Analysis of Amelogenin Gene & Short Tandem Repeat(STR) locus F13A01, LPL from dentin of the endodontic treated teeth

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I. INTRODUCTION

Individual identification is necessary in many social, forensic and medical circumstance. Recent advances in molecular genetic technology have led to its application to individual identification [46,52,55]. This has prompted the investigation of various human tissues as potential sources of genetic evidentiary material. Genetic characterization of individuals for identity testing is increasingly being performed at the deoxyribonucleic acid(DNA) level[28,33,34,79].

The polymerase chain reaction(PCR)[30,50,69,92] is the method of amplifying small quantities of relatively short target sequences of DNA using sequence-specific oligonucleotide primers and thermostable Taq. DNA polymerase. PCR does not require native high molecular weight DNA in order to amplify target sequences. It is possible to amplify if the target sequence itself is intact. Thus, PCR can amplify partially degraded and/or denatured DNA.

Genetic markers known as STR(or microsatellites)[58] are ideal for use in linkage mapping and DNA fingerprinting. The STR systems are presented according to their chromosomal location, repeat unit, product size, primer sequences and fluorophore tags[17]. The markers - short sequences(1-5bp) that are repeated in a tandem fashion - are abundant, highly polymorphic and uniformly distributed throughout the human genome[58].

DNA analysis has been used for such forensic purposes as individual identification. Sex determination of DNA samples has been performed by amplification of sex chromosomal genes, such as Y chromosomal repetitive DYZ1 sequence[2,3] in the distal Y chromosome long arm, centromeric α-satellite repeats of X and Y chromosomes, and the X-Y homologous amelogenin gene[1,2,53,80].

Recently, forensic studies using the teeth have been described and applied it to individual identification[77,78,89]. Teeth are good material for DNA analysis, since the dental hard tissue physically encloses the pulp, offering an anatomic configuration of great durability[12,30,75,77,88,92,93]. Most DNA analysis of the teeth made use of pulp. DNA analysis of endodontic treated teeth is not reported. The most characteristic feature of dentin is the closely packed dentinal tubules that traverse its entire thickness and occupied for part or all of their length by odontoblastic process. The bodies of the odontoblasts are arranged in a layer on the pulpal surface of the dentin[8,82].

Theoretically, if the odontoblastic layer on the pulpal surface of the dentin is removed, it is impossible to find nucleus after the teeth has undergone endodontic therapy. So dentin does not contain genomic DNA. But it is in existence the possibility that blood permeates into the dentin by endodontic therapy and trauma. Therefore DNA in blood can be detected from the dentin layer.

The purpose of this investigation is to evaluate the possibility of individual identification after detection of DNA in the dentin of endodontic treated teeth. The authors performed to amplified fragment length polymorphisms(Amp-FLPs) by PCR from dentin of the endodontic treated teeth and detected of X-Y homologous amelogenin gene for sex determination and STR locus F13 A01, LPL detection.

Ⅱ. MATERIALS AND METHODS

1. Subjects

The 40 endodontic treated teeth were selected for this study. The teeth were unrelated with one another(male:21, female:19). The ages ranged from 20 to 70 years.

2. DNA extraction

The crown and root apex of all samples were removed. The roots of teeth were used. Adherent bone, periodontal ligaments, gingiva, blood component, calculus, nycotin, pigments and cementum of root surface were removed using high speed dental bar. The teeth were rinsed with distilled water and sectioned along the vertical axis and seperated with surgical chisel. The filling materials in pulp chamber and root canal, pulpal wall were removed.

The teeth were crushed with a hammer until resulting fragments were 0.1mm or less in diameter. The dentin powder was placed in 15mL eppendorf tube and dispersed 100-200$\mu\ell$ distilled water. The samples were submitted to a conventional DNA extraction method including an overnight incubation at 55℃ in nucleolysis buffer (0.5% S.D.S., 10mM Tris. Cl, 0.1M EDTA, pH 8.0), and proteinase K. Each sample was spun at 10,000 rpm in a centrifuge for 10 minutes to further purify the DNA and discarded the supernatant. Added ethanol precipitated(5M NaCl and 100% ethanol), washed in 70% ethanol, dried, overnighted incuvation at 37℃ for 24 hours, and resuspended in distilled water. The concentration of genomic DNA was determined at 260 nm and 280 nm with UV visible spectrophotometer. Purity of samples were determined by evaluating the A_{260}/A_{280} ratios.

3. X-Y homologous amelogenin gene detection.

The PCR mixture contained follows; 50$\mu\ell$ reaction containing 2-4$\mu\ell$ template DNA, buffer (100mM Tris-HCl(pH 9.0), 500mM KCl, 1.0% Triton) (POSCO), 1.5mM $MgCl_2$, 0.2μM primers (5'-CCCTGGGCTCTGTAAAGAATAGTG-3' and 5'-ATCAGAGCTTAAACTGGGAAGCTG-3') according to Sullivan et al[80], 200μM dNTPs and

1.5 unit of Taq. DNA polymerase. The PCR was performed under condition of 95℃ for 10 sec, 1 cycles; 94℃ for 60 sec, 60℃ for 60 sec, 72℃ for 60 sec, 33 cycles; 72℃ for 10 min, 1 cycles in PCR thermocycler(480 model, Perkin Elmer Cetus).

The PCR products(10㎕) were loaded onto 12% natual polyacrylamide gel, 1mm thickness using vertical electrophoresis unit(GIBCO BRL Ins.). Electrophoresis was carried out at 80V for 3 hours in TBE buffer(90mM Tris-Borate, 2mM EDTA). The gel was subsequently stained with Ethidium Bromide(0.5㎍/㎖).

4. STR locus F13A01, LPL Detection.

The PCR mixture contained follows; 25㎕ reaction containing 25-50ng template DNA, 10X Taq. DNA poymerase buffer(500mM KCl, 100mM Tris- HCl[pH 9.0 at 25℃], 1% Triton® X-100, 15mM $MgCl_2$, 2mM dNTPs, Promega®), 1uM의 F13A01 locus specific primer(5'-GAGGTTGCAC TCCAGCCTTTGCAA-3' ; AAAG strand, 5'-T TCTGAATCATCCCAGAGCCACA-3' ; CTTT strand) or LPL locus specific primer(GZ-14 = ATCTGACCAAGGATAGTGGGATATA, GZ-15 = CCTGGGT AACTGAGCGAGACTGTGTC), 0.01 unit of Taq. DNA polymerase/ul(Promega®).

The PCR conditions of F13A01 locus were 96℃ for 2 min, 1 cycle; 94℃ for 60 sec, 64℃ for 60 sec, 70℃ for 90 sec, 10 cycles; 90℃ for 60 sec, 64 ℃ for 60 sec, 70℃ for 90 sec, 20 cycles.

The PCR conditions of LPL locus were 96℃ for 2 min, 1 cycle; 94℃ for 60 sec, 60℃ for 60 sec, 70℃ for 90 sec, 10 cycles; 90℃ for 60 sec, 60 ℃ for 60 sec, 70℃ for 90 sec, 20 cycles, 60℃ for 30 min, 1 cycle.

The PCR products(2.5㎕) were loaded onto 4% denaturing polyacrylamide gel (acrylamide : bis-acylamide = 19 : 1) containing 7M urea (Promega®) after mixed 2.5㎕ of 2X loading solution(10mM NaOH, 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol FF, Promega®).

Electrophoresis was carried out at 40W for 1 hour 30 min.

The silver staining[15] procedure were as follows;

Step	Solution	Time
a. fixing	fix/stop solution	20 min
b. washing	deionized H_2O	2 min ×3
c. staining	staining solution	30 min
d. washing	deionized H_2O	10 sec
e. developing	developer solution (4-10℃)	5 min
f. stoping	fix/stop solution	5 min
g. washing	deionized H_2O	2 min
h. dry		

Ⅲ. RESULTS

1. Concentration of DNA by Spectrophotometer

DNA could be found in 19 samples out of 40. The DNA concentration of samples was estimated 8.7-69.6 ng/㎕ and purity was 0.29-1.10 (Table 1).

2. Detection of X-Y homologous amelogenin gene.

X-Y homologous amelogenin gene was detected for sex determination. Male and female samples have two bands(106 bp and 112 bp), one band(106 bp) respectively. It was observed 5 cases out of 21 in male, 7 cases out of 19 in female(Table 2).

Table 1. DNA concentrations extracted from the endodontic treated teeth

Sample No.	Sex	DNA concntrations(ng/㎕)	DNA Purity	Sample No.	Sex	DNA concntrations(ng/㎕)	DNA Purity
1	M	47.8	1.10	21	F	39.1	0.90
2	M	69.6	1.07	22	F	8.7	0.40
3	M	0	0	23	M	8.7	6.70
4	F	0	0	24	F	0	0
5	F	0	0	25	F	0	0
6	M	26.1	0.75	26	F	13.0	0.38
7	M	26.1	0.75	27	F	8.7	0.29
8	M	0	0	28	M	0	0
9	F	0	0	29	M	0	0
10	F	0	0	30	F	0	0
11	M	17.4	0.57	31	F	13.0	0.50
12	F	30.4	0.70	32	M	13.0	0.60
13	M	0	0	33	F	0	0
14	M	0	0	34	M	0	0
15	M	0	0	35	M	0	0
16	F	52.1	0.80	36	F	30.4	0.60
17	M	26.0	0.75	37	F	17.4	0.60
18	M	21.7	0.50	38	F	21.7	0.60
19	M	0	0	39	M	0	0
20	M	0	0	40	F	0	0

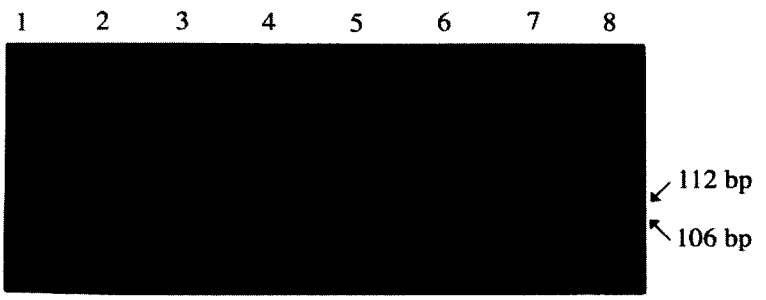

Figure 1. Results of the detection of X-Y homologous amelogenin gene by PCR from the endodontic treated teeth
Lane 1 : size marker Psi 1,
Lane 2 : female control, Lane 3 : male control,
Lane 4, 7, 8, 9 : male teeth, Lane 5, 6 : female teeth.

Table 2. Results of the detection of X-Y homologous amelogenin gene by PCR from the endodontic treated teeth

Sample No.	Sex	Detection	Sample No.	Sex	Detection
1	M	-	21	F	+
2	M	-	22	F	-
3	M	-	23	M	++
4	F	-	24	F	++
5	F	++	25	F	++
6	M	-	26	F	-
7	M	++	27	F	-
8	M	-	28	M	-
9	F	-	29	M	-
10	F	-	30	F	-
11	M	-	31	F	-
12	F	-	32	M	-
13	M	-	33	F	-
14	M	-	34	M	-
15	M	-	35	M	-
16	F	-	36	F	++
17	M	-	37	F	++
18	M	++	38	F	++
19	M	++	39	M	-
20	M	++	40	F	-

+ : A faint band was observed
++ : A distinct band was observed
- : No specific band was observed

3. Detection of STR locus F13A01, LPL

The F13A01 locus was observed in 6 samples out of 40 and 4 alleles were 3.2, 4, 5, 6 and 5 genotypes were 3.2-3.2, 3.2-4, 3.2-5, 4-5, 6-6 (Table 3). The LPL locus was observed in 7 samples out of 40 and 3 alleles were 10, 11, 12 and 3 genotypes were 10-10, 10-12, 11-12(Table 4).

Table 3. Alleles & genotypes of F13A01 locus from the endodontic treated teeth

Allele	Number of alleles observed	Genotype	Number observed
3.2	5	3.2 - 3.2	1
4	3	3.2 - 4	2
5	2	3.2 - 5	1
6	2	3.2 - 6	0
7	0	4 - 4	0
8	0	4- 5	1
9	0	4- 6	0
10	0	5- 5	0
11	0	5- 6	0
12	0	6- 6	1
13	0		
14	0		
15	0	homozygote	2
16	0	heterozygote	4
all	12	total sample	6

Table 4. Alleles & genotypes of LPL locus from the endodontic treated teeth

Allele	Number of alleles observed	Genotype	Number observed
7	0	10-10	3
8	0	10-11	0
9	0	10-12	2
10	8	11-11	0
11	2	11-12	2
12	4	12-12	0
13	0	homozygote	3
14	0	heterozygote	4
all	14	total sample	7

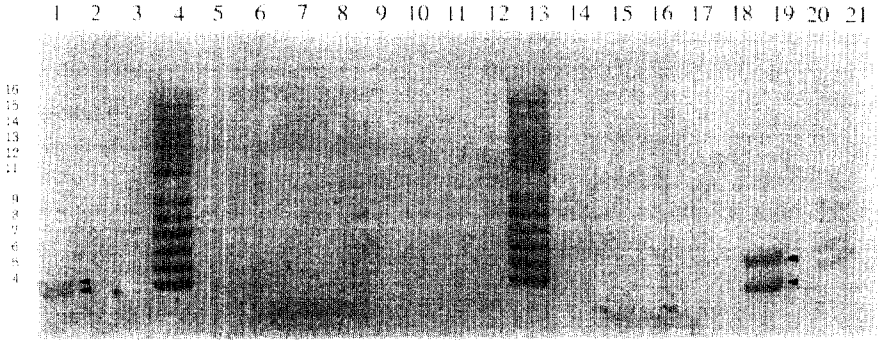

Figure 2. Bands of F13A01 locus from the endodontic treated teeth
Lane 1 : genotype 3.2-4(arrow), Lane 19 : genotype 3.2-5(arrow)
Lane 2, 3, 5-12, 14-18, 20 : genotype was not observed.
Lane 4, 13, 21 : allelic ladder(F13A01 ladder, Promega)

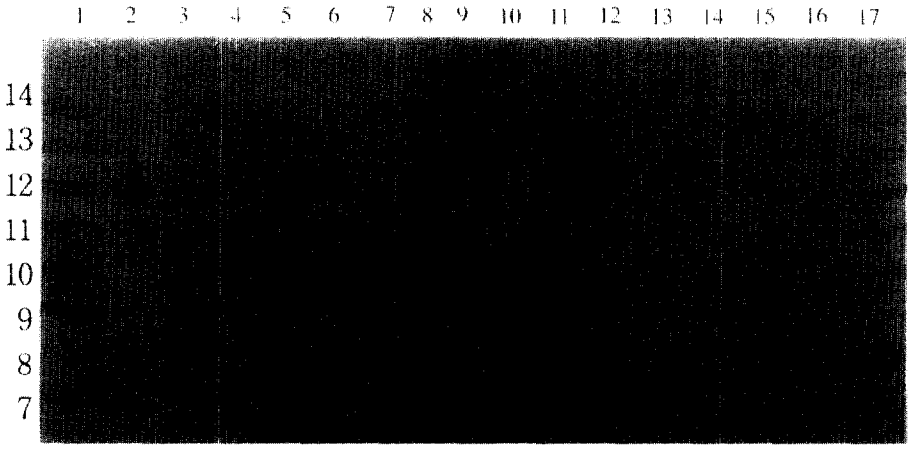

Figure 3. Bands of LPL locus from the endodontic treated teeth
Lane 1, 9, 11, 17 : allelic ladder(LPL ladder, Promega)
Lane 5, 7 : genotype 10-10(distinct-arrow)
Lane 6 : genotype 10-12(faint-arrow)
Lane 2, 3, 4, 8, 10, 12-16 : genotype was not observed.

IV. DISCUSSION

Individual identification using the teeth has very significant in forensic odontological field. A tooth is the hardest tissue of the human body, pulp is surrounded by enamel, dentin and cementum, so it is well protected. The tooth is distinct anatomic location where DNA is found[25,77,88,92].

DNA polymorphisms is size differences of DNA fragments after digestion of DNA by restriction endonuclease(restriction fragment length polymorphisms, RFLPs)[26,66]. It can be visualized by Southern blotting and DNA hybridization [19,20,34,35,40,54,92]. Some genetic markers of DNA polymor-

phisms are used in forensic science. Particularly the "DNA fingerprint"[49] technique, which was first described by Jeffreys et al. [19,27,33-36], is very useful for individual identification. This is because the resulting Southern- blot profile consists of a complex set of large and highly variable DNA fragments and has extraordinary individualizing power. The DNA fingerprint technique has also been used in paternity determination, based on the fact that each fragment shown by this technique is segregated in Mendelian fashion.

However, RFLPs analysis fails to detect a large fraction of mutations and polymorphisms and has other limitations such as sensitivity, analysis time or the impossibility of typing highly degraded samples.

The introduction of the PCR[1,2,66] has raised the possibility that variable number of tandem repeats(VNTRs) and other DNA polymorphisms might be analyzed without these difficulties.

DNA amplification by the PCR has become a useful approach for typing VNTRs loci[42]. These regions are analysed according to their length using the Southern blot hybridization method which requires 0.1-1.0μg DNA. The development of the PCR by Saiki et al.[67] requiring 1-10ng has made it possible to analyse DNA from small amounts of forensic sample. In the field of forensic science, the so-called Amp-FLPs[43,70] method has been developed, which involves allele amplification followed by allele length analysis. Loci, such as ApoB[73], D17S5[6], D17S30[32] or D1S80[5,7,10,13,37,38,68,70,72,78,83], have been typed by amplification of DNA by PCR.

Although the sensitivity of Amp-FLPs is suitable for analysing forensic samples, most VNTR locus alleles are too long to be analysed as Amp-FLPs; for example, D1S8(MS32)[81,90,91] on chromosome 1 consists of a tandem array of 29 bp repeat units, the array varying from 0.3 to 20 kb in length. Amp-FLPs from degraded DNA samples may fail to obtain target bands[70]. With the advent of PCR, STR marker polymorphisms [24,45,65] replaced VNTRs as the markers of choice. It is estimated that the human genome contains approximately 500,000 STRs(6,000 to 10,000 trimeric and tetrameric repeats)[15,23,29]. These abundant repeats may be detected using the PCR and polyacrylamide gel electrophoresis. Loci, such as TH01[22,25], CD4[16], CSF1PO, vWA, D1S53, D21S11, MBP, F13A01[59,64], F13B[56], FES/FPS, hTPO, HPRT, HMG14, TRMI[62], CRYG1, CYP19[63], vWF[39], LPL[94], have been typed by amplification of DNA by PCR.

The F13AO1 locus[59,64], one of STR locus, is $[AAAG]_n$ polymorphism and present within the 5′ untranslated region of the human coagulation factor XIII A subunit gene on chromosome 6p24-p25 and has 3.2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 alleles. These alleles vary in length from 181 through 231 base pair. The LPL locus is $[TTTA]_n$ polymorphism and human lipoprotein lipasegene on chromosome 8p22 and has 7, 9, 10, 11, 12, 13, 14 alleles.

Sex determination of forensic DNA samples has been performed by amplification of sex chromosomal genes, such as Y chromosomal repetitive DYZ1 sequence[2,3] in the distal Y chromosome long arm, centromeric α-satellite repeats of X and Y chromosomes, and the X-Y homologous amelogenin gene[1,2,53,80]. An advantage of the DYZ1 locus analysis has been that its repetitive nature increases the sensitivity of the assay, reducing the need for template DNA. However, this method lacks an internal control. Therefore, the absence of amplified DYZ1 material could be interpreted as due to either the female sex of the template DNA or amplification failure. Additionally, autosomal homologues of the DYZ1 family are co-amplified resulting in nonspecific amplified fragments in female samples[1,2,3,18,31,41,76,84,85], counterparts were detected at the same time.

The Y counterpart of the gene has a deletion within one of the introns, making it possible to differentiate between X and Y counterparts based on the difference in size. This difference in the size was attributed to that of an intron, and the X-fragment acted as an internal control that validated the analytical procedures.

Enamel is the most highly mineralized tissue, consisting of 96% inorganic material and 4% organic material and water. Dentin is chemically composed of a approximately 70% inorganic material and 20% organic material and 10% water. The central portion of the pulp is circumscribed by the specialized odontogenic region composed of the dentin-forming cells, the odontoblasts, the cell-free zone, and the cell-rich zone[8,82].

The most characteristic feature of dentin is the closely packed dentinal tubules that traverse its entire thickness and contains the odontoblastic process which once formed the dentin and now maintain it.

Dentinal tubules are small, canal-like spaces within the dentin filled with tissue fluid and occupied for part or all of their length by odontoblastic process. The processes are cytoplasmic extensions of the odontoblasts. They extend through the entire thickness of the dentin from the dentinoenamel junction to the pulp and their codfiguration indicates the course taken by the odontoblast during dentinogenesis. The processes are larger near the junction with the cell body of the odontoblasts[8,82].

The dentinal tubules are tapered in outline, measuring approximately 2.5㎛ in diameter near the pulp, 1.2㎛ in the midportion of the dentin, and 0.9㎛ near the dentinoenamel junction. In coronal dentin, there are approximately 20,000 tubules per square millimeter near the enamel and 45,000 per square millimeter near the pulp.

Because of many cell body and odontoblastic process in the dentin near the pulp, nuclear DNA can be found. But in the endodontic treated teeth, the dentin near the pulp was removed, so it should be considered that dentin did not contain nuclear DNA.

However, blood and pulp component can permeate into the dentinal tubules by endodontic therapy or trauma. Thus, there is possiblility to detect of DNA in dentin.

In this study, DNA could be found from 19 dentin out of 40. The DNA concentration of samples was estimated from 8.7ng/㎕ to 69.6 ng/㎕(Table 1) and purity was from 0.29 to 1.10(Table 2). It suggested that the nuclear DNA infiltrated dentin.

Sex determination by X-Y homologous amelogenin gene was possible in 12 samples out of 40. The 106 bp and 112 bp band were observed in 5 male samples out of 21 and the 106 bp in the 7 female samples out of 19.

The F13A01 locus was observed in the 6 samples out of 40 and observed alleles were 3.2, 4, 5, 6 and the most frequently observed allele was 3.2. The observed genotypes were 3.2-3.2, 3.2-4, 3.2-5, 4-5, 6-6 and the most frequently observed genotype was 3.2-4. The LPL locus was observed in the 7 samples out of 40 and observed alleles were 10, 11, 12 and the most frequently observed allele was 10. The observed genotypes were 10-10, 10-12, 11-12 and the most frequently observed genotype was 10-10. Although DNA was extracted from the sample, it failed to reveal STR gene from some of them. Perhaps it is expressed that teeth did contain degraded DNA or RNA or other protein.

This study demonstrated the possibility that endodontic treated teeth were suitable for DNA extraction, sex determination, amplification of STR locus F13A01, LPL. However, additional assays of more variable VNTR, STR locus is needed in the future. Isolation of maximum DNA before assay is also impotant in forensic samples. Thus, the variable method must be studied to

isolate maximum DNA from endodontic treated teeth.

V. CONCLUSIONS

Author isolated the human DNA from 40 endodontic treated teeth, performed AmpFLPs by PCR and electrophoresed for detection of STR locus F13A01, LPL gene and X-Y homologous amelogenin gene.

The following results were obtained.

1) DNA extraction was possible in 19 endodontic treated teeth out of 40.
2) Sex determination was possible in 12 endodontic treated teeth out of 40 by detection of X-Y homologous amelogenin gene
3) F13A01 locus was detected in 6 endodontic treated teeth out of 40, observed 4 alleles(3.2, 4, 5, 6) and 5 genotypes(3.2-3.2, 3.2-4, 3.2-5, 4-5, 6-6).
4) LPL locus was detected in 7 endodontic treated teeth out of 40, observed 3 alleles(10, 11, 12) and 3 genotypes(10-10, 10-12, 11-12).

From the above results, DNA extraction, sex determination, amplification of STR locus F13A 01, LPL gene were possible in the endodontic treated teeth and it was suggested that endodontic treated teeth were useful and applicatable as molecular biological samples for individual identification.

REFERENCES

1. Akane, A., Shiono, H., Matsubara, K., Nakamura, H., Hasegawa, M., and Kagawa, M. : Purification of forensic specimens for the polymerase chain reaction(PCR) analysis, J. Forensic Sci., 38(3), 691-701, 1993.
2. Akane, A. et al : Sex identification of forensic specimens by polymerase chain reaction(PCR) : Two alternative methods, Forensic Science International, 49, 81-88, 1991
3. Akane, A., Seki, S., Shiono, H., Nakamura, H., Hasegawa, M., Matsubara, K., Nakahori, Y., Nagafuchi, S., and Nakagome, Y. : Sex determination of forensic samples by dual PCR amplification of an X-Y homologous gene, Forensic Science International, 52, 143-148, 1992.
4. Alford, R. L., Hammond, H. A., Coto, I. and Caskey, C. T. : Rapid and Efficient Resolution of Parentage by Amplifcation of Short Tandem Repeats. Am. J. Hum. Genet., 55, 190~195, 1994.
5. Baechtel, F. S., Smerick, J. B., Presley, K. W. and Budowle, B : Multigenerational amplification of a reference ladder for alleles at locus D1S80, J. Forensic Sci., 38(5), 1176-1182, 1993
6. Balazs, I., Baird, M., Clyne, M. and Meade, E. : Human population genetic studies of five hypervariable DNA loci, Am. J. Hum. Genet., 44, 182-190, 1989.
7. Barros, F., Lareu, M. V. and Carracedo, A. : Detection of polymorphisms of human DNA after polymerase chain reaction by miniaturized SDS- PAGE . Forensic Science International, 55, 27-36, 1992
8. Bhaskar, S. N. : Oral Histology and Embryology, 8th ed., USA : The C.V. Mosby Company, 105~109, 1976.
9. Bodfish, P., Warne, D., Nyberg, K. and Spurr, N. K. : Dinucleotide Repeat Polymorphism at the Human Erythroid Alpha-Spectrin (SPTA1) mRNA Gene Detected Using PCR. Human Molecular Genetics, 1(4), 89, 1992.
10. Budowle, B. et al : Analysis of the VNTR locus DIS80 by the PCR followed by high-resolution PAGE, Am. J. Hum. Genet., 48, 137-144, 1991.
11. Budowle, B., Waye, J. S., Shutler, G. G. and Baechtel, F. S. : Hae Ⅲ-A suitable restriction endonuclease for restriction fragment length polymorphism analysis of biological evidence samples, J. Forensic Sci., 35(3), 530- 536, 1990
12. Choi J. H., Han M. S., Sun M. S., Kim C. Y. : Experimental study for DNA fingerprint from teeth of charred body. The Journal of Korean Academy of Oral Medicine, 21(2), 351~367, 1996.
13. Chuah, S. Y., Tan, W. F., Yap, K. H., Tai, H. E.,

and Chow, S. T. : Analysis of the D1S80 locus by amplified fragment length polymorphism technique in the Chinese, Malays and Indians in Singapore, Forensic Science International, 68, 169-180, 1994.
14. Cooke, H. J. : Repeated sequences specific to human males, Nature, 262, 182-186, 1976.
15. Edwards, A., Civitello, A., Hammond, H. A. and Caskey, T. : DNA Typing and Genetic Mapping with Trimeric and Terameric Tandem Repeats. Am. J. Hum. Genet., 49, 746~756, 1991.
16. Edwards, M. C., Clemens, P. R., Tristan, M., Pizzuti, A. and Gibbs, R. A. : entanucleotide Repeat Length Polymorphism at the Human CD4 Locus. Nucleic Acids Research, 19(19), 5450, 1991.
17. Frégeau, C. J. and Fourney, R. M. : DNA Typing with Fluorescently Tagged Short Tandem Repeat : A Sensitive and Accurate Approach to Human Identification. BioTechniques, 15(1), 100~119, 1993.
18. Fukushima, H., Hasekura, H. and Nagai, K. : Identification of male bloodstains by dot hybridization of hyman Y chromosome-specific DNA probe, J. Forensic Sci., 33, 621-627, 1988.
19. Gill, P., Jeffreys, A. J., and Werrett, D. J. : Forensic application of DNA 'fingerprints', Nature, 318, 577-579, 1985.
20. Gill, P., Sullivan, k., and Werrett, D. J. : The analysis of hypervariable DNA profiles : problems associated with the objective determination of the probability of a match, Hum. Genet., 85, 75-79, 1990.
21. Granqvist, M., Xiang, K., Seino, M., Fukumoto, H. and Bell, G. I. : Dinucleotide Repeat Polymorphism in Human GLUT2/liver Facilitative Glucose Transporter Gene on Chromosome 3. Nucleic Acids Research, 19(17), 4791, 1991.
22. Han M. S., Lee Y. H., Park K. W., Seon M. S., Choi S. K. : The genetic analysis of STR HUMTHO1 locus using AMP-FLP applied to human identification. Annual report of N.I.S.I., 26, 149~152, 1994.
23. Han M. S., Lee Y. H., Seon M. S., Park K. W., Choi S. K. : The genetic analysis of STR vWA locus and frequency distribution in Korean population. Annual report of N.I.S.I.. 27, 180~187, 1995
24. Hammond, H. A., Jin, L., Zhong, Y., Caskey, T. and Chakraborty, R. : Evaluation of 13 Short Tandem Repeat Loci for Use in Personal Identification Applications. Am. J. Hum. Genet., 55, 175~189, 1994.
25. Hearne, C. M. and Todd, J. A. : Tetranucleotide Repeat Polymorphism at the HPRT Locus. Nucleic Acids Research, 19(19), 5450, 1991.
26. Hearne, C. M. and Todd, J. A. : Trinucleotide Repeat Polymorphism at the CRYG1 Locus. Nucleic Acids Research, 19(19), 5450, 1991.
27. Hill, A. V. S., and Jeffreys, A. J. : Use of minisatellite DNA probes for determination of twin zygosity at birth, Lancet, 21(28), 1394-1395, 1985.
28. Huang, T. H-M., Hejtmancik, J. F., Edwards, A., Pettigrew, A. L., Herrera, C. A., Hammond, H. A., Caskey, C. T., Zoghbi, H. Y. and Ledbetter, D. H. : Linkage of the Gene for an X-linked Mental Retardation Disorder to a Hypervariable $(AGAT)_n$ Repeat Motif within the Human Hypoxanthine Phosphoribosyltransferase (HPRT) Locus (Xq26). Am. J. Hum. Genet., 49, 1312~1319, 1991.
29. Hubert, R., Weber, J. L., Schmitt, K., Zhang, L. and Arnheim, N. : A New Source of Polymorphic DNA Markers for Sperm Typing : Analysis of Microsatellite Repeats in Single Cells. Am. J. Hum. Genet., 51, 985~991, 1992.
30. Hur W., Yoon C. L. : Camparison of 4 methods of DNA extraction for sex determination and D1S80 locus detection in teeth. The Journal of Korean Academy of Oral Medicine, 20(2), 497~512, 1995.
31. Ishizu, H. : Special communication 1-sex identification of forensic biological materials, Jpn. J. Legal Med., 47(6), 423-424, 1993.
32. Ivey, J. N., Atchison, B. A. and Georgalis, A. M. : Assessment of PCR of the D17S30 locus for forensic identification, J. Forensic Sci., 39(1), 52-63, 1994.
33. Jeffreys, A. J., Brookfield, J. F. Y., and Semeonoff, R. : Positive identification of an immigration test-case using human DNA fingerprints, Nature, 317, 818-819, 1985.
34. Jeffreys, A. J., Turner, M. and Debenham, P. : The Efficiency of Multilocus DNA Fingerprint Probes for Individualization and Establishment of Family Relationships, Determined from Extensive Casework. Am. J. Hum. Genet., 48, 824~840, 1991.
35. Jeffreys, A. J., Wilson, V., and Thein, S. L. : Individual-specific 'fingerprints' of DNA, Nature, 316, 76-81, 1985.
36. Jeffreys, A. J., Wilson, V., and Thein, S. L. : Hypervariable 'minisatellite' regions in human

DNA, Nature, 314, 67-73, 1985.
37. Kasai, K., Nakamura, Y., and White, R. : Amplification of a variable number of tandem repeats (VNTR) locus(pMCT118) by the polymerase chain reaction(PCR) and its application to forensic science, J. Forensic Sci., 35(5), 1196-1200, 1990.
38. Kim C. H., Myeong H. K., Hong Y. P., Hwang J. J. : Estimates of allelic frequencies and genetic diversity at VNTR D1S80 locus revealed genetic homogeneity in Korean. The Korean Journal of Legal Medicine, 18(1), 60-70, 1994.
39. Kimpton, C., Walton A. and Gill, P. : A Further Tetranucleotide Repeat Polymorphism in the vWF Gene. Human Molecular Genetics, 1(4), 289, 1992.
40. Kim Y. S., Hur W., Yoon C. L. : Analysis of Short Tandem Repeat(STR) locus F13B by polymerase chain reaction in Korean. The Journal of Korean Academy of Oral Medicine, 21(2), 243~253, 1996.
41. Kobayashi, R. et al : Sex identification in fresh blood and dried bloodstains by a nonisotopic DNA analyzing technique, J. Forensic Sci., 33, 613-620, 1988.
42. Kwak M. J., Myeong H. K., Lee H. S., Hwang J. J. : Allele frequencies of COL2A1 locus in Koreans detected by polymerase chain reaction. The Korean Journal of Legal Medicine, 18(2), 1-8, 1994.
43. Kwon K. H., Park C. J., Lee H. S., Lee H. R., Song E. S., Hwang J. J. : Genetic diversities of three Short Tandem Repeats(STRs) loci in Korean amplified by multiplex PCR. The Korean Journal of Legal Medicine, 20(1), 24~36, 1996.
44. Lee H. S., Lee H. R., Park H. K., Song E. S., Hwang J. J. : Mitochondrial DNA polymorphism in the Korean Population. The Korean Journal of Legal Medicine, 20(1), 47~65, 1996.
45. Lee K., Lee H. S., Park H. K., Song E. S., Hwang J. J. : Genetic diversities of DXS52 locus in Korean population amplified by polymerase chain reaction. The Korean Journal of Legal Medicine, 20(1), 37~46, 1996.
46. Lee S. D., Shin C. H., Kim K. B., Choi Y. T., Lee Y. S., Lee J. B. : Individual identification using polymorphism in control region of mtDNA in case of mass disaster. The Korean Journal of Legal Medicine, 20(1), 1~11, 1996.
47. Lee Y. S., Yoon C. L. : Genotype and alle frequency of the Short Tandem Repeat(STR) F13A01 locus by polymerase chain reaction in Korean. The Journal of Korean Academy of Oral Medicine, 21(2), 317~329, 1996.
48. Lorente, J. A., Lorente, M., Budowle, B., Wilson, M. R.. and Villanueva, E. : Analysis of Short Tandem Repeat(STR) HUMVWA in the Spanish Population. Forensic Science International, 65, 169~175, 1994.
49. Ludes, B., pfitzinger, H., and Mangin, P. : DNA fingerprinting from tissues after var iable postmortem period, J. Forensic Sci., 38(3), 686-690, 1993
50. Mullis, K. B., and Faloona, F. A. : Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction, Methods in Enzymology, 155, 335-350, 1987.
51. Nagai, A., Yamada, S., Watanabe, Y., Bunai, Y. and Ohya, I. : Analysis of the STR loci HUMF1301, HUMFXIIIB, HUMLIPOL, HUMTH01, HUMTPOX and HUMVWFA31 in a Japanese Population. Int. J. Legal Med., 109, 34~36, 1996.
52. Nagao, M., Yamada, Y., Zhang, W. D., Kobayashi, M., Oono, T., Iwadate, K., Yagi, K., and Takatori, T., : Personal identification from human remains using morphological characteristics and DNA analysis, Jpn. J. Legal Med., 48(2), 87-91, 1994.
53. Naito, E., Dewa, K., Yamanouchi, H., and Kominami, R. : Sex typing of forensic DNA samples using male-and female-specific probes, J. Forensic Sci., 39(4), 1009-1017, 1994.
54. Nakahori, Y., Mitani, K., Yamada, M., and Nakagome, Y. : A human Y- chromosome specific repeated DNA family(DYZ 1) consists of a randem array of pentanucleotides, Nucleic Acid Res., 14, 7569-7580, 1985.
55. Nam Y. S., Lee H. S., Lee H. R., Kim H. S., Kim K. H., Hwang J. J. : DNA typing analysis for the identification of bone fragments from Sampoong disaster. The Korean Journal of Legal Medicine, 20(1), 12~23, 1996.
56. Nishimura, D. Y. and Murray, J. C. : A Tetranucleotide Repeat for the F13B Locus. Nucleic Acids Research, 20(5), 1167, 1992.
57. Nishimura, D. Y., Leysens, N. J. and Murray, J. C. : A Dinucleotide Repeat for the D1S53 Locus. Nucleic Acids Research, 20(5), 1167, 1992.
58. Perlin, M. W., Lancia, G. and See-Kiong Ng : Toward Fully Automated Genotyping : Genotyping

Microsatellite Markers by Deconvolution. Am. J. Hum. Genet., 57, 199~1210, 1995.
59. Polymeropoulos, M. H., Rath, D. S. and Merril, C. R. : Tetranucleotide Repeat Polymorphism at the Human Coagulation Factor XIII A Subunit Gene(F13A1). Nucleic Acids Research, 19(15), 4306, 1991.
60. Polymeropoulos, M. H., Xiao, H., Rath, D. S. and Merril, C. R. : Dinucleotide Repeat Polymorphism at the Human Non-Histone Chromosomal Protein HMG14 Gene. Nucleic Acids Research, 19(13), 3753, 1991.
61. Polymeropoulos, M. H., Xiao, H., Rath, D. S. and Merril, C. R. : Tetranucleotide Repeat Polymorphism at the Human Tyrosine Hydroxylase Gene (TH). Nucleic Acids Research, 19(13), 3753, 1991.
62. Polymeropoulos, M. H., Xiao, H., Rath, D. S. and Merril, C. R. : Trinucleotide Repeat Polymorphism at the Human Met-tRNA-i Gene 1(TRMI). Nucleic Acids Research, 19(15), 4306, 1991.
63. Polymoropoulos, M. H., Xiao, H., Rath, D. S. and Merril, C. R. : Tetranucleotide Repeat Polymorphism at the Human Aromatase Cytochrome P-450 Gene(CYP19). Nucleic Acids Research, 19(1), 195, 1991.
64. Puers, C., Hammond, H. A., Caskey, T., Lins, A. M., Sprecher, C. J., Brinkmann, B. and Schumm, J. W. : Allelic Ladder Characterization of the Short Tandem Repeat Polymorphism Located in the 5′ Flanking Region to the Human Coagulation Factor XIII A Subunit Gene. Genomics, 23, 260~264, 1994.
65. Puers, C., Hammond, H. A., Jin, L., Caskey, T. and Schumm, J. W. : Identification of Repeat Sequence Heterogeneity at the Polymorphic Short Tandem Repeat Locus HUMTH01[AATG]$_n$ and Reassignment of Alleles in Population Analysis by Using a Locus-Specific Allelic Ladder. Am. J. Hum. Genet., 53, 953~958, 1993.
66. Reynolds, R., and Sensabaugh, G. : Analysis of genetic markers in forensic DNA samples using the polymerase chain reaction, Anal. Chem., 63, 2-15, 1991.
67. Saiki, R. K. et al : Enzymatic amplification of β -globulin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia, Science, 230, 1350-1354, 1985.
68. Sajantila, A. et al : PCR amplification of alleles at the D1S80 locus : Comparison of a finnish and a north american caucasian population sample, and forensic casework evaluation, Am. J. Hum. Genet., 50, 816-825, 1992.
69. Sajantila, A., Pacek, P., Lukka, M., Syvänen, A. C., Nokelainen, P., Sistonen, P., Peltonen, L. and Budowle, B. : A Microsatellite Polymorphism in the von Willebrand Factor Gene : Comparison of Allele Frequencies in Different Population Samples and Evaluation for Forensic Medicine. Forensic Science International, 68, 91~102, 1994.
70. Sajantila, A., Ström, M., Budowle, B., Karhunen, P. J. and Peltonen, L. : The Polymerase Chain Reaction and Post-Mortem Forensic Identity Testing : Application of Amplified D1S80 and HLA-DQα Loci to the Identification of Fire Victims. Forensic Sceince International, 51, 23~34, 1991.
71. Schmitt, C., Schmutzler, A., Prinz, M. and Staak, M. : High Sensitive DNA Typing Approaches for the Analysis of Forensic Evidence : Comparison of Nested Variable Number of Tandem Repeats (VNTR) Amplification and a Short Tandem Repeats(STR) Polymorphism. Forensic Science International, 66, 129~141, 1994.
72. Schnee-Griese, J. et al : Frequency distribution of D1S80 alleles in the German population, Forensic Science International, 59, 131-136, 1993.
73. Schnee-Griese, j. and Teifel-Greding, J. : DNA length polymorphism of the APO B 3′ region : Frequency distribution of the alleles in the German population, Forensic Science International, 51, 173-178, 1991.
74. Schnee, G. J., and Teifel, G. J. : Frequency distribution of D1S80 alleles in the German population, Forensic Science International, 59, 131-136, 1993.
75. Schwartz, T. R., Schwartz, E. A., Mieszerski, L., McNally, L., and Kobilinsky, L., : Characterization of deoxyribonucleic acid(DNA) obtained from teeth subjected to various environmental conditions, J. Forensic Sci., 36(4), 979-990, 1991.
76. Semba, S., Yamamoto, Y. and Ishizu, H. : Sex determination from blood and bloodstains by polymerase chain reaction(PCR), Jpn. J. Legal Med., 48(1), 7-18, 1994.
77. Smith, B. C., Fisher, D. L., Weedn, V. W., Warnock,

G. R., and Holland, M. M., :A systematic approach to the sampling of dental DNA, J. Forensic Sci., 38(5), 1194-1209, 1993.

78. Song E. S., Jung J. A., Lee H. S., Hwang J. J. : Valid conditions on the analysis of the D1S80 locus using PCR. The Korean Journal of Legal Medicine, 18(1), 45-59, 1994.
79. Stewart, E. A., White, A., Tomfohrde, J., Osborne-Lawrence, S., Prestridge, L., Bonne-Tamir, B., Scheinberg, I. H., St George-Hyslop, P., Giagheddu, M., Kim, J. W., Seo, J. K., Lo, W. H.-y., Ivanova-Smolenskaya, I. A., Limborska, S. A., Cavalli-Sforza, L. L., Farrer, L. A. and Bowcock, A. M. : Polymorphic Microsatellites and Wilson Disease (WD). Am. J. Hum. Genet., 53, 864~873, 1993.
80. Sullivan, K. M., Mannucci, A., Kimpton, C. P., and Gill, P. : A rapid and quantitative DNA sex test : Fluorescence-based PCR analysis of X-Y homologous gene ame logenin, BioTechniques, 15(4), 636-642, 1993.
81. Tamaki, K. et al : DNA typing and analysis of the D1S8(MS32) allele in the Japanese population by the minisatellite variant repeat(MVR) mapping by polymerase chain reaction(PCR) assay, Jpn. J. Legal Med., 46(6), 474-482, 1992.
82. Ten Cate, A. R. : Oral Histology, 2nd ed., USA:The C.V.Mosby Company, 146~152, 1985.
83. Thymann, M., Nellemann, L. J., Masumba, G., Irgens, L. M., and Morling, N. : Analysis of the locus D1S80 by amplified fragment length polymorphism technique(AMP-FLP). Frequency distribution in Danes. Intra and Inter laboratory reproducibility of the technique, Forensic Science International, 60, 47-56, 1992.
84. Tyler, M. G., Kirby, L. T., Wood, S. and Vernon, S. : Human blood stain identification and sex determination in dried blood stains using recombinant DNA technique, Forensic Sci. Int., 31, 267-272, 1986.
85. Verbovaya, L. V. and Ivanov, P. L. : "Sexing" deoxyribonucleic acid (DNA) on DNA fingerprint gel : An internal control for DNA fingerprint evidence. J. Forensic Sci., 36(4), 991-998, 1991,
86. Watkins, C., Bodfish, P., Warne, D., Nyberg, K. and Spurr, N. K. : Dinucleotide Repeat Polymorphism in the Human Alpha-Cardiac Actin Gene, Intron IV (ACTC), Detected Using the Polymerase Chain Reaction. Nucleic Acids Research, 19(24), 6980, 1991.
87. Watson, J. D. and Crick, F. H. C. : Molecular Structure of Nucleic Acids : A Structure for Deoxyribonucleic Acid. Nature, 171, 737~738, 1953.
88. Yamada, Y., Yamanoto, K., Yoshi, T., and Ishiyama, I. : Analysis of DNA from tooth and application to forensic dental medicine, Jpn. J. Legal Med., 43(5), 420-423, 1989.
89. Yamamoto, K. : Molecular biological studies on teeth inquests, Jpn. J. Legal Med., 46(6), 349-355, 1992.
90. Yamamoto, T. et al : DNA typing of the D1S8 (MS32) locus by rapid detection minisatellite variant repeat(MVR) mapping usingpolymerase chain reaction(PCR) assay, Forensic Science International, 66, 69-75, 1994.
91. Yamamoto, T., Tamaki, K., Kojima, T., Uchihi, R., and Katsumata, Y. : Potential forensic applications of minisatellite variant repeat(MVR) mapping using the polymer ase chain reaction(PCR) at D1S8, J. Forensic Sci., 39(3), 743-750, 1994.
92. Yoon C. L., Kim C. Y. : Individual identification by analysis of DNA from the teeth. The Journal of Korean Academy of Oral Medicine, 20(1), 229-245, 1995.
93. Yoon C. L., Kim C. Y. : Forensic Dental Medicine, Seoul:EEWOO MUNHWA Co., 151-194, 1995
94. Zuliani G. and Hobbs, H. H. : Tetranucleotide Repeat Polymorphism in the LPL Gene. Nucleic Acids Research, 18(16), 4958, 1990.

국문초록

근관치료된 치아상아질에서 Amelogenin Gene 및 Short Tandem Repeat(STR) 유전좌위 F13A01, LPL에 대한 분석

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치아는 성별과 연령의 추정은 물론 혈형 검사와 유전자 검사까지 가능하게 하는 중요한 법의치과학적 자료이다. 대부분 치아를 이용한 연구는 핵 DNA가 들어있는 치수에서의 연구로 치수내에는 풍부한 혈액 및 세포가 분포해 있어 핵 DNA가 다량 함유되어 있다. 그러나 순수 상아질에는 핵이 없고 따라서 핵 DNA도 없는 것으로 알려졌지만 치수내에 존재하는 핵 DNA가 상아세관을 통하여 상아질내로 침투할 가능성이 있고 실제 근관치료가 되어 있는 무수치를 감정하게 되는 경우도 있다. 본 연구에서는 이러한 치아중에서도 근관치료를 받은 무수치에서 개인식별에 활용되는 유전자가 검출되는지 여부를 확인하고자 하였다.

40개의 근관치료된 치아상아질에서 DNA를 추출하고 중합효소반응을 이용하여 증폭절편다형(Amp-FLPs)을 실시하고 X-Y homologous amelogenin gene과 STR 유전좌위 F13A01, LPL를 검색하여 다음과 같은 결과를 얻었다.

1. 40개의 근관치료된 치아중 19개에서 DNA가 추출되었다.
2. X-Y homologous amelogenin gene 검색으로 40개의 근관치료된 치아에서 21개의 남자치아중 5개, 19개의 여자치아중 7개 등 모두 12개 치아에서 성별검사가 가능하였다.
3. F13A01 유전자는 40개의 근관치료된 치아중 6개의 치아에서 검색되었으며, 4개의 대립유전자 및 5개의 유전자형을 관찰하였다.
4. LPL 유전자는 40개의 근관치료된 치아중 7개의 치아에서 검색되었으며, 3개의 대립유전자 및 3개의 유전자형을 관찰하였다.

이상의 결과를 종합하여 볼 때 근관치료된 치아상아질에서 중합효소반응을 이용한 성별검사 및 STR 유전좌위의 검색은 일부 치아에서만 가능하였으나, 근관치료된 치아들도 개인식별을 위한 법의치과학적 자료로서 유용할 것으로 사료된다.