			X-	7.51
SN 2-15-2	F	M	XY	16.6
			-Y	
			-Y	11.2
SN 2-19-2	F	B	X-	11.5
			X-	14.1
SH 2-10-2	F	B	X-	35.0
			X-	24.2
EP C-10-1	M	B	--	0.34
			--	Undetected
EP 188-2	F	B	X-	11.3
			X-	15.1
SN 1-2	M	B	XY	16.5
			XY	13.7

Table 12. Cases not-confirmed by amelogenin gene analysis. Both the cultural and anatomical approaches were showed perfectly matching results between them.

Samples	Sex Estimation Methods			Quantifiler (pg/ul)
	Cultural	Anatomical	Amelogenin (aDNA)	
SN 1-1-1	M	M	--	Undetected
			--	Undetected
SN 1-1-2	F	F	--	1.48
			--	0.78
SN 3-7-1	M	M	--	1.96
			--	Undetected
SN 3-7-2	F	F	--	Undetected
			--	Undetected
SN 4-25-1	M	M	--	3.50
			--	Undetected
SH 2-3-1	M	M	--	Undetected
			--	Undetected
SH 2-3-2	F	F	--	2.81
			--	Undetected
SH 2-14-1	M	M	--	Undetected
			--	Undetected
SH 2-14-2	F	F	--	Undetected
			--	Undetected
EP C-10-2	F	F	--	Undetected
			--	Undetected
EP 2-43-1	M	M	--	0.20
			--	0.08
EP 2-43-2	F	F	--	0.34
			--	Undetected
EP 379-1	M	M	--	0.27
			--	0.87
EP 379-2	F	F	--	Undetected
			--	Undetected
GJ 1-1-1	M	M	--	0.34
			--	2.44
GJ 1-1-2	F	F	--	0.27
			--	Undetected

Table 13. Anatomical data for sex determination

Name	Major Criteria						Minor Criteria				
	Greater sciatic notch	Pre-auricular sulcus	Subpubic angle	Ischiopubic ramus	Subpubic concavity	Ventral arc	Nuchal crest	Matoid process	Supraorbital margin	Glabella	Mental eminence
SN 1-1-1	4	X	X	X	X	X	5	4	X	X	4
SN 1-1-2	1	present	X	X	X	X	1	1	X	X	2
SN 2-15-1	1	present	X	X	X	X	1	1	1	1	2
SN 2-15-2	4	absent	X	broad	absent	absent	2	4	1	2	1
SN 2-19-1	4	absent	narrow	broad	absent	absent	2	4	3	5	4
SN 2-19-2	1	present	wide	sharp	**absent**	present	1	1	2	1	3
SN 3-7-1	4	absent	narrow	broad	absent	absent	5	4	4	3	4
SN 3-7-2	2	present	X	X	X	X	4	2	X	2	3
SN 4-18-1	5	absent	X	broad	absent	absent	2	3	X	X	2
SN 4-18-2	2	X	X	X	X	X	X	1	2	1	2
SN 4-25-1	5	absent	X	X	X	X	3	2	X	X	X
SN 4-25-2	1	present	X	X	X	X	1	1	2	X	1
EP C-10-1	5	**present**	X	X	X	X	3	3	X	X	4
EP C-10-2	1	present	wide	sharp	present	present	1	1	X	x	1
EP 2-43-1	5	absent	narrow	broad	absent	absent	2	4	4	2	3
EP 2-43-2	1	present	wide	sharp	present	present	X	1	2	2	2
EP 188-1	5	absent	X	broad	absent	absent	3	1	X	X	2
EP 188-2	**3**	**absent**	X	X	X	X	2	1	1	1	2
EP 379-1	4	absent	X	X	X	X	3	3	2	2	3
EP 379-2	2	present	X	X	X	X	1	2	1	1	2

Table 13. continued

Name	Greater sciatic notch	Pre-auricular sulcus	Subpubic angle	Ischiopubic ramus	Subpubic concavity	Ventral arc	Nuchal crest	Matoid process	Supraorbital margin	Glabella	Mental eminence
SH 2-3-1	5	absent	X	X	X	X	2	3	X	X	X
SH 2-3-2	1	present	X	X	X	X	X	1	X	X	1
SH 2-10-1	5	absent	narrow	broad	absent	absent	2	4	2	2	3
SH 2-10-2	2	present	X	X	**absent**	**absent**	2	1	X	X	3
SH 2-14-1	5	absent	X	X	X	X	2	X	2	3	3
SH 2-14-2	2	present	X	X	X	X	4	1	X	X	2
GJ 1-1-1	5	absent	narrow	broad	absent	absent	4	3	4	2	3
GJ 1-1-2	2	present	wide	sharp	present	present	3	3	1	2	1
SN 1-2	5	**present**	X	X	X	X	1	4	2	1	2
SN PK	5	absent	X	broad	absent	absent	4	3	3	4	4
Yongin	2	present	wide	sharp	present	present	2	2	1	1	2
Hadong	2	present	wide	sharp	present	present	2	1	1	1	X
Seocheon	2	present	X	X	X	X	1	1	2	1	3
Waegwan	4	absent	narrow	broad	absent	absent	3	4	2	3	3

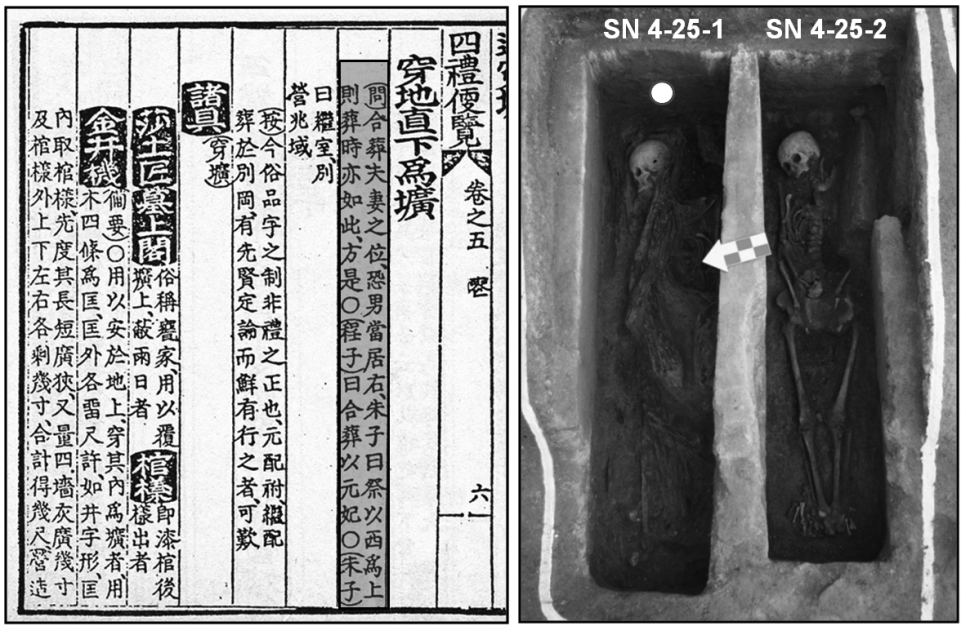


Figure 11. Relationship between historical documents and funeral rite. **(Left)** The provision for the reciprocal locations of wife and husband's coffins (shaded in red) described in the book for funeral ceremony (Saryepyeonlam published in 1844) of Joseon Dynasty. When the wife and husband's dead bodies were laid together in the same burial pit, the husband's coffin must have been situated in the right side of wife's coffin. **(Right)** For example, SN 4-25-1 was for a husband; SN 4-25-2 was for a wife. Husband (marked by a red dot) was buried in the right side of his wife.

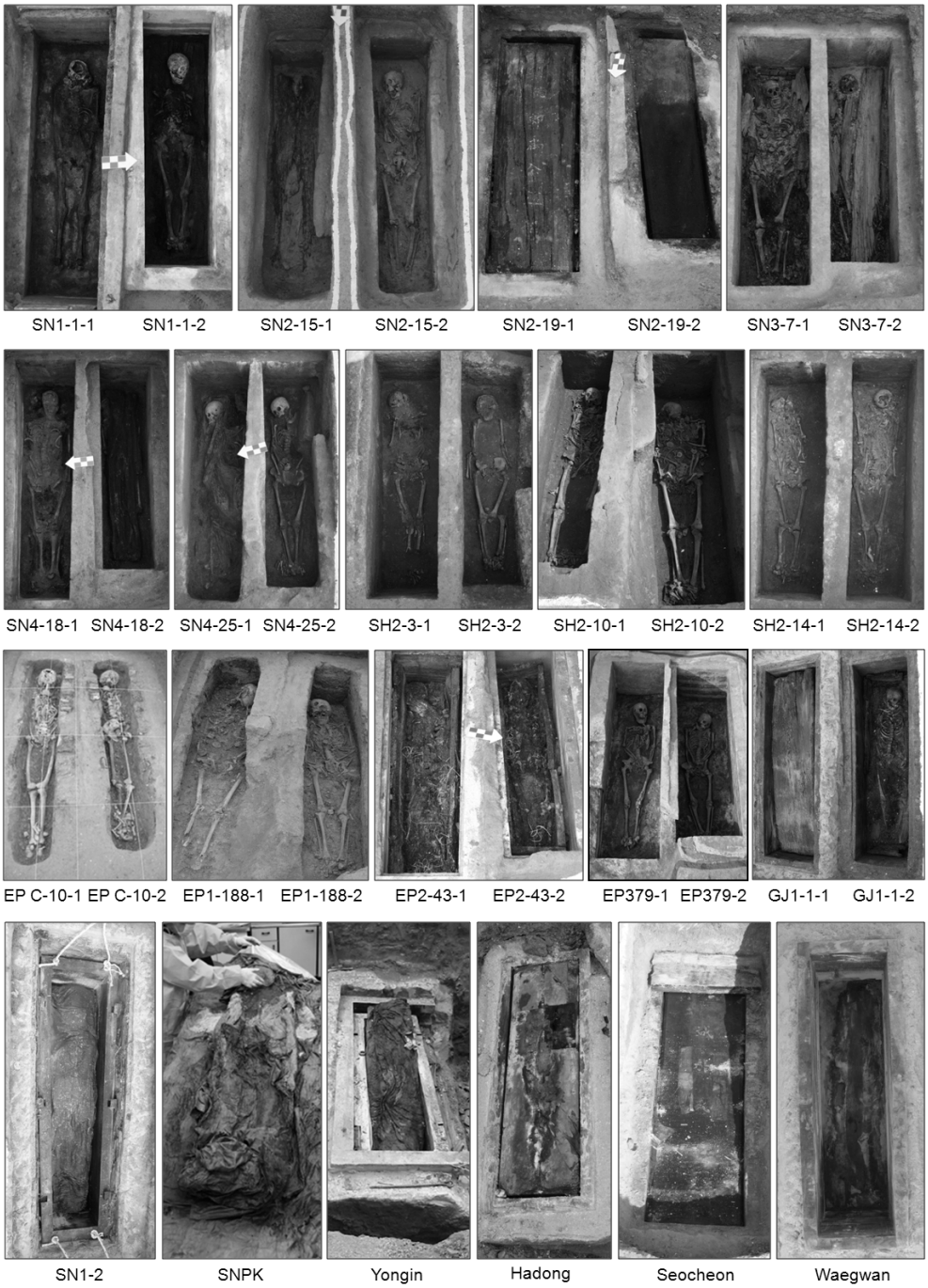


Figure 12. The tombs examined in this study. Archaeologists determined the sexes of skeletons based on the reciprocal

Figure 12. continued

locations of the coffins in the burial pit (the tombs in the first to third rows). Sexes of the other cases (in the fourth row) were determined by cultural remains (e.g. clothes) discovered from the coffin.

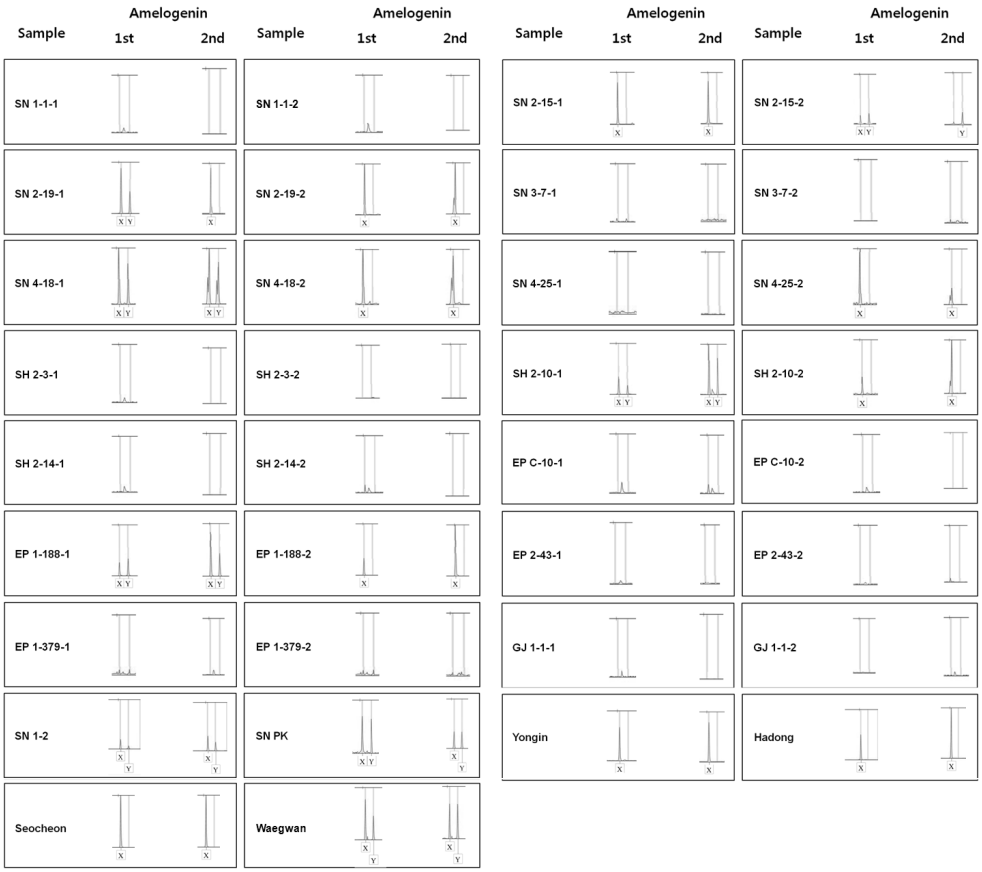


Figure 13. Results of amelogenin gene analysis used for sex determination. Experiments were repeated two times.

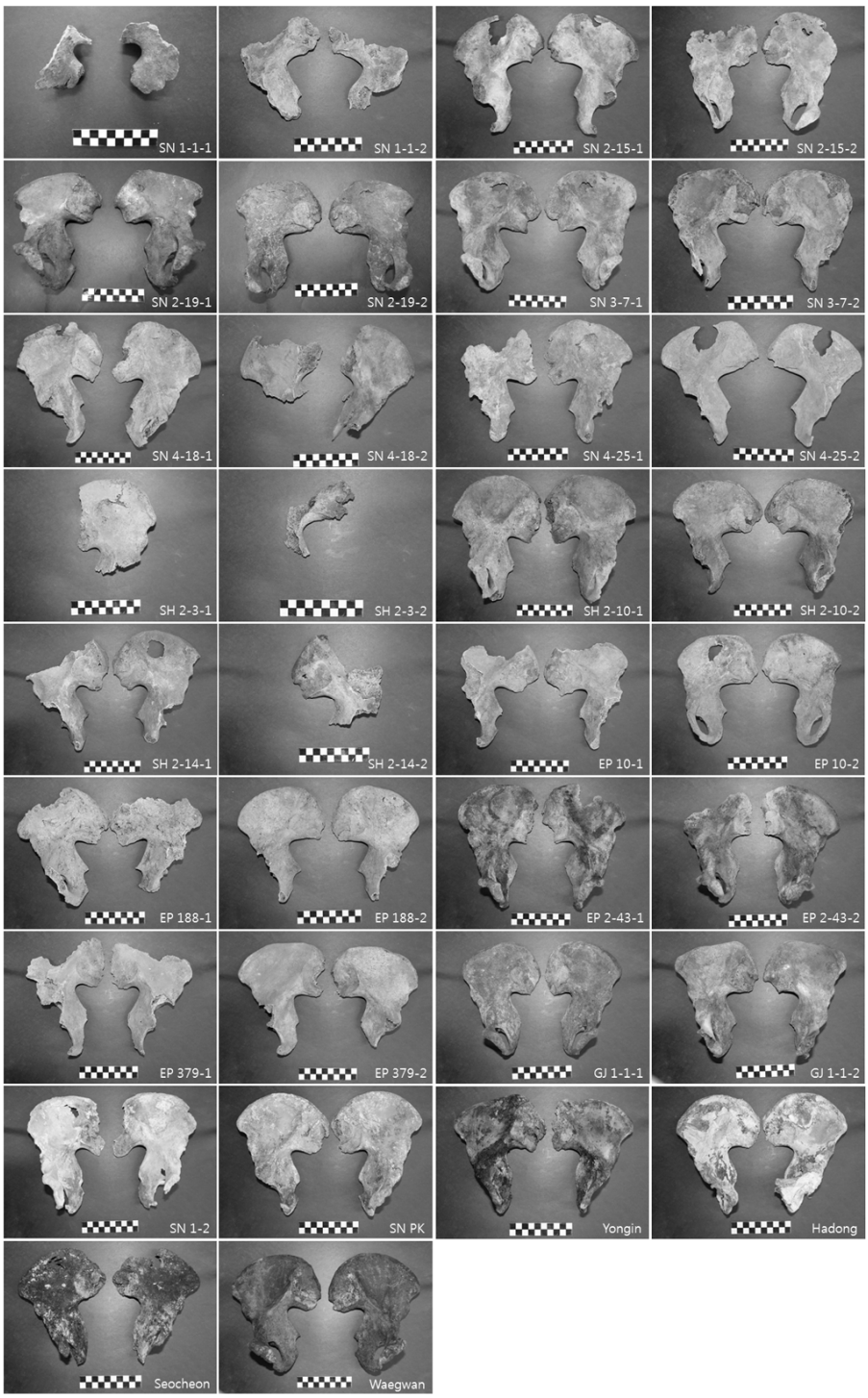


Figure 14. Morphologies of pelvic bones used for sex determination

Figure 15. A case that the result of sex determination does not match each other. Left-side coffin (SN 2-15-2) was actually the longer of the two. Anatomical examination showed that hip bone of SN 2-15-2 might be a male's. Analysis of amelogenin gene also confirmed that SN 2-15-1 was XX; and SN 2-15-2 was XY.

Discussion

In this study, I employed molecular techniques to confirm the culturally and anatomically determined sexes of archaeological human remains buried during Joseon Dynasty. Although 32.3% of the samples showed fully concordant results between the two sex-determination methods, the positive aDNA results necessary for corroboration of those findings were sometimes not obtained. Amelogenin aDNA could be successfully amplified by PCR only for about 50% of the samples.

PCR amplification of DNA extracted from ancient bones is generally known to have a relatively low success rate, due to DNA degradation by hydrolysis and oxidation occurring over time (Paabo, 1989; Hoss et al., 1996; Willerslev and Copper, 2005). However, the success rate of the amelogenin assay in the present study, even compared with other aDNA studies, was thought to be relatively low. As for the 700-year-old North American skeletons noted earlier, the success rate of the DNA-based sex determination was as high as 95% (19 out of 20 individuals; Stone et al., 1996). The other above-noted aDNA analysis of skeletal remains, the Israeli study, showed an 80% success rate (12 out of 15 cases).

The relatively unsuccessful amelogenin assay performed in the

present study might be explained by the poor preservation condition of archaeological sites in Korea. In fact, archaeological investigations undertaken over the past several decades have revealed that ancient Korean skeletons typically have been found in very poor states of preservation, even when compared with those discovered in the other East Asian countries. Even if confirmation will have to await additional scientific investigation, researchers suspect that the cause might be the acidic pH of the soil around organic remains in Korea (Jee et al., 2008).

Actually, the poor preservation status of the amplifiable DNA in the samples was well demonstrated in Quantifiler analysis as well. There, the amelogenin gene could be successfully analyzed only in the samples showing better Quantifiler results (Tables 10 and 11). Therefore, although sex determination by the amelogenin assay might be as sensitive as those based on cultural or anatomical clues, I should admit the relatively limited role that should be expected of aDNA analysis in confirmations of skeleton sex, especially skeletons discovered at Korean archaeological sites.

Notwithstanding, I can recite some merits of the use of the amelogenin assay in ancient-skeleton sex determinations conducted in Korea. Above all, I must note that, with regard to the Table 10 data, the discordances among the anatomical, cultural and

molecular sex determinations were very minor in cases where the amelogenin assay results could be obtained by PCR. Also, as the Table 11 data shows, aDNA analysis can play the decisive role in sex determinations where anatomical and cultural indications prove discordant or where samples, according to anatomical criteria, are borderline cases. Taken together, where conclusions could be drawn by the molecular method, the pertinent data could be very helpfully complementary to the findings made using the anatomical and/or cultural techniques.

CHAPTER 4

A study on the funeral rites of Joseon society using the aDNA analysis to bone and teeth found in the same coffin

Introduction

Korean mummies have been discovered in a very unique type of tomb constructed during the Joseon Dynasty (1392–1910 CE). News of Korean mummies first surfaced in 1968; however, it was not until serious interdisciplinary collaboration began in 2001 among anatomy, physical anthropology, archaeology, history, forensic medicine, radiology and parasitology researchers, and others, that significant strides were made in unraveling the mysteries of Korean mummies (Shin et al., 2003, 2010, 2011, 2012, 2013; Chang et al., 2006a, 2006b, 2008; Kim et al., 2006, 2008; Lee et al., 2007, 2009a, 2009b, 2013; Lim et al., 2008, 2010; Seo et al., 2008; Oh et al., 2011, 2013; Kahila Bar-Gal et al., 2012). Indeed, Korean mummies have been found to be so nearly perfectly preserved that they have yielded medical clues to the health and disease status of Joseon populations.

However, in Joseon tombs, Korean archaeologists have also discovered, besides the mummified human remains, various types of cultural artifacts. Well preserved clothing, documents, and other textiles and objects relating to funeral rites were collected, and have been proved very significant to historians' evidentiary

reconstructions of Joseon culture. The funeral ceremony at that time was very thoroughly codified, strictly stipulated and consistently observed by Joseon society. And certainly, in the examinations of seemingly stereotypical artifacts obtained from Joseon tombs (Lee et al., 2013), I always have been amazed at how sincerely burial customs were followed.

Nonetheless, in my experience there were also cases, even if they did not occur commonly, in which archaeological findings did not exactly follow the common patterns of Joseon funeral rites as outlined in the texts. For instance, the texts suggest that husbands and wives, when buried together, should be positioned with the husband to the wife's right hand side. However, analysis of skeletal remains of paired individuals shows that this is not always the case. Such exceptions, however rare, have sometimes sparked serious academic disputes about the proper interpretation of anthropological findings from Joseon mummies (Kim et al., 2011).

Recently, I became aware of one such very interesting case. In the course of the examination of a mummy found in a several-hundred-year-old Joseon tomb, archaeologists discovered many teeth contained in a pouch. And correspondingly, the historical literature on the subject of Joseon funerals includes accounts and descriptions of pouches into which an individual's lifetime's

worth of lost teeth, clipped hair and/or nails were gathered, and left in the coffin with the body (Fig. 16). In spite of this documentary evidence, it was not easy for historians and archaeologists to accept that those teeth in fact belonged to the mummy that shared the same tomb. They considered that the teeth were too perfect to have remained buried for so many years after their loss. The conventional archaeological methods could not answer the question either affirmatively or negatively. In fact, as there was no available scientific evidence that such a provision in historical literature had been adhered to by Joseon society, aDNA analysis techniques became, as if by default, the final arbiter.

Materials and methods

On October of 2007, in Sinnae Dong of Seoul, a Korean mummy was discovered by archaeologists of the Hangang Institute of Cultural Heritage. The mummy was found in a Joseon tomb (SN1-2) that was completely sealed by lime-soil mixture barrier (LSMB). By a tree-ring dating analysis, the tomb was thought to have been constructed anytime between 1605 and 1733 CE (Hangang Institute of Cultural Heritage, 2009). The archaeological information on SN1-2 tomb is summarized in Figure 17. Briefly, when the LSMB was broken, cross bars were found upon the coffin lid (Fig. 17A). After the cross bars were removed, the coffin could be identified (Fig. 17B). The mummy was discovered in the coffin, heavily wrapped with clothing (Fig. 17C). When the coffin was lifted out of the burial pit (Fig. 18A), I saw that the lateral walls and bottom are also fortified by LSMB (Fig. 17D).

At first, there was not any information about who the dead person was. However, during the investigation of tomb, archaeologists found a banner draped upon the coffin, in which the clan name of mummy was inscribed. According to it, the individual belonged to Munhwa Yu clan, and worked for King's court as a high-level official (Gaeuidaebu). Since the post was reserved only for men, the

sex of the dead person was deemed likely to have been a male, subject to anthropological confirmation. The mummy was then moved to bioanthropology laboratory for more scientific studies.

Next, clothing removal was performed by the textile historians, wearing sterilized gowns, head caps, gloves and masks (Fig. 18B). The tools to be employed for clothing removal or biological sampling were sterilized before use. Suitable precautions were taken at every step of the process so as to minimize modern-DNA contamination. The clothing-removal procedures were recorded by textile historians. Nobody was permitted to contact with the clothing or human samples without permission.

During the clothing removal, I discovered the teeth within a pouch that was tethered to the lateral side of the body bundle (Fig. 19A and B). In the pouth, 32 permanent teeth (8 incisors; 4 canines; 8 premolars; and 12 molars) and one deciduous tooth (right mandibular M2) were founded (Fig. 19C). The mummy's mandible and maxilla were edentulous. Furthermore, since every alveolar socket was completely remodeled, the individual must have lost his teeth long before his death (Fig. 20).

Anthropological examination of the mummy was done after clothing removal, during which biological sampling was also performed for ancient DNA analysis. Stature was estimated by

Fujii' s method (1960). The dental-attrition age of the teeth obtained from the pouch was estimated by Takei' s method (1984). Histomorphometric analysis was undertaken on femoral bone following Han et al. (2009).

Brain, femur and teeth samples from SN1-2 Korean mummy were used for aDNA analysis in this study. Actually, since preservation status of deciduous teeth was not appropriate for aDNA analysis, only two permanent teeth of 49.53±7.0 years (Tooth A, left maxillary M3; Tooth B, left mandibular M3) and one permanent teeth of under 20 years (Tooth C, right maxillary M2) were used for aDNA analysis. The surfaces of the bone samples were scraped using a sterilized blade. After UV-irradiation for 20 minutes, the samples were immersed in 5.4% (w/v) sodium hypochlorite and subsequently air-dried in a clean space. The bones were then pulverized into a fine powder using a SPEX 6750 Freezer/Mill (SPEX SamplePrep, Metuchen, NJ, USA) (O' Rourke et al., 2000; Kemp and Smith, 2005). In case of tooth, after the neck of tooth was cut, dentine sample in the part of tooth root was obtained by pulverization with dental drill (Gilbert et al., 2004; Rohland and Hofreiter, 2007).

The brain tissue (0.2-0.3g), bone (0.3-0.5g) and tooth powder (0.1-0.2g) was incubated in 1 ml of lysis buffer (EDTA 50 mM, pH

8.0; 1 mg/ml of proteinase K; SDS 1 %; 0.1M DTT), at 56℃ for 24 hours. Total DNA was extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1), and then treatment with chloroform/isoamyl alcohol (24:1). DNA isolation and purification was performed using a QIAquick PCR purification kit (QIAGEN, Hilden, Germany). The purified DNA was eluted in 50 μl of EB buffer (QIAGEN, Hilden, Germany) (Yang et al., 1998; Casas et al., 2006; Blow et al., 2008; Calvignac et al., 2008).

We amplified the samples' extracted DNA using the Quantifiler™ kit (Applied Biosystems, Foster City, CA, USA), and estimated the total amount of amplifiable human DNA remaining in each. The Quantifiler results were analyzed by the 7000 system with SDS software version 1.2.3 (Applied Biosystems, Foster City, CA, USA). Autosomal STR analyses were repeated multiple times for each 10 μl sample (brain samples: 7 times; bone and teeth samples: 5 times) using the AmpF*l*STR MiniFiler kit (Applied Biosystems, Foster City, CA, USA). Then, the amplified products were analyzed on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Every procedure was performed in accordance with the respective manufacturer's instructions.

When STR allele profiles were reproducible in at least five of the

seven replicates (for brain) or three of the five replicates (for bone and teeth), they were regarded authentic, consensus profiles (Budowle et al., 2009). Peak height threshold for the MiniFiler was set at 50 relative fluorescence unit (RFU). To determine if the samples had incurred any modern DNA contamination, the autosomal STR profiles of the researchers involved in this study were determined (under the permission of Institutional Review Board of Seoul National University, H-0909-049-295), and then compared with the ancient sample STR profiles.

aDNA lab facilities were set up according to the Hofreiter et al. (2001) protocol: the DNA extraction/PCR preparation rooms were equipped with isolated ventilation as well as laminated flow hood. In addition, rooms completely separate from the main PCR lab were set up for aDNA extraction and PCR preparation, respectively. The other criteria for authentic aDNA analysis (Willerslev and Cooper, 2005) also were followed.

Results

In the CT radiography taken before clothing removal, I could see that some internal organs of the mummy were still preserved (Fig. 21A). Within the skull, I could find mummified brain, the size of which was remarkably reduced (Fig. 21B). Very interesting was small radiopaque structures placed beside the head (Fig. 21B to E). Although they looked like teeth, the exact nature could be only confirmed when the structures were directly examined after removal of clothing.

After clothing removal was finished, anthropological studies on the dead body started. I confirmed that individual was a male, based upon non-metric features of skull and hip bone, corroborating the archaeologists' opinion based on the text inscribed on the banner. By Fujii's method (1960), the stature was estimated to be 168.65 cm (left femur length=45.5 cm). The quality of mummification fell short of my expectation, as many parts of body were already skeletonized even though skin, lung, brain, and intestine were successfully mummified (Fig. 22).

By histomorphometric analysis on anterior cortex of the femur (Han et al., 2009), his age at death was estimated to be 67.5±7.0 years. On the other hand, when the age was estimated by the dental

attrition (following Takai' s 1994 method), each permanent tooth from the pouch did not show the identical age. The right permanent maxillary first and second molars might have been lost when he was yet under 20 because their root apices were still open. Except for these, the rest of permanent teeth must have been lost around the same age (49.53±7.0 years) because root lengths in the teeth were nearly identical to each other. I note the difference in the age estimation by femur histomorphometry (67.5±7.0 yrs) and by teeth attrition (49.53±7.0 years; under 20 years; deciduous). The most reasonable explanation for the difference is that the individual kept every tooth for the rest of his life after he lost his teeth at different ages. This suggestion can be tested using scientific analysis.

In order to test the hypothesis that Joseon funeral custom, as documented by historical texts, was strictly stipulated by Joseon society, particularly that individuals should keep their teeth after loss and that the teeth should be buried with them, I performed aDNA analysis on the remains. To confirm the texts, I must show if the teeth showing different age estimation (49.53±7.0 years; under 20 years; and deciduous) originated from the same person who buried in the tomb. To prove this, I tried to see if aDNA profiles obtained from brain and skeletons were identical to those of teeth with different estimated ages.

First of all, I performed MiniFiler analyses on brain, femur and teeth samples. The overall results of the analyses are summarized in Table 14. Based on the amelogenin gene (AMEL) analysis on the extracted aDNA, the dead person was a male, corroborating the sex estimations by cultural or anatomical evidences (Phenice, 1969; Hangang Institute of Cultural Heritage, 2009). In the autosomal STR analyses, STR profiles from the brain sample were obtained in 48 out of 56 STR loci (85.7%); from the femur sample in 30 out of 40 STR loci (75%); the tooth A sample in 33 out of 40 STR loci (82.5%); the tooth B sample in 31 out of 40 STR loci (77.5%); and tooth C sample in 26 out of 40 STR loci (65%). Consensus profiles were obtained in 100% (8/8, brain), 62.5% (5/8, femur), 100% (8/8, tooth A), 87.5% (7/8, tooth B), and 87.5% (7/8, tooth C) of STR loci, respectively. Specifically, full concordance between the STR locus profiles of the brain, femur, teeth A, B and C was observed in 4 STR loci (D13S317, D16S539, D18S51 and CSF1PO). The difference observed in 3 STR loci (D7S820, D21S11 and FGA) were caused by allelic drop-outs, possibly as a result of processing only a small number of starting DNA templates for PCR (Table 15).

The authenticity of the STR profiles obtained from the brain, femur and teeth samples could be proven by checking the concordance between them and those of the researchers

participating in the research. Since there were no identical locus profiles between them (Table 16), I confirmed that the autosomal STR profiles obtained from ancient samples must have been endogenous.

Assuming the consensus profile obtain from brain sample is the full profile of the mummy, the random match probability of the sample from brain is 3.3×10^{-11} with the allele frequency data from Yoo et al., (2011). For the three teeth samples, allele drop-out events were observed. The allele drop-out rate varies with the quantity and quality of input DNA, and varies across the loci. Since the DNA quantities of the experiments for each tooth were close, the allele drop-out rate of each locus for each tooth could be estimated by the numbers of allele dropped out divided by the total number of alleles in all experiments for this tooth. For example, the allele drop-out rate at D21S11 for Tooth A is 4/10 = 40%. There is one allele dropped out at D21S11 for Tooth A. Therefore, the likelihood ratio (LR) of observing the genotype profiles, given the hypothesis that the first tooth and the brain are from the same individual compared with the hypothesis that these two samples are from different individuals, is 1.3×10^{9}. In the calculation, the LR of the locus without allele dropout is the inverse of the random match probability. For the locus D21S11, the LR is Pd/[P302+2P30(1-

P30)], where Pd is the allele drop-out rate (e.g., 40%) and P30 is the frequency of allele 30 in the modern Korean population (Yoo et al., 2011). With the same approach, the LRs for Tooth B and C are 1.3×10^{9} and 9.5×10^{5}, respectively. Combining other non-genetic evidence, such as the samples were collected from the same tomb, the LR should be higher. Therefore, it is highly likely these teeth belong to the mummy.

When I performed aDNA analysis on human mitochondrial hypervariable region 1 (HVR1), every mtDNA amplicons (PS1 and PS2) for HVR1 were successfully amplified with brain, femur and teeth samples (Fig. 23). I determined the consensus mtDNA sequence by cloning and sequencing of PCR amplicons (Fig. 24). The consensus mtDNA sequences from the brain, femur, and teeth were 100% identical to each other for the HVR1 regions (Fig. 25). The possible modern DNA contamination was ruled out by comparison of mtDNA haplotypes from SN1-2 samples and researchers who participated in this study. Since there were not any identical mtDNA haplotypes between them, contamination by modern DNA could be successfully ruled out (Table 17). Since the haplotype frequency of this mtDNA sequence is 0.0034 in Korean population (Lee et al., 2006, 2008), the LR, given the sequences the same maternal lineage instead of difference lineages in Korean

population, is 294.1 (LR=1/0.0034). Considering that the brain, femur, and teeth were collected from the same tomb, the chance that the brain, femur, and teeth examined in this study were from the very person who buried in the tomb is very high.

Table 14. STR analysis of brain, femur and teeth samples in this study

	Labs	Cycle #	PCR	AMEL	D13 S317	D7 S820	D2 S1338	D21 S11	D16 S539	D18 S51	CSF1PO	FGA	Quantifler (pg/ul)
Brain	A	30	1	X,Y	8,10	11,–	17,19	31,–	11,–	15,–	12,–	–	71.8
		30	2	X,Y	8,10	–	17,19	30,–	–	15,–	12,–	18,–	
		30	3	X,Y	8,10	–	17,19	30,31	–	15,–	12,–	18,23	67.03
		30	4	X,Y	8,10	11,–	17,19	31,–	11,13	15,–	12,–	18,23	
		30	5	X,Y	8,10	11,–	17,19	30,31	11,13	15,–	12,–	18,23	76.2
	B	30	1	X,Y	8,10	11,–	17,19	30,31	–	15,–	12,–	18,23	65.6
		30	2	–,Y	8,10	–	17,19	30,31	11,13	15,–	12,–	_	
	Cons			X,Y	8,10	11,–	17,19	30,31	11,–	15,–	12,–	18,23	
Femur	A	35	1	–,Y	8,10	–	17,19	29,30	11,–	15,–	–	18,–	16.5
		35	2	X,Y	8,–	–	17,19	–	11,–	15,–	12,–	23,–	13.9
		35	3	X,Y	8,10	11,–	17,–	–	11,–	15,–	12,–	–	11.1
	A	35	1	X,Y	8,–	–	17,19	–	11,–	15,–	12,–	23,–	10.5
		35	2	–	8,10	11	17,–	29,30	11,–	–	–	18,–	
	Cons			X,Y	8,10	–	17,19	–	11,–	15,–	12,–	–	
Tooth A	A	35	1	X,Y	8,10	–	17,19	31,–	–	15,–	12,–	18,23	26.3
		35	2	X,Y	8,10	11,–	17,19	29,30,31	–	15,–	10,12	18,23	
	A	35	1	X,Y	8,10	11,–	–	31,–	11,–	15,–	12,–	18,23	20.3
		35	2	X,Y	–	11,–	17,19	30,–	11,13	–	12,–	18,–	
		35	3	X,Y	8,10	11,–	17,–	31,–	11,13	15,–	–	18,–	
	Cons			X,Y	8,10	11,–	17,19	31,–	11,–	15,–	12,–	18,23	
Tooth B	A	35	1	X,Y	8,10	11,–	17,19	30, –	–	15,–	12,–	18,23	19.0
		35	2	X,Y	8,10	11,–	17,19	–	11,–	15,–	12,–	23,–	
	A	35	1	X,Y	8,–	–	17,–	–	–	15,–	12,–	18,–	18.4
		35	2	X,Y	–	11,–	17,–	–	11,–	15,–	–	23,–	
		35	3	X,Y	8,10	11,–	17,19	31,–	11,13	–	12,–	18,–	
	Cons			X,Y	8,10	11,–	17,19	–	11,–	15,–	12,–	18,23	
Tooth C	A	35	1	X, –	8, 10	–	17,19	–	–	–	12,–	–	8.4
		35	2	–	8,10	–	17, –	29	11,–	15,–	–	23,–	
	A	35	1	X,Y	8,10	11,–	17, –	–	–	15,–	12, –	–	16.4
		35	2	X,Y	8,10	11,–	17, 19	–	11,–	–	12,–	23,–	
		35	3	X,Y	–	11,–	17, –	30,–	11,–	15,–	12,–	23,–	
	Cons			X,Y	8,10	11,–	17, –	–	11,–	15,–	12,–	23,–	

Table 15. Comparison of STR analyses of brain, femur and teeth samples

Samples	PCR	AMEL	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
Brain	Cons	X,Y	8,10	11,–	17,19	30,31	11,13	15,–	12,–	18,23
Femur	Cons	X,Y	8,10	–	17,19	–	11,–	15,–	12,–	–
Tooth(A)	Cons	X,Y	8,10	11,–	17,19	31,–	11,–	15,–	12,–	18,23
Tooth(B)	Cons	X,Y	8,10	11,–	17,19	–	11,–	15,–	12,–	18,23
Tooth(C)	Cons	X,Y	8,10	11,–	17,–	–	11,–	15,–	12,–	18,–

Table 16. STR profiles of the researchers joining in the study

Researcher	AMEL	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
1	X,–	8,12	8,12	17,19	29,31	9,12	16,17	12,–	24,–
2	X,–	9,–	11,–	17,23	30,32.2	9,10	11,17	10,14	21,25
3	X,–	11,12	9,10	23,–	29,–	9,11	14,17	11,12	19,24
4	X,–	12,13	9,11	19,25	29,–	12,13	15,–	11,12	23
5	X,Y	8,11	11,–	20,25	29,31	9,12	15,–	10,–	22,23
6	X,Y	8,12	8,12	22,23	29,32.2	12,–	17,21	10,12	19,22
7	X,Y	10,12	10,–	21,25	30,31.2	9,11	13,14	11,12	22,24
8	X,Y	11,12	10,12	18,–	29,30	9,12	16,19	11,12	22,23
9	X,Y	8,13	11,–	18,20	30,31	10,11	15,–	11,12	22,24

Table 17. Comparison of mtDNA haplotypes from SN1-2 samples and researchers

Subject	Hypervariable Region 1 (16026-16390)
SN 1-2 Brain	16126C 16163G 16223T 16234T 16235G 16264T 16274A 16290T 16319A
SN 1-2 Femur	16126C 16163G 16223T 16234T 16235G 16264T 16274A 16290T 16319A
SN 1-2 Teeth(A)	16126C 16163G 16223T 16234T 16235G 16264T 16274A 16290T 16319A
SN 1-2 Teeth(B)	16126C 16163G 16223T 16234T 16235G 16264T 16274A 16290T 16319A
SN 1-2 Teeth(C)	16126C 16163G 16223T 16234T 16235G 16264T 16274A 16290T 16319A
Researcher 1	16183C 16189C 16220C 16254G 16298C 16362C
Researcher 2	16172C 16174T 16223T 16362C
Researcher 3	16126C 16231C 16266T 16319A 16362C

Figure 16. A histological document of ritual custom. In ritual books of Joseon Dynasty (Saryepyeonram, published in 1844, by Jae Yi), there was a provision about the preparation of a pouch in which teeth, hairs and nails would be contained for funeral ceremony. Every tooth lost must not discard at once, but should be collected in the small pouch, being buried within the coffin during funeral ceremony.

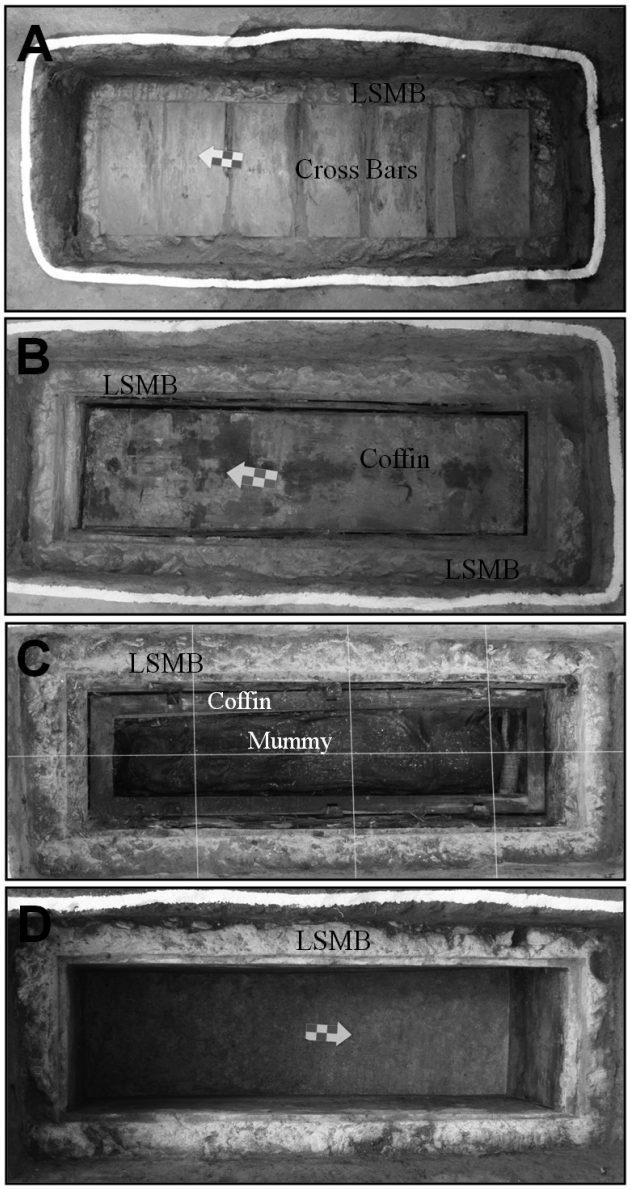


Figure 17. The archaeological information of SN1-2 tomb. (A) Cross bars are exposed. LSMB, lime soil mixture barrier. (B) Coffin lid is exposed after cross bars were removed. (C) Within coffin, mummy could be identified. (D) When the coffin was lifted out of the burial pit, the bottom made of LSMB could be also identified.

Figure 18. A mummified human remains wrapped with clothes. (A) SN1-2 coffin lifted out of the burial pit (B) Textile historians wore sterilized gowns, head caps, gloves and masks during removal of clothing from mummy.

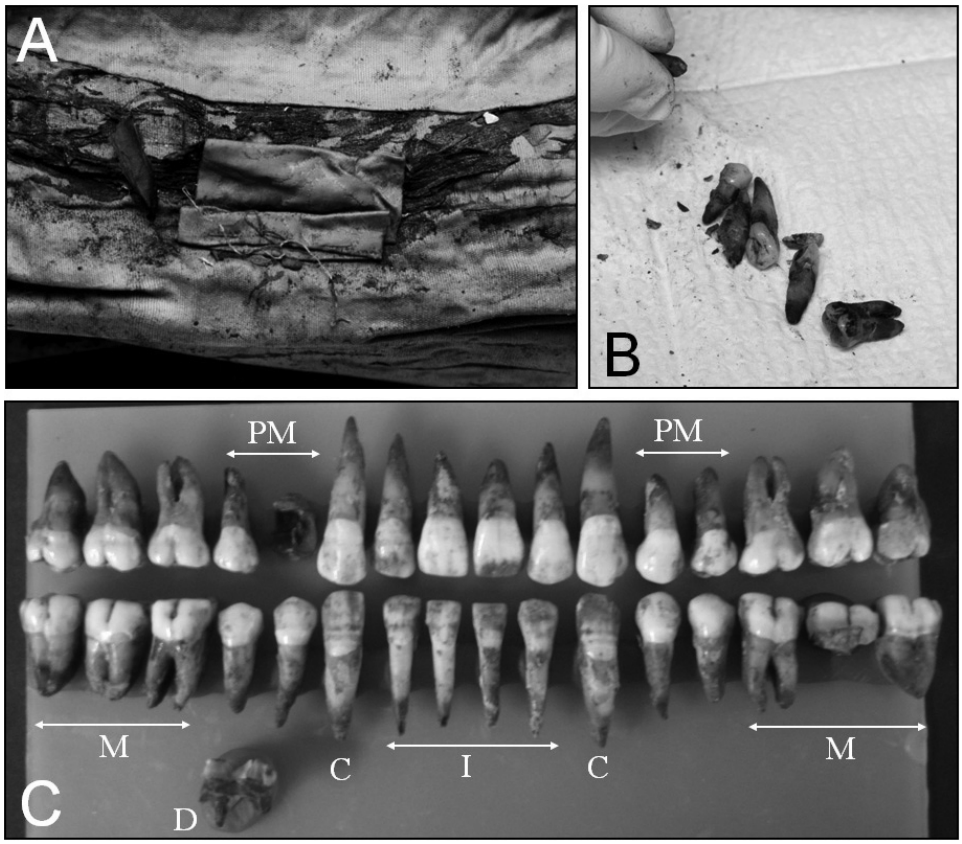


Figure 19. Teeth found in pouch. (A) A pouch discovered during removal of clothing. (B) The teeth were found within the pouch. (C) 32 permanent teeth were discovered without any missing. M, molar; PM, premolar; C, canine; I, incisor; D, deciduous tooth.

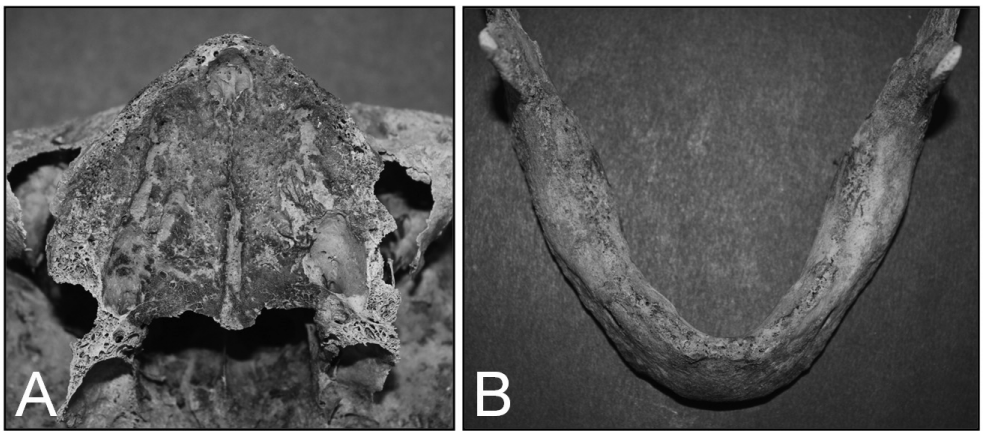


Figure 20. The (A) maxilla and (B) mandible picture of SN1-2 mummy. Every alveolar socket was filled already.

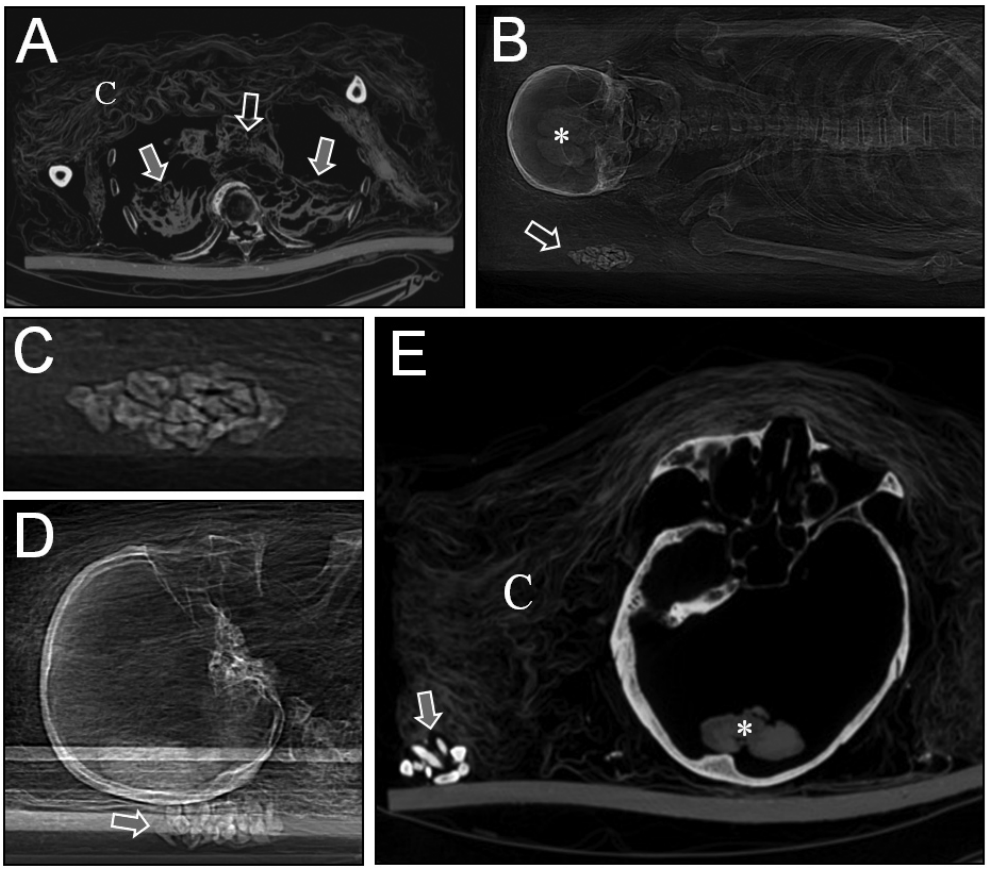


Figure 21. CT radiography taken before clothing removal. (A) Axial view of thorax. Mummified lungs are indicated by gray arrows. Heart is indicated by blank arrow. C, clothing wrapped around the body. (B) Brain (asterisk) could be seen in radiograph (P−A view). Small radiopaque structures (arrow) identified. (C) Magnified image of (B). The radiopaque structures looked like teeth. (D) Radiopaque structures (arrow) could be seen in lateral view. (E) Axial view of CT image. Radiopaque structures (arrow) could be clearly seen. Mummified brain (asterisk) is remained within skull.

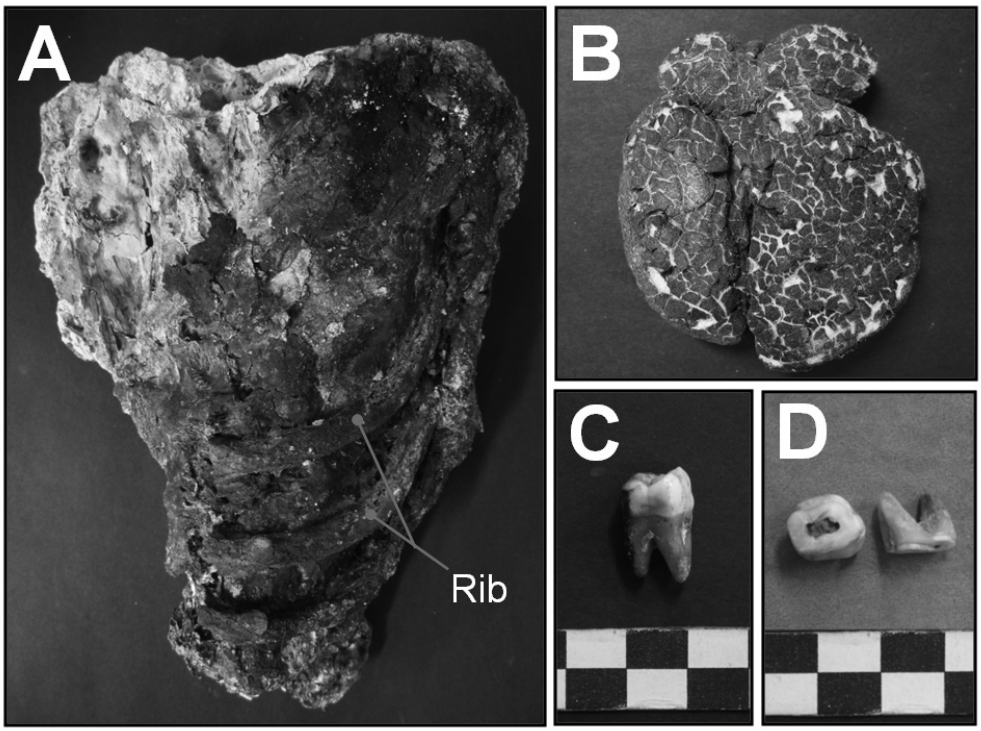


Figure 22. Mummified lung and brain of SN1-2 mummy, and teeth found in the same coffin. (A) and (B) Mummified internal organs of SN1-2 case. (A) Right lung. (B) Brain. Both samples are used for aDNA analysis in this study. (C) Left maxillary first molar. (D) Cut through the neck of the tooth for aDNA sampling.

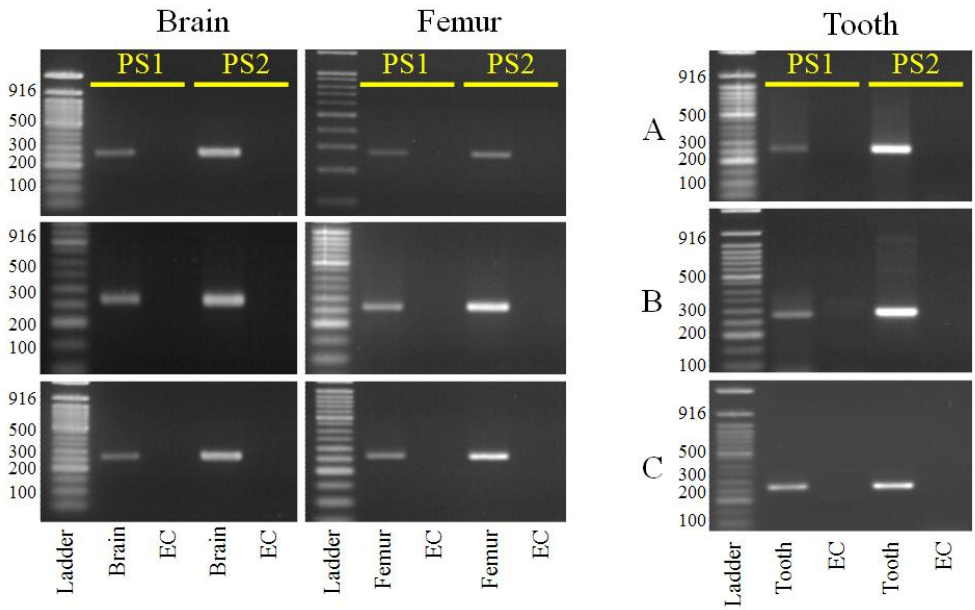
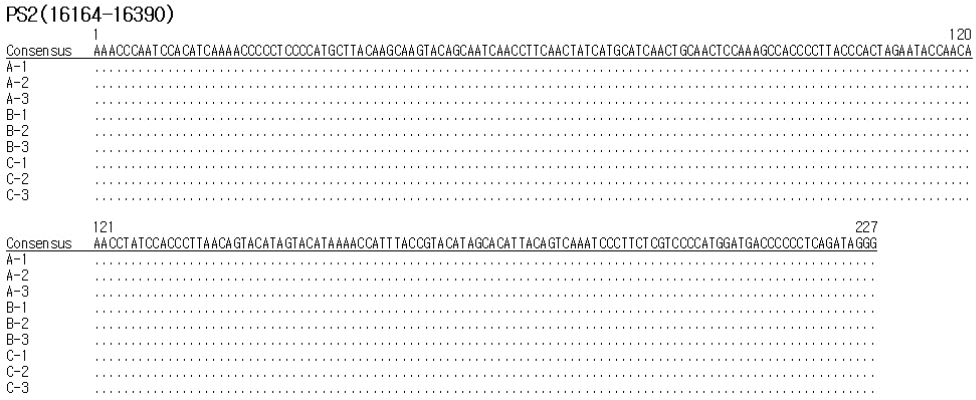


Figure 23. mtDNA amplicons from brain, femur and tooth samples (Lab A). The sizes of amplicons: PS1, 267 bp; PS2, 267 bp.

SN1-2 Brain

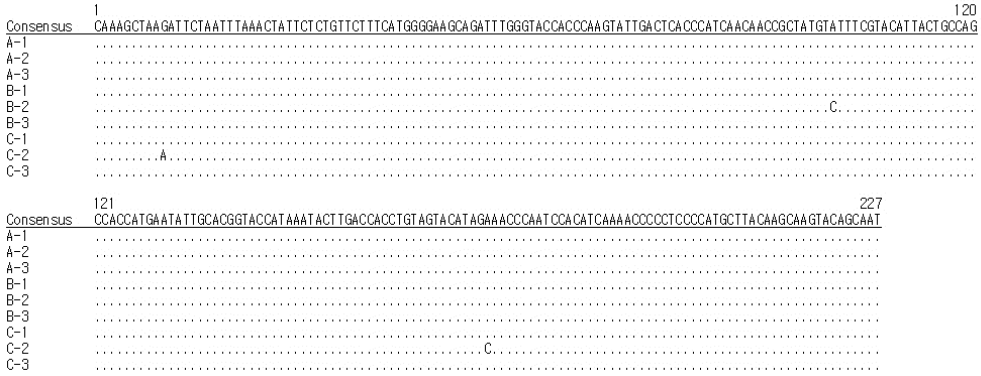
PS1(15991-16217)

PS2(16164-16390)

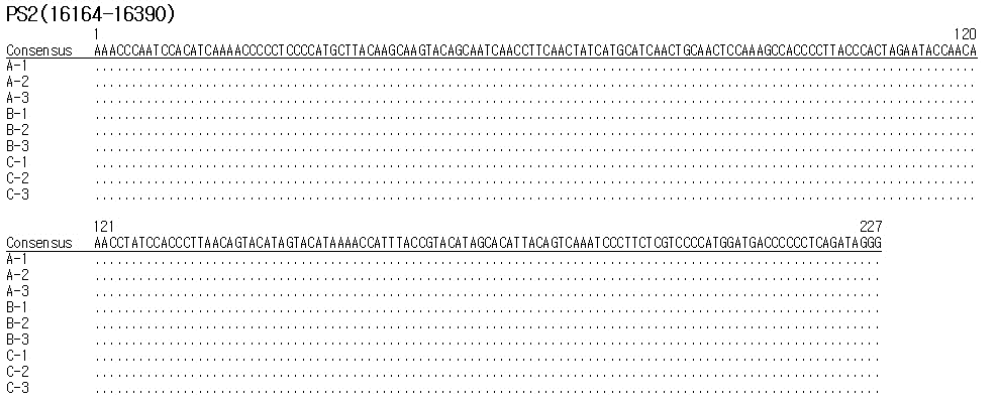


SN1-2 Femur

PS1(15991-16217)



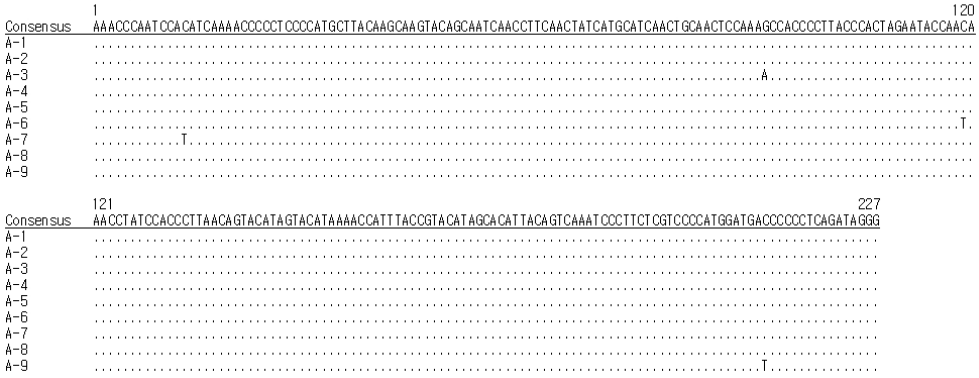
PS2(16164-16390)



SN1-2 Teeth (A)

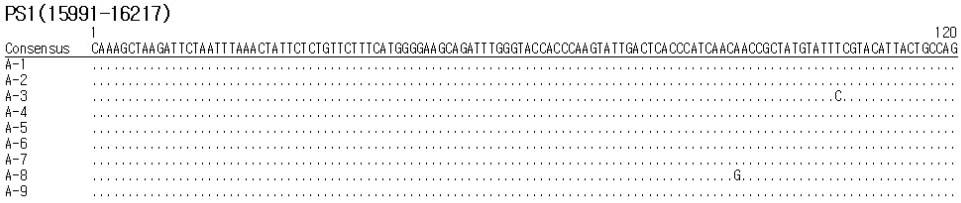
PS1(15991-16217)

PS2(16164-16390)

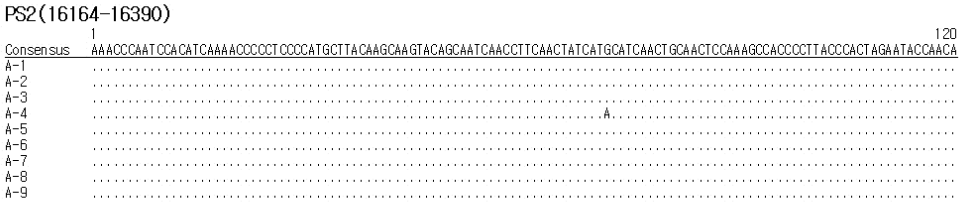


SN1-2 Teeth (B)

PS1(15991-16217)



PS2(16164-16390)



SN1-2 Teeth (C)

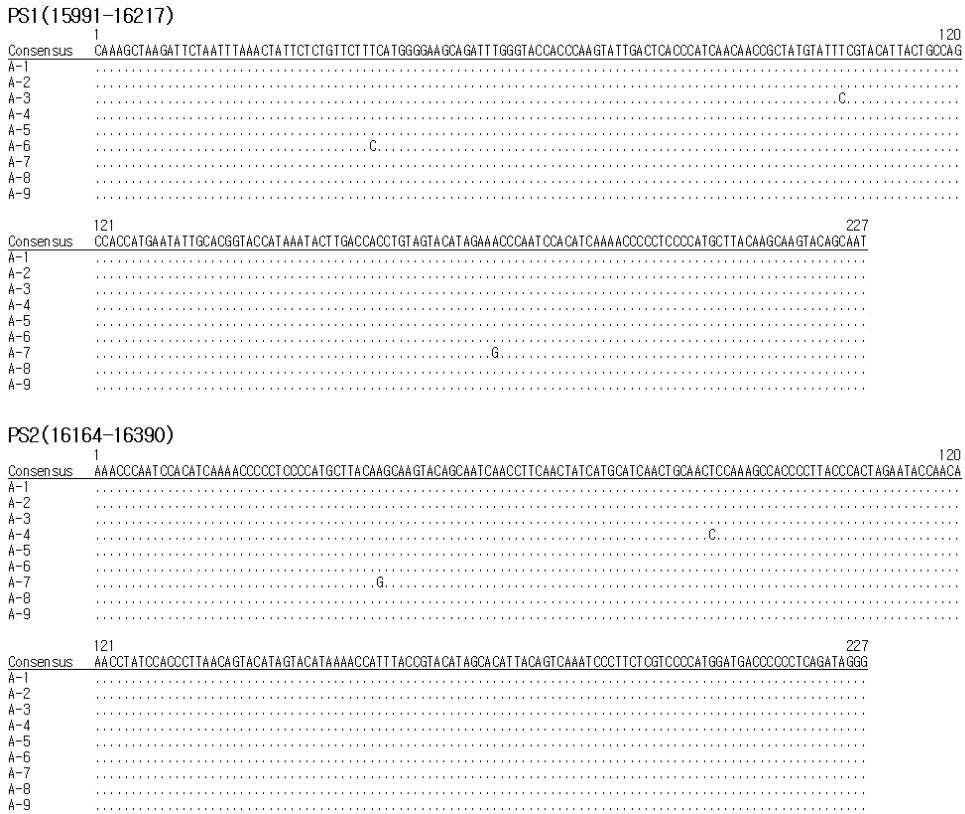


Figure 24. Consensus mtDNA sequence by cloning and sequencing of PCR amplicons for brain, femur and three tooth (A to C).

Figure 25. Comparison of the consensus mtDNA sequence from the brain, femur and teeth. Since they were 100% identical to each other, the tooth A, B and C must have originated from the very person who buried in the tomb.

Discussion

Recent developments in DNA typing techniques have widened their applications to include parentage and kinship testing, disaster victim identification (DVI), mass-graves investigations, missing-person cases, and still other areas (Butler, 2010). Similarly, aDNA analysis has begun to make significant contributions to the study of archaeologically obtained human samples (Marota and Rollo, 2002). The role of aDNA analysis, moreover, is not restricted to the realm of simple biology. aDNA typing has proved particularly attractive to scientists owing to its provision of genetic information on ancient human remains that is not easily or possibly obtainable by other techniques (Iwamura et al., 2004).

Significantly, aDNA analysis has also played a key role in the assessment of historical texts. As discussed above, cultural artifacts in large overall numbers have been discovered in Joseon tombs of Korea. Detailed examinations of those have revealed the intricacies of Joseon funeral rites and the solemnity with which they were adhered to by society. For instance, bodies in Joseon tombs were heavily clad preparatory to interment. In fact, in just one Joseon tomb, as many as 70 garments were collected for examination by textile specialists (Lee et al., 2013). These kinds of

investigations have yielded, and continue to expand on, a detailed and comprehensive history of Joseon clothing. Textile historians are beginning to learn that the numbers and types of shrouds discovered in the tombs had been strictly stipulated by the relevant ritual guidebooks of Joseon society. In every Korean mummy case studied thus far (Lee et al., 2009, 2013), the clothing discovered within the coffin has been arranged in a very fixed and deliberate manner, according to the standards of Joseon funerals. In the current case (SN1-2) likewise, many ritualistically arrayed clothing articles were found within the tomb.

However, as work on this case proceeded, I was amazed to discover a complete set of teeth, possibly lost at different ages, contained in a small pouch. According to Joseon texts, such pouches were maintained by individuals throughout their lifetime. Not only teeth but also hair or nail clippings were kept therein as they became available; and finally stored post-mortem within the coffin. This means that if those teeth actually had belonged to the mummy, he must have kept them for a long while, at least 20 years or more. However, it is also conceivable that the teeth belonged to a loved one or someone else important to the individual. In the absence of direct tests, neither conclusion can be definitely accepted.

Indeed, as mentioned in the introduction, my experience already

includes cases in which anthropological findings do not correspond well with the documentary evidence from on purportedly sacrosanct Joseon funeral rites. For example, according to the pertinent Joseon texts, when wives and husbands were buried together in the same tomb, the husband should be situated to the wife's right. But in some of the tombs investigated, the skeleton on the left side (archaeologically female) showed male characteristics on osteological examination and/or aDNA analysis (Kim et al., 2011). The lesson here was that healthy skepticism should be exercised whenever documentary evidence is applied to the interpretation of archaeological findings on Joseon mummies. Additionally, and even more importantly, my experience made clear that a supplementary analytical tool was needed for making hypotheses more reliable. Since DNA typing of ancient samples was for several years already established as an indispensable process in Korean mummy studies, I naturally turned to aDNA analysis to satisfy that need.

As in forensic DNA typing, aDNA analysis requires comparison between multiple samples: questioned (Q) samples and known (K) samples. In crime scene investigation (CSI) cases, Q samples (crime scene evidence) are always compared to a single or multiple K samples from suspects. Since my study was designed to reveal whether the teeth originated from the person who was buried in the

tomb, teeth (Q) samples were directly compared with reference DNA obtained from the SN1-2 mummy's brain and bone (K) samples.

However, Q-K comparison in the field of archaeology is complicated by the fact that the quality of samples is, at least in many cases, compromised. In the autosomal STR result, only four STR loci (D13S317, D16S539, D18S51, and CSF1PO) showed full concordance of loci profiles, and differences due possibly to allelic drop-outs were observed in an additional three loci (D7S820, D21S11, and FGA). Notably too, allelic drop-outs were commonly observed in previous studies (Oh et al., 2012; Lee et al., 2013). Still, whereas STR typing did not show full concordance between the two samples, the statistical calculation showed that the teeth in the pouch were highly likely to belong to the mummy under examination. Q-K comparison of the mtDNA sequence results, furthermore, showed almost 100% matches between the samples.

Conclusions drawn by the aDNA analysis can be supportive of, or contradictory to, archaeological or historical hypotheses (Kim et al., 2011). Certainly, this study is a successful example of aDNA analysis data having confirmed the historical texts which suggest that, individuals kept their own teeth for such a long time after their loss. As far as the current aDNA data are concerned, it is highly

likely that the teeth contained in the pouch originated from the mummy in the tomb. The outcome of the current study is significant not only to biological anthropologists, but also to archaeologists and historians.

Finally, it is important to note that DNA typing must be performed very carefully if reliable results are to be obtained (Butler, 2010). The authenticity of aDNA studies is seriously challenged by modern DNA contamination (Marota and Rollo, 2002). To help researchers avoid this pitfall, stringent protocols of aDNA analysis have been recommended by a number of pioneering researchers (Hofreiter et al., 2001). Even before aDNA analysis begins, sample collection and preservation are very vital steps, regardless of the DNA typing adopted. When ancient samples are not handled properly in these initial steps, final results are rendered inauthentic, and a lot of hard work is lost (Butler, 2010).

In this regard, an advantage of Korean mummy studies, particularly from the perspective of the collection and storage of ancient samples for authentic aDNA study, should be mentioned herewith. Joseon tombs offer near-perfect preservation owing to the presence and maintained integrity of a LSMB against contamination from the outside; correspondingly, Joseon mummies are, almost without exception, spectacularly well preserved. Hence

it is very important that clothes wrapped around a Korean mummy be removed by well-trained technicians wearing sterilized clothes and using disposable tools under well-controlled lab conditions. In this way, and keeping to the minimum the number of researchers contacting samples, modern-DNA contamination of ancient samples can be avoided (Lee et al., 2013).

Conclusion

aDNA analysis is based on key techniques developed over the past few decades in the fields of molecular biology and archaeological science. In that time, aDNA study has emerged as a new discipline, having enabled great achievements in the fields of human and animal evolution, human migration, and even paleopathological studies. As the public interest in archaeological science has grown, so too has interest in aDNA study increased, particularly among anthropologists and archaeologists around the world.

Nowadays, aDNA analysis is a major field within the discipline of archaeology, and it is expected to prove useful as a complementary method for solving problems unsolvable by traditional techniques. In this regard, my thesis will be meaningful to concerned researchers interested in aDNA analysis on archaeological samples. In this work, I have clarified the typical preservation state of aDNA within Joseon samples and have explained the established aDNA analysis techniques. Additionally, I applied the technique established in this study to controversial samples difficult to resolve with other archaeological methods, and showed that disputes of those kinds can be resolved by aDNA analysis.

Considering that aDNA analysis is still relatively novel in Korea and that related analytic techniques are not well established, I hope that my thesis will be helpful to archaeological scientists interested in Korean aDNA studies.

Bibliography

Akane A, Seki S, Shiono H, Nakamura H, Hasegawa M, Kagawa M, Matsubara K, Nakahori Y, Nagafuchi S, Nakagome Y. 1992. Sex determination of forensic samples by dual PCR amplification of an X-Y homologous gene. Forensic Sci Int 52: 143-148.

Alaeddini R, Walsh SJ, Abbas A. 2010. Forensic implications of genetic analyses from degraded DNA--a review. Forensic Sci Int Genet 4(3):148-157.

Alenizi MA, Goodwin W, Hadi S, Alenizi HH, Altamar KA, Alsikel MS. 2009. Concordance between the AmpFlSTR MiniFiler and AmpFlSTR Identifiler PCR amplification kits in the Kuwaiti population. J Forensic Sci 54:350-352.

Amory S, Keyser C, Crubézy E, Ludes B. 2007. STR typing of ancient DNA extracted from hair shafts of Siberian mummies. Forensic Sci Int 166:218-229.

Andelinović S, Sutlović D, Erceg Ivkosić I, Skaro V, Ivkosić A, Paić F, Rezić B, Definis-Gojanović M, Primorac D. 2006. Twelve-year experience in identification of skeletal remains from mass graves. Croat Med J 46:530-539.

Anderson TJC 2001. The dangers of using single locus markers in

parasite epidemiology: *Ascaris* as a case study. Trends Parasitol 17: 183-188.

Applied Biosystems. 2006. AmpFlSTR® MiniFiler™ PCR Amplification Kit User Guide. Foster City, California: Applied Biosystems.

Applied Biosystems. 2006. Quantifiler™ Human DNA Quantification Kit, User's Manual. Foster City, California: Applied Biosystems.

Ask-Upmark E. 1960. A case of brain tumor (parasagittal meningioma) in ancient Egypt. Sven Lakartidn 57:2249-2253.

Aufderheide AC, Rodriguez-Martin C. 1998. The Cambridge Encyclopedia of Human Paleopathology. Cambridge University Press, UK.

Aufderheide AC, Salo W, Madden M, Streitz J, Buikstra J, Guhl F, Arriaza B, Renier C, Wittmers Jr. LE, Fornaciari G, Allison M. 2004. A 9000-year record of Chagas' disease. Proc Natl Acad Sci USA 101: 2034-2039.

Baca M, Doan K, Sobczyk M, Stankovic A, Węgleński P. 2012. Ancient DNA reveals kinship burial patterns of a pre-Columbian Andean community. BMC Genet 13:30.

Bailey DM, Affara NA, Ferguson-Smith MA. 1992. The X-Y homologous gene amelogenin maps to the short arms of both the X and Y chromosomes and is highly conserved in primates.

Genomics 14, 203-205.

Barry JM, O' Rourke FJ. 1967. *Ascariasis* in pig and in man. Sci Proc R Dublin Soc Ser A 3: 39-55.

Blow MJ, Zhang T, Woyke T, Speller CF, Krivoshapkin A, Yang DY, Derevianko A, Rubin EM. 2008. Identification of ancient remains through genomic sequencing. Genome Res 18:1347-1353.

Bozzola JJ, Russell LD. 1992. Electron microscopy. In Principles and Techniques for Biologists (ed. Bozzola JJ, Russell LD). Jones & Bartlett Publishers Inc, Boston, pp. 14–62.

Brown K. 2000. Ancient DNA applications in human osteoarchaeology: achievements, problems and potential. In Human Osteology in Archaeology and Forensic Science. Cox M, Mays, Medical Media, Greenwich, pp. 455-472.

Budowle B, Eisenberg AJ, van Daal A. 2009. Validity of low copy number typing and applications to forensic science. Croat Med J 50:207-217.

Buikstra JE, Ubelaker DH. 1994. Standards for data collection from human skeletal remains. Arkansas Archaeological Survey Research Series No. 44. Arkansas Archeological Survey, Arkansas.

Butler JM. 2005. Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers (2nd Edition). Elsevier Academic Press,

New York.

Butler JM, Shen Y, McCord BR. 2003. The development of reduced size STR amplicons as tools for analysis of degraded DNA. J Forensic Sci 48:1054-1064.

Butler JM. 2010. Fundamentals of Forensic DNA Typing. San Diego: Elsevier Academic Press.

Callen EO, Cameron TWM. 1960. A prehistoric diet as revealed in coprolites. New Sci 8: 35-40.

Calvignac S, Hughes S, Tougard C, Michaux J, Thevenot M, Philippe M, Hamdine W, Hänni C. 2008. Ancient DNA evidence for the loss of a highly divergent brown bear clade during historical times. Mol Ecol 17:1962-1970.

Casas MJ, Hagelberg E, Fregel R, Larruga JM, González AM. 2006. Human mitochondrial DNA diversity in an archaeological site in al-Andalus: genetic impact of migrations from North Africa in medieval Spain. Am J Phys Anthropol 131(4):539-551.

Chang BS, Uhm CS, Park CH, Kim HK, Jung HS, Ham JH, Lee GY, Kim DH, Lee KJ, Bang IS, Oh CS, Shin DH. 2008. Ultramicroscopic investigation of the preservation status of hair collected from a full-term, intrauterine baby mummy of the Joseon Dynasty, Korea. Int J Osteoarchaeol 18:624-631.

Chang BS, Uhm CS, Park CH, Kim HK, Lee GY, Cho HH, Chung YH,

Shin DH. 2006a. Ultramicroscopic study on the hair of newly found 15th century mummy in Daejeon, Korea. Ann Anat 188:439-445.

Chang BS, Uhm CS, Park CH, Kim HK, Lee GY, Cho HH, Kim MJ, Chung YH, Song KW, Lim do S, Shin DH. 2006b. Preserved skin structure of a recently found fifteenth-century mummy in Daejeon, Korea. J Anat 209:671-680.

Chung J. 1994. A Study on Funeral Customs in the Early Chosun Dynasty. Seoul: Chung-Ang University (in Korean).

Cipollaro M, Di Bernardo G, Galano G, Galderisi U, Guarino F, Angelini F, Cascino A. 1998. Ancient DNA in Human Bone Remains from Pompeii Archaeological Site. Biochem. Biophys Res Commun 247: 901-904.

Cipollaro M, Galderisi U, Di Bernardo G. 2005. Ancient DNA as a multidisciplinary experience. J Cell Physiol 202(2):315-322.

Clisson I, Keysr C, Francfort HP, Crubezy E, Samashev Z, Ludes B. 2002. Genetic analysis of human remains from a double inhumation in a frozen Kurgan in Kazakhstan (Berel site, Early 3rd Century BC). Int J Legal Med 116:304-308.

Coble MD, Butler JM. 2005. Characterization of new miniSTR loci to aid analysis of degraded DNA. J Forensic Sci 50:43-53.

Coble MD, Loreille OM, Wadhams MJ, Edson SM, Maynard K, Meyer

CE, Niederstätter H, Berger C, Berger B, Falsetti AB, Gill P, Parson W, Finelli LN. 2009. Mystery solved: the identification of the two missing Romanov children using DNA analysis. PLoS One 4(3):e4838.

Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. 2004. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR Amplification Kit. J Forensic Sci 49(6):1265-1277.

Criscione CD, Anderson JD, Sudimack D, Peng W, Jha B, Williams-Blangero S, Anderson TJ 2007. Disentangling hybridization and host colonization in parasitic roundworms of humans and pigs. Proc Biol Sci 274: 2669-2677.

Crompton DWT 1989. Biology of *Ascaris lumbricoides*. In DWT Crompton, MC Nesheim, ZS Pawlowski (eds), *Ascariasis* and its prevention and control, Taylor & Francis, London, p.10-44.

Dabney J, Meyer M, Pääbo S. 2013. Ancient DNA damage. Cold Spring Harb Perspect Biol 5(7). pii: a012567.

de Pancorbo MM, Castro A, Alonso S, Fernández-Fernández I, Barbero C, García-Orad A, Izaguirre N, Iriondo M, de la Rúa C. 1995. Genetic typing with HUMTH01, HUMVWA31A and HUMFES/FPS short tandem repeat loci, D1S80 variable number

tandem repeat locus and HLA-DQ alpha of recent and from XII-XIII centuries spongy bone. Electrophoresis 16:1612-1616.

Dent BB, Forbes SL, Stuart BH. 2004. Review of human decomposition processes in soil. Environmental Geology 45:576-585.

Di Nunno N, Saponetti SS, Scattarella V, Emanuel P, Baldassarra SL, Volpe G, Di Nunno C. 2007. DNA extraction: an anthropologic aspect of bone remains from sixth- to seventh-century ad bone remains. Am J Forensic Med Pathol 28:333-341.

Doran GH, Dickel DN, Ballinger WE Jr., Agee OF, Laipis PJ, Hauswirth WW. 1986. Anatomical, cellular and molecular analysis of 8,000-yr-old human brain tissue from the Windover archaeological site. Nature 323:803-806.

Drábek J, Chung DT, Butler JM, McCord BR. 2004. Concordance study between Miniplex assays and a commercial STR typing kit. J Forensic Sci 9:859-860

Drancourt M, Raoult D. 2005. Palaeomicrobiology: current issues and perspectives. Nat Rev Microbiol 3:23-35.

Eklektos N, Dayal MR, Manger PR. 2006. A forensic case study of a naturally mummified brain from the Bushveld of South Africa. J Forensic Sci 51:498-503.

Faerman M, Filon D, Kahila G, Greenblatt CL, Smith P, Oppenheim A.

1995. Sex identification of archaeological human remains based on amplification of the X and Y amelogenin alleles. Gene 167:327-332.

Ferreira LF, Araújo A, Confalonieri U, Chame M, Gomes DC, 1991. Trichuris eggs in animal coprolites dated from 30,000 years ago. J Parasitol 77:491-493.

Fujii A. 1960. On the relation of long bone lengths of lower limb to stature. Bulletin of the School of Physical Education, Juntendo University 3:49-61.

Gerszten PC, Martinez AJ. 1995. The neuropathology of South American mummies. Neurosurgery 36:756-761.

Gilbert MT, Cuccui J, White W, Lynnerup N, Titball RW, Cooper A, Prentice MB. 2004. Absence of Yersinia pestis-specific DNA in human teeth from five European excavations of putative plague victims. Microbiology 150:341-354.

Gilbert MTP, Rudbeck L, Willerslev E, Hansen AJ, Smith C, Penkman KEH, Prangenberg K, Nielsen-Marsh CM, Jans ME, Arthur P, Lynnerup N, Turner-Walker G, Biddle M, Kjølye-Biddle B, Collins MJ. 2005. Biochemical and physical correlates of DNA contamination in archaeological human bones and teeth excavated at Matera, Italy. J Arch Sci 32:785-793.

Gill P. 2001. Application of low copy number DNA profiling. Croat

Med J 42(3):229-232. Review.

Gill P, Ivanov PL, Kimpton C, Piercy R, Benson N, Tully G, Evett I, Hagelberg E, Sullivan K. 1994. Identification of the remains of the Romanov family by DNA analysis. Nat Genet 6:130-135.

Gonçalves MLC, Araújo A, Ferreira LF. 2003. Human intestinal parasites in the past: New findings and a review. Mem Ins Oswaldo Cruz 98:103-118.

Grubwieser P, Mühlmann R, Berger B, Niederstätter H, Pavlic M, Parson W. 2006. A new "miniSTR-multiplex" displaying reduced amplicon lengths for the analysis of degraded DNA. Int J Legal Med 120:115-120.

Guhl F, Jaramillo C, Vallejo GA, Yockteng R, Cárdenas-Arroyo F, Fornaciari G, Arriaza B, Aufderheide AC. 1999. Isolation of *Trypanosoma cruzi* DNA in 4,000-year-old mummified human tissue from northern Chile. Am J Phys Anthropol 108: 401-407.

Haak W, Gruber P, Rühli FJ, Böni T, Ulrich-Bochsler S, Frauendorf E, Burger J, Alt KW. 2005. Molecular evidence of HLA-B27 in a historical case of ankylosing spondylitis. Arthritis Rheum 52:3318-3319.

Haas-Rochholz H, Weiler G. 1997. Additional primer sets for an amelogenin gene PCR-based DNA-sex test. Int J Legal Med 110:312-315.

Han SH, Kim SH, Ahn YW, Huh GY, Kwak DS, Park DK, Lee UY, Kim YS. 2009. Microscopic age estimation from the anterior cortex of the femur in Korean adults. J Forensic Sci 54:519-522.

Hangang Institute of Cultural Heritage. 2009. Excavation Report of Shinnae-dong, in Seoul, Korea. Hangang Institute of Cultural Heritage, Seoul.

Hangang Institute of Cultural Heritage. 2009. Shinnae-dong Site, Seoul: Research Report 4. Hangang Institute of cultural Heritage: Seoul.

Herrmann B, Hummel S. 1994. Ancient DNA: Recovery and Analysis of Genetic Material from Paleontological, Archaeological, Museum, Medial, and Forensic Specimens. Springer-Verlag, New York.

Hess MW, Klima G, Pfaller K, Künzel KH, Gaber O. 1998. Histological investigations on the Tyrolean Ice Man. Am J Phys Anthropol 106:521-532.

Hill CR, Kline MC, Mulero JJ, Lagacé RE, Chang CW, Hennessy LK, Butler JM. 2007. Concordance study between the AmpFlSTR MiniFiler PCR amplification kit and conventional STR typing kits. J Forensic Sci 52:870-873.

Hofreiter M, Jaenicke V, Serre D, von Haeseler A, Pääbo S. 2001. DNA sequences from multiple amplifications reveal artifacts

induced by cytosine deamination in ancient DNA. Nucleic Acids Res 29(23):4793-4799.

Hofreiter M, Serre D, Poinar HN, Kuch M, Pääbo S 2001. Ancient DNA. Nat Rev Genet 2: 353-359.

Holland MM, Huffine EF. 2001. Molecular analysis of the human mitochondrial DNA control region for forensic identity testing. Curr Protoc Hum Genet Chapter 14:Unit 14.7.

Höss M, Dilling A, Currant A, Pääbo S. 1996. Molecular phylogeny of the extinct groundsloth *Mylodon darwinii*. Proc Natl Acad Sci USA 93:181-185.

Höss M, Jaruga P, Zastawny TH, Dizdaroglu M, Pääbo S. 1996. DNA damage and DNA sequence retrieval from ancient tissues. Nucleic Acids Res 24(7):1304-1307.

Hummel S, Schultes T, Bramanti B, Herrmann B. 1999. Ancient DNA profiling by megaplex amplications. Electrophoresis 20:1717-1721.

Hayat MA. 1970. Embedding. In Principles and Techniques of Electron Microscopy, Biological Applications 1. van Nostrand Reinhold Company, New York, pp. 109–179.

Iñiguez AM, Reinhard K, Carvalho Gonçalves ML, Ferreira LF, Araújo A, Paulo Vicente AC 2006. SL1 RNA gene recovery from *Enterobius vermicularis* ancient DNA in pre-Columbian human

coprolites. Int J Parasitol 36:1419–1425.

Iñiguez AM, Reinhard KJ, Araújo A, Ferreira LF, Vicente ACP. 2003. *Enterobius vermicularis*: ancient DNA from North and South American Human Coprolites. Mem Inst Oswaldo Cruz 98:67–69.

Iwamura ES, Soares–Vieira JA, Munoz DR. 2004. Human identification and analysis of DNA in bones. Revista do Hospital das Clinicas; Faculdade de Medicina da Universidade de Sao Paulo 59: 383–388.

Jee SH, Jung YJ, Seo MS. 2008. Archaeogenetic Research of Excavated Human Bones from the Ancient Tombs. Mun Hwa Jae 41:99–108.

Kahila Bar–Gal G, Kim MJ, Klein A, Shin DH, Oh CS, Kim JW, Kim TH, Kim SB, Grant PR, Pappo O, Spigelman M, Shouval D. 2012. Tracing hepatitis B virus to the 16th century in a Korean mummy. Hepatology 56:1671–1680.

Kemp BM, Smith DG. 2005. Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. Forensic Sci Int 154:53–61.

Kim AJ, Kim K, Choi JH, Choi EH, Jung YJ, Min NY, Lkhagvasuren G, Rhee S, Kim JH, Noh M, Park AJ, Kim KY, Kang Y, Lee KH, Kim KC. 2010. Mitochondrial DNA analysis of ancient human bones excavated from Nukdo island, S. Korea. BMB Rep

43(2):133-139.

Kim JH, Oh JH, Song JH, Jeon JT, Han SH, Jung YH, Oh MY. 2005. Molecular genetic analysis of ancient cattle bones excavated from archaeological sites in Jeju, Korea. Mol Cells 20(3):325-330.

Kim MJ, Oh CS, Lee IS, Lee BH, Choi JH, Lim D-S, Yi YS, Han W-J, Kim Y-S, Bok GD, Lee SD, Shin DH. 2008. Human mummified brain from a medieval tomb with lime-soil mixture barrier of the Joseon Dynasty, Korea. Int J Osteoarchaeol 18:614-623

Kim NY, Lee HY, Park MJ, Yang WI, Shin KJ. 2011. A genetic investigation of Korean mummies from the Joseon Dynasty. Mol Biol Rep 38:115-121.

Kim YH, Yoon JH, Jung BG, Zhang YS, Kwak HK. 2004. pH Buffer Capacity and Lime Requirement of Korean Acid Soils. J Korean Soc Soil Sci Fert 37:378-382.

Kim SB, Shin JE, Park SS, Bok GD, Chang YP, Kim J, Chung YH, Yi YS, Shin MH, Chang BS, Shin DH, Kim MJ. 2006. Endoscopic investigation of the internal organs of a 15th-century child mummy from Yangju, Korea. J Anat 209:681-688.

Kim YS, Oh CS, Lee SJ, Park JB, Kim MJ, Shin DH. 2011b. Sex determination of Joseon people skeletons based on anatomical, cultural and molecular biological clues. Ann Anat 193:539-543.

Kim YS, Lee IS, Jung GU, Kim MJ, Oh CS, Yoo DS, Lee WJ, Lee E,

Cha SC, Shin DH. 2014. Radiological diagnosis of congenital diaphragmatic hernia in 17th century Korean mummy. PLoS One 9(7):e99779.

Kolman CJ, Tuross N. 2000. Ancient DNA analysis of human populations. Am J Phys Anthrop 111:5-23.

Konomi N, Lebwohl E, Mowbray K, Tattersall I, Zhang D. 2002. Detection of mycobacterial DNA in Andean mummies. J Clinic Microbiol 40:4738-4740.

Krogman WM, Iscan MY. 1986. The human skeleton in forensic Medicine, 2nd ed. Charles C. Thomas, Illinois.

Lawlor DA, Dickel CD, Hauswirth WW, Parham P. 1991. Ancient HLA genes from 7,500-year-old archaeological remains. Nature 349:785-788.

Lee E-J, Oh CS, Yim SG, Park JB, Kim Y-S, Shin MH, Lee SD, Shin DH. 2013. Collaboration of archaeologists, historians and bioarchaeologists during removal of clothing from Korean mummy of Joseon dynasty. Int J Histor Archaeol 17:94-118.

Lee E-J, Shin DH, Yang HY, Spigelman M, Yim SG. 2009a. Eung Tae's tomb: a Joseon ancestor and the letters of those that loved him. Antiquity 83:145-156.

Lee HY, Song I, Ha E, Cho SB, Yang WI, Shin KJ. 2008. mtDNAmanager: a Web-based tool for the management and

quality analysis of mitochondrial DNA control-region sequences. BMC Bioinformatics 9:483

Lee HY, Yoo JE, Park MJ, Chung U, Shin KJ. 2006. Mitochondrial DNA control region sequences in Koreans: identification of useful variable sites and phylogenetic analysis for mtDNA data quality control. Int J Legal Med 120:5-14.

Lee HY, Yoo JE, Park MJ, Kim CY, Shin KJ. 2008. Genetic characterization and assessment of authenticity of ancient Korean skeletal remains. Hum Biol 80:239-250.

Lee IS, Kim MJ, Yoo DS, Lee YS, Park SS, Bok GD, Han SH, Chung YH, Chang BS, Yi YS, Oh CS, Shin DH. 2007. Three-dimensional reconstruction of medieval child mummy in Yangju, Korea, using multi-detector computed tomography. Ann Anat 189:558-568.

Lee IS, Lee EJ, Park JB, Baek SH, Oh CS, Lee SD, Kim YS, Bok GD, Hong JW, Lim DS, Shin MH, Seo M, Shin DH. 2009. Acute traumatic death of a 17th century general based on examination of mummified remains found in Korea. Ann Anat 191:309-320.

Leles D, Araújo A, Ferreira LF, Vicente ACP, Iñiguez AM. 2008. Molecular paleoparasitological diagnosis of Ascaris sp. from coprolites: new scenery of ascariasis in pre-Columbian South America times. Mem Inst Oswaldo Cruz 103:106-108.

Leles D, Araújo A, Vicente AC, Iñiguez AM 2010. ITS1 intra-

individual variability of Ascaris isolates from Brazil. Parasitol Int 59(1):93-96.

Lim D-S, Oh CS, Lee SJ, Shin DH. 2010. Auto-fluorescence emitted from the cell residues preserved in human tissues of medieval Korean mummies. J Anat 217:67-75.

Lim DS, Lee IS, Choi KJ, Lee SD, Kim YS, Oh CS, Kim, YS, Bok GD, Kim MJ, Yi YS, Lee EJ, Shin DH. 2008. The potential for non-invasive study of mummies: validation of the use of computerized tomography by post factum dissection and histological examination of a 17th century female Korean mummy. J Anat 213:482-495.

Liu W-Q, Liu J, Zhang J-H, Long X-C, Lei J-H, Li Y-L. 2007. Comparison of ancient and modern *Clonorchis sinensis* based on ITS1 and ITS2 sequences. Acta Tropica 101: 91-94.

Loreille O, Bouchet F. 2003. Evolution of ascariasis in humans and pigs: a multi-disciplinary approach. Mem Inst Oswaldo Cruz 98: 39-46.

Loreille O, Roumat E, Verneau O, Bouchet F, Hänni C. 2001. Ancient DNA from *Ascaris*: extraction amplification and sequences from eggs collected in coprolites. Int J Parasitol 31:1101-1106.

Lovejoy CO, Meindl RS, Pryzbeck TR, Mensforth RP. 1985. Chronological metamorphosis of the auricular surface of the ilium:

a new method for the determination of adult skeletal age at death. Am J Phys Anthropol 68:15-28.

Marota I, Rollo F 2002. Molecular paleontology. Cell Mol Life Sci 59: 97-111.

Michaud CL, Foran DR. 2011. Simplified field preservation of tissues for subsequent DNA analyses. J Forensic Sci 56(4):846-852.

Mulero JJ, Chang CW, Lagacé RE, Wang DY, Bas JL, McMahon TP, Hennessy LK. 2008. Development and validation of the AmpFlSTR MiniFiler PCR Amplification Kit: a MiniSTR multiplex for the analysis of degraded and/or PCR inhibited DNA. J Forensic Sci 53(4):838-852.

Nakahori Y, Takenaka O, Nakagome Y. 1991. A human X-Y homologous region encodes amelogenin. Genomics 9:264-269.

O'Connor S. 1999. Do we need a brain? Abstracts from a one-day forum held at the Tempest Anderson Hall, York on 17 November 1999. University of York, York.

O' Rourke DH, Hayes MG, Carlyle SW. 2000. Ancient DNA studies in physical anthropology. Annu Rev Anthropol 29:217-242.

Oh CS, Lee SJ, Lee SD, Kim MJ, Kim Y-S, Lim D-S, Shin DH. 2013. Amplification of DNA remnants in mummified human brains from medieval Joseon tombs of Korea. Anthropol Anz 70:57-81.

Oh CS, Lee SJ, Park JB, Lee SD, Seo SB, Kim HY, Kim J, Kim YS, Shin DH. 2012. Autosomal short tandem repeat analysis of ancient DNA by coupled use of mini- and conventional STR kits. J Forensic Sci 57:820-825.

Oh CS, Lee SY, Lee IS, Kim Y-S, Koh KS, Shin DH. 2011. Differential findings in post-factum dissections of medieval Korean mummies which exhibited similar preservation patterns on computerized tomography images. Ann Anat 193:544-549.

Oh CS, Seo M, Chai JY, Lee SJ, Kim MJ, Park JB, Shin DH. 2010. Amplification and sequencing of *Trichuris trichiura* ancient DNA extracted from archaeological sediments. J Arch Sci 37:1269-1273.

Pääbo S, Gifford JA, Wilson AC. 1988. Mitochondrial DNA sequences from 7,000 year old brain. Nucleic Acids Res 16:9775-9787.

Pääbo S. 1989. Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. Proc Natl Acad Sci USA 86:1939-1943.

Pedersen S, Murrell KD. 2001. Whipworms-nutrition interaction. Trends in Parasitology 17:470.

Peng W, Yuan K, Hu M, Zhou X, Gasser RB. 2005. Mutation scanning-coupled analysis of haplotypic variability in

mitochondrial DNA regions reveals low gene flow between human and porcine *Ascaris* in endemic regions of China. Electrophoresis 26: 4317–4326.

Perper JA. 2006. Time of death and changes after death. In: Spitz, W.U. (Ed.). Spitz and Fisher's medico legal investigation of death. fourth ed. Charles C Thomas Publisher Ltd. pp. 107–108.

Phenice TW. 1969. A newly developed visual method of sexing the os pubis. Am J Phys Anthropol 30:297– 301.

Poinar HN, Höss M, Bada JL, Pääbo S. 1996. Amino acid racemization and the preservation of ancient DNA. Science 272(5263):864–866.

Poinar HN, Stankiewicz BA. 1999. Protein preservation and DNA retrieval from ancient tissues. Proc Natl Acad Sci USA 96(15):8426–8431.

Previgliano CH, Ceruti C, Reinhard J, Araoz FA. Diez JG. 2003. Radiologic evaluation of the Llullaillaco mummies. Am J Roentgenol 181:1473–1479.

Radanov S, Stoev S, Davidov M, Nachev S, Stanchev N, Kirova E. 1992. A unique case of naturally occurring mummification of human brain tissue. Int J Legal Med 105:173–175.

Reinhold S. 2003. Traditions in transition: Some thoughts on Late Bronze Age and Early Iron Age burial costumes from the

Northern Caucasus. J Eur Archaeol 6:25-54.

Ricaut FX, Bellatti M, Lahr MM. 2006a. Ancient mitochondrial DNA from Malaysian hair samples: some indications of Southeast Asian population movements. Am J Hum Biol 18:654-667.

Ricaut FX, Fedoseeva A, Keyser-Tracqui C, Crubézy E, Ludes B. 2005a. Ancient DNA analysis of human neolithic remains found in northeastern Siberia. Am J Phys Anthropol 126:458-462.

Ricaut FX, Keyser-Tracqui C, Cammaert L, Crubézy E, Ludes B. 2004. Genetic analysis and ethnic affinities from two Scytho-Siberian skeletons. Am J Phys Anthropol 123:351-360.

Ricaut FX, Keyser-Tracqui C, Crubézy E, Ludes B. 2005b. STR-genotyping from human medieval tooth and bone samples. Forensic Sci Int 151:31-35.

Ricaut FX, Kolodesnikov S, Keyser-Tracqui C, Alekseev AN, Crubézy E, Ludes B. 2006b. Molecular genetic analysis of 400-year-old human remains found in two Yakut burial sites. Am J Physic Anthropol 129:55-63.

Ricaut FX, Kolodesnikov S, Keyser-Tracqui C, Alekseev AN, Crubézy E, Ludes B. 2006. Molecular genetic analysis of 400-year-old human remains found in two Yakut burial sites. Am J Phys Anthropol 129:55-63.

Roberts C, Ingham S. 2008. Using ancient DNA analysis in

palaeopathology: a critical analysis of published papers, with recommendations for future work. Int J Osteoarchaeol 18:600–613.

Rohland N, Hofreiter M. 2007. Ancient DNA extraction from bones and teeth. Nat Protoc 2:1756–1762.

Sallares R, Gomzi S 2001. Biomolecular archaeology of malaria. Anc Biomol 3: 195–213.

Seo M, Shin DH, Guk SM, Oh CS, Lee EJ, Shin MH, Kim MJ, Lee SD, Kim YS, Yi YS, Spigelman M, Chai JY. 2008. Gymnophalloides seoi eggs from the stool of a 17th century female mummy found in Hadong, Republic of Korea. J Parasitol 94:467–472.

Shin DH, Choi YH, Shin KJ, Han GR, Youn M, Kim CY, Han SH, Seo JC, Park SS, Cho YJ, Chang BS. 2003. Radiological analysis on a mummy from a medieval tomb in Korea. Ann Anat 185:377–382.

Shin DH, Lee IS, Kim MJ, Oh CS, Park JB, Bok GD, Yoo DS. 2010. Magnetic resonance imaging performed on a hydrated mummy of medieval Korea. J Anat 216:329–334.

Shin DH, Oh CS, Chai J-Y, Lee H-J, Seo M. 2011. Enterobius vermicularis Eggs Discovered in Coprolites from a Medieval Korean Mummy. Korean J Parasitol 49:323–326.

Shin DH, Oh CS, Lee HJ, Chai JY, Lee SJ, Hong D-W, Lee SD, Seo M. 2013. Ancient DNA analysis on *Clonorchis sinensis* eggs

remained in samples from medieval Korean mummy, J Archaeol Sci 40:211-216.

Shin DH, Oh CS, Lee SJ, Lee E-J, Yim SG, Kim MJ, Kim Y-S, Lee SD, Lee YS, Seo M. 2012. Ectopic paragonimiasis from 400 year old female mummy of Korea. J Archaeol Sci 39:1103-1110.

Shin DH, Youn M, Chang BS. 2003. Histological analysis on the medieval mummy in Korea. Forensic Sci Int 137:172-182.

St Hoyme LE, Iscan MY. 1989. Determination of sex and race: accuracy and assumptions. In Iscan M.Y. and Kennedy K.A.R. (eds.): Reconstruction of Life from the Skeleton. New York: Alan R Liss, pp. 53-93.

Stone AC, Milner GR, Pääbo S, Stoneking M. 1996. Sex determination of ancient human skeletons using DNA. Am J Phys Anthropol 99:231-238.

Sullivan KM, Mannucci A, Kimpton CP, Gill PA. 1993. Rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. Biotechniques 15:636-638.

Takei T. 1984. Age estimation from dental attrition and state of dental treatment — by application of the theory of quantification type I. J Nihon Oniv Sch Dent 26:119-132.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using

maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731-2739.

Thali MJ, Yen K, Schweitzer W, Vock P, Ozdoba C, Dirnhofer R. 2003. Into the decomposed body-forensic digital autopsy using multislice-computed tomography. Forensic Sci Int 134(2-3):109-114.

Tkocz I, Bytzer P. Bierring F. 1979. Preserved brains in medieval skulls. Am J Phys Anthropol 51:197-202.

Tsukada K, Takayanagi K, Asamura H, Ota M, Fukushima H. 2002. Multiplex short tandem repeat typing in degraded samples using newly designed primers for the TH01, TPOX, CSF1PO, and vWA loci. Leg Med (Tokyo) 4:239-245.

Ubelaker DH. 1999. Human Skeletal Remains: Excavation, Analysis, Interpretation. 3rd ed. Manuals on Archeology, vol. 2. Taraxacum, Washington.

Vanek D, Saskova L, Koch H. 2009. Kinship and Y-chromosome analysis of 7th century human remains: novel DNA extraction and typing procedure for ancient material. Croat Med J 50:286-295.

von Wurmb-Schwark N, Harbeck M, Wiesbrock U, Schroeder I, Ritz-Timme S, Oehmichen M. 2003. Extraction and amplification of nuclear and mitochondrial DNA from ancient and artificially aged bones. Leg Med (Tokyo) 5(Suppl 1):S169-172.

von Wurmb-Schwark N, Schwark T, Harbeck M, Oehmichen M. 2004. A simple Duplex-PCR to evaluate the DNA quality of anthropological and forensic samples prior short tandem repeat typing. Leg Med (Tokyo) 6:80-88.

Wiegand P, Kleiber M. 2001. Less is more-length reduction of STR amplicons using redesigned primers. Int J Legal Med 114:285-287.

Willerslev E, Cooper A 2005. Ancient DNA. Proc Biol Sci 272: 3-16.

Wurmb-Scwark NV, Harbeck M, Wiesbrock U, Schroeder I, Ritz-Timme S, Oehmichen M. 2003. Extraction and amplification of nuclear and mitochondrial DNA from ancient and artificially aged bones. Leg Med (Tokyo) 5, S169-S172.

Yang DY, Eng B, Waye JS, Dudar JC, Saunders SR. 1998. Technical note: improved DNA extraction from ancient bones using silica-based spin columns. Am J Phys Anthropol 105:539-543.

Yoo SY, Cho NS, Park MJ, Seong KM, Hwang JH, Song SB, Han MS, Lee WT, Chung KW. 2011. A large population genetic study of 15 autosomal short tandem repeat loci for establishment of Korean DNA profile database. Mol Cell 32:15-19.

Zhu X, Chilton NB, Jacobs E, Boes J, Gasser RB 1999. Characterization of *Ascaris* from human and pig hosts by nuclear

ribosomal DNA sequences. Int J Parasitol 29: 469–478.

국문초록

서론: 사람을 대상으로 한 고DNA (aDNA) 연구는 고고학 발굴현장에서 수습된 백골화 또는 미라화된 사람의 사체에서 유전자를 추출/분석함으로서, 이전 시대에 살았던 사람들의 건강상태, 기생충 또는 바이러스와 같은 감염체의 탐색, 또는 인류의 기원, 이동 및 진화와 같은 연구에 과학적 증거를 제시하는데 그 목적이 있다. 하지만 우리나라의 경우 사체가 빠르게 부패하기 쉬운 환경조건을 갖고 있기 때문에 오랜 기간 무덤 안에 매장 되어있던 사체는 DNA의 보존상태가 좋지 못할 것으로 판단되며, 이러한 DNA 시료의 분석을 위해 현대 시료를 대상으로 하는 실험 기법을 그대로 적용할 경우 분석에 실패할 가능성이 매우 높다. 따라서, 본 논고에서는 조선시대 출토된 고인골 및 미라 시료를 대상으로 성공적인 aDNA 분석을 위한 조건을 탐색하고, aDNA 분석이 실제 우리나라의 고고 과학 분야에서 유용하게 적용될 수 있는지를 확인해 보았다.

방법: 본 연구를 위해 사용한 샘플은 조선시대 회곽묘에서 발굴된 인골 또는 미라를 이용하였으며, 피장자의 성별은 고고학, 해부학 또는 분자생물학적인 방법으로 판정하였다. 샘플에서 DNA를 추출하기 위하여 넙다리뼈, 치아 또는 미라화된 뇌조직을 이용하였으며, phenol을 이용한 추출법을 사용하여 DNA를 분리하고 정량 하였다. 시료에서 추출된 DNA는 핵유전자의 경우 Minfiler kit 또는 Identifiler kit를

이용하여 분석하였고 미토콘드리아 유전자의 경우 과변이부위에 대한 염기서열 분석을 실시 하였다.

결과: 인골의 넙다리뼈에서 추출한 DNA를 이용하여 샘플에 내재되어 있던 DNA의 보존상태를 Identifiler와 Minifiler kit를 이용한 STR분석을 통해 확인하였다. 분석결과 고인골에서 추출한 DNA의 보존상태는 Identifiler kit를 이용한 분석을 할 수 없을 정도로 좋지 않은 보존상태를 나타내었다. 하지만 Identifiler kit에서 증폭산물의 크기가 큰 8개의 유전자위(locus)를 대상으로 증폭산물의 크기를 작게 만들도록 고안된 Minifiler kit를 이용한 분석에서는 같은 유전좌위에 대하여 Identifiler kit 보다 약 2배나 많은 분석 결과를 얻을 수 있었다. 이 연구를 통해, 회곽묘에서 출토된 조선시대 인골을 대상으로 aDNA 분석 시 같은 증폭산물의 크기를 작게 만들수록 분석 성공률을 높일 수 있다는 것을 확인할 수 있었다.

다음으로, 형태학적으로 보존상태가 좋은 미라화된 뇌가 aDNA분석을 위한 샘플로 가치가 있는지를 조사하기 위하여 일반적으로 많이 사용되는 넙다리뼈와 함께 비교연구를 진행하였다. 실험결과 미라화된 뇌에서 추출한 DNA가 넙다리뼈에서 추출한 것 보다 Minifiler kit를 이용한 STR 분석 성공률이 더 높다는 것을 확인할 수 있었다. 또한 미토콘드리아 DNA를 대상으로 한 분석에서도 넙다리뼈에서 추출한 DNA보다 미라화된 뇌에서 추출한 DNA가 더 큰 크기의 증폭 산물을 만들어 내는 것을 확인할 수 있었다. 이 연구를 통해, 미라화된 뇌에 내재성 DNA가 매우 좋은 상태로 보존되어 있다는 것이 확인되어

aDNA 분석에 유용하게 사용될 수 있다는 것을 증명 하였다.

다음으로 피장자의 성별 분석에 aDNA 분석이 도움이 될 수 있는지를 확인해 보았다. 이를 위해 조선시대 회곽묘에서 출토된 총 34개체의 고인골과 미라 샘플을 대상으로 성별 판정을 실시 하였으며, 이중 5 개체는 해부학적으로 성별을 판정할 수 없는 경우가 발생하였고 2 개체에서는 고고학적인 분석 결과와 해부학적인 분석 결과가 서로 다르게 나타났다. 이에 대하여 Minfiler kit를 이용한 아멜로제닌 유전자의 분석을 진행한 결과, 해부학적으로 성별 판정을 할 수 없었던 5개체는 유전자 분석에 실패한 1케이스를 제외하고 여자 3명, 남성 1명으로 확인되었고, 고고학적, 그리고 해부학적으로 성별 판정 결과가 서로 반대로 나타난 두 케이스의 경우 해부학적인 성별의 판정이 정확 했다는 것이 확인되었다.

마지막으로, 관 내에서 피장자 곁에 놓여있던 주머니에 안에 담아져 있던 치아들이 피장자의 것 인지를 확인하는 연구가 진행하였다. 우선 Minifiler kit를 이용하여 STR 유전좌위에 있는 대립유전자를 분석한 결과, 피장자의 미라화된 뇌와 넙다리뼈에서 확인된 대립유전자가 치아에서 확인된 것과 거의 일치하는 것이 확인되었다. 또한 미토콘드리아 과변이부위의 염기서열을 서로 비교한 결과, 미라화된 뇌, 넙다리뼈 및 치아에서 확인된 염기서열이 서로 완전히 일치하는 것이 확인되었다. 이러한 분석 결과를 고려할 때, 주머니에서 발견된 치아의 소유자가 같은 관에 있었던 피장자의 것이 될 가능성이 가장 높았다.

결론: 이 연구를 통해, 나는 조선시대 회곽묘에서 출토된 사람 시료에

남아있는 DNA의 양과 보존상태를 확인하였으며, 또한 aDNA 분석을 진행하기 위한 연구 방법도 수립 할 수 있었다. 그리고 이 aDNA 연구 기법을 이용하여 고고학 사람 시료에 대한 성별의 판정과 개인 식별이 성공적으로 수행 될 수 있음을 확인하였다. 우리나라에서 고인골 및 미라에 대한 aDNA 연구가 비교적 새로운 기술임을 감안할 때, 나는 이 논문이 aDNA 연구에 관심이 있는 연구자들에게 도움이 되기를 바란다.

키워드: 고DNA, 인골, 미라, 조선, STR, 성별분석

학번: 2008-30570