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STR LOCI D19S216, D20S502 AND D20S842 ANALYSIS IN THE SERBIAN POPULATION USING DENTIN DNA

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Abstract - Dentin provides a protective enclosure for genomic and mitochondrial DNA. In the present study, DNA was obtained from pulverized or ground teeth. The quality of the DNA extracted from the teeth of 70 unrelated individuals was tested in the context of assessing the allelic and genotypic frequencies of autosomal loci D19S216, D20S502 and D20S842, and calculating a number of parameters of population genetics and forensic interest. This study illustrates that teeth can be a convenient tissue to extract DNA from large numbers of individuals for population genetic studies as well as for forensic case work.

Key words: Forensic science, population genetics, STR, dentin

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INTRODUCTION

A suitable approach in forensic and medical DNA typing is the use of genetic material from hard tissues including bones and teeth. As a result of their high resistance to external physical or chemical insult, these tissues allow for the recovery of DNA after prolonged time (Di Nunno et al., 2007, Ricaut et al., 2005). Teeth are actually the most stable structures in the body, though the quantity of dental DNA will depend on its location within the tooth (Malaver and Yunis, 2003). Cementoblasts and odontoblasts are embedded in the mineral matrix of the dental structure and thus protected from many environmental degradation forces. Yet, due to the hardness of the tissue, DNA extraction can often be technically challenging. Dental specimens have been used as a source of DNA in forensic analysis with various degrees of success, depending on the type of polymorphic markers employed and the extraction method utilized (Trivedi et al., 2002, Yamamoto, 1996). Microsatellites or short tandem repeat (STR) markers are important genetic tools used for human identity testing. STR loci are currently widely used in generating population databases for human DNA profiling (Rowold and Herrera, 2005a). STR polymorphisms are very important markers for human identification and phylogenetic studies due to their hypervariable nature (Rowold and Herrera, 2003b). In this study, we aimed to establish the usefulness of dental tissue as a source of DNA for genotyping the hypervariable STR loci D19S216, D20S502 and D20S842, which are of medicinal, forensic and population genetic interest.

MATERIAL AND METHODS

DNA extraction

Teeth extracted for therapeutic purposes from 70 unrelated individuals living in Serbia were prepared for DNA isolation following storage for 2 years at ambient temperature and humidity (50 teeth), in sand (10 teeth) and water (10 teeth). To remove surface contamination, the teeth were successively washed in a mild detergent and subsequently treated with 5% bleach, rinsed with three changes of sterile distilled water and 100% ethanol, each, and then allowed to air-dry. The thoroughly dried samples were crushed with a mortar and pestle (20 teeth) or pulverized (50 teeth) using a sterilized high-speed handpiece (Dentsply, Maillefer, Switzerland) and then placed into separate tubes. Genomic DNA was extracted from the crushed or pulverized dental tissues after a decalcification step with EDTA. This was accomplished by subjecting the dental powder or crushed teeth to decalcification by treating with a 0.5M EDTA solution for one week. The upper phase was decanted and the dental powder was incubated with extraction buffer (10mM Tris-HCl, pH 8.0, 100nM NaCl, 2% sodium dodecyl sulfate, 10mM EDTA and proteinase K) for 2 days at 56°C. The DNA was isolated by phenol-chloroform isoamyl extraction and ethanol precipitation (Minaguchi, 1999).

PCR amplification of STRs

All PCR reactions were performed utilizing 100 ng of DNA, 2.5 μl Taq buffer (10xPCR Buffer II, Fermentas, Vilnius, Lithuania), 0.2 μl of 25 μM $MgCl_2$, 0.5 μl dNTPs mix (10 μM of each nucleotide), 0.5 μM of each primer and 1 unit of Taq polymerase in a volume of 25 μl. PCR amplification conditions were specific for each analyzed STR loci, with annealing temperatures of 62°C (D20S502), 58°C (D19S216) and 50°C (D20S842). The amplified products were separated by 12% vertical nondenaturating polyacrylamide gel electrophoresis for 6 h on constant power of 600 V, visualized by silver staining and genotyped according to known fragment sizes utilizing a 50 bp ladder size ladder.

Population data calculation

Allele frequencies were estimated for each locus using the gene counting method. Unbiased estimates of the expected heterozygosity were calculated according to the method described by Edwards and colleagues and compared to the observed heterozygosity (Edwards et al., 1992). Hardy-Weinberg equilibrium expectations (HWEs) were assessed using the exact test based on 1000 shuffling (Guo and Thompson, 1992, Weir, 1990). Determinations of statistical parameters of forensic interest such as expected heterozygosity, power of discrimination, average probability of exclusion, polymorphism information content and typical paternity index, were performed using the Cervus statistical software (Marshall, 1998).

RESULTS

DNA extraction

The quantity and quality of human DNA obtained from each tooth were compared. The purity and concentration of DNA, as determined by spectrophotometry, obtained from crushed and pulverized teeth were assessed. The 260/280 ratio was found to be between 1.8 and 2.0. The quantity of dentin material used for DNA extraction varied between 1.5 and 2.2 g. The DNA concentrations ranged from 40 to 200 ng/μl. The relative yield was higher in teeth conserved at ambient temperature/humidity and in sand than in those kept in water. The yield was also higher in powdered teeth than in crushed, but no statistically significant difference in DNA concentrations was found between any of the tested groups ($p>0.05$).

STR Analysis

The microsatellite loci D20S502 and D20S842, in the Serbian population sample (n=70), were in Hardy-Weinberg equilibrium, whilst D19S216 did not meet the criteria for HWE. All analyzed parameters are given in Table 1. Only one or two alleles were detected per locus in all the individuals examined. Considering the hypervariable nature of these three loci, the lack of three or more alleles indicates that the decon-

Table 1. Allele frequencies and forensic data for three STR loci in Serbian population (n=70)

Allele frequencies			
Allele	D20S502	D19S216	D20S842
a	0.28	0.20	0.08
2	0.27	0.16	0.09
3	0.20	0.26	0.16
4	0.07	0.13	0.18
5	0.07	0.12	0.14
6	0.03	0.05	0.20
7	0.02	0.02	0.06
8	0.02	0.02	0.04
9	-	-	0.02
Forensic data			
H_o	0.871	0.500	0.914
H_e	0.793	0.777	0.862
P_m	0.08	0.009	0.038
PD	0.92	0.91	0.962
PIC	0.756	0.736	0.839
PE	0.411	0.376	0.546
PI	2.89	2.44	3.75
P*	0.7773	0.000	0.2742

H_o, observed heterozygosity; H_e, expected heterozygosity; P_m, probability of match; PD, power of discrimination; PIC, polymorphism information content; PE, power of paternity exclusion; PI, typical paternity index; P*, exact test.

tamination procedure was successful and no foreign DNA was introduced during the extraction.

DISCUSSION

Several studies have focused their interest on optimizing the efficiency of teeth decontamination and DNA retrieval from dental tissue for forensic purposes (Kemp and Smith, 2005, Lijnen and Willems, 2001, Gaytmenn and Sweet, 2003). The elimination of contaminant DNA from the surface of teeth is an important step and is routinely accomplished by immersion in at least 3.0% sodium hypochlorite for at least 15 min. As demonstrated by Kemp and Smith (2005), endogenous DNA proved to be quite stable, even to extreme sodium hypochlorite treatment (6.0% for 21 h), suggesting that DNA adsorbs to hydroxyapatite in bone, allowing for the preservation of DNA (Lijnen and Willems, 2001). With respect to DNA retrieval, including mtDNA from dental tissues, different techniques (sliced, crushed, ground teeth, shorter/longer decalcification periods and various digestion buffers) and tooth parts (dentin, cement, pulp, whole tooth) have been employed, with various degrees of success (Gaytmenn and Sweet, 2003, Mörnstad et al., 1999, Pfeiffer et al., 1998, Trivedi et al., 2002).

In this study, we implemented a simple approach to isolate DNA from whole teeth consisting of a short process of decontamination using bleach, followed by crushing or pulverizing of the tissue, decalcification, and digestion in EDTA with proteinase K. The method gave acceptable amounts of DNA suitable for PCR amplification.

DNA was extracted from the teeth of 70 unrelated individuals to attempt to demonstrate that this procedure is applicable and practical for analyzing groups of individuals in population genetic studies. Furthermore, the capacity of the extracted DNA to serve as targets for PCR amplification was tested with three autosomal STR loci of population genetics and forensic interest.

Two loci were found to be informative and useful for forensic identity testing. No linkage disequilibrium was observed among them. Genotyping this number of individuals demonstrated the usefulness of this method for population genetic studies. The genotypic data obtained allowed us to determine that the collection of individuals of Serbian origin are in Hardy-Weinberg equilibrium. In addition, a number of population genetics and forensic indices were calculated from the allelic and genotypic frequencies, demonstrating the integrity of the isolated DNA and enriching a database rather scant in

terms of polymorphic loci for the Serbian population (Puzović et al., 2006,a, Puzović et al., 2009b). The results indicate that for the analyzed sample population, the two STR loci met Hardy-Weinberg expectations and there was no evidence of linkage disequilibrium among the loci. Thus, two out of three selected markers examined in this study represent an additional set of highly polymorphic STR markers with applications in population genetic studies and medical association studies as well as in forensic case work.

REFERENCES

Di Nunno, N., Saponetti, S.S., Scattarella, V., Emanuel, P., Baldassarra, S.L., Volpe, G., and C.Di Nunno (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.

Edwards, A., Hammond, H., Jin, L., Caskey, C.T. and *R. Chakraborty* (1992). Genetic variation at five trimeric and terameric repeat loci in four human population groups. *Genomics.* **12**, 241-253.

Gaytmenn, R., and *D. Sweet* (2003). Quantification of forensic DNK from various regions of human teeth. *J Forensic Sci*. **48,** 622-625.

Guo, S.W., and *E.A. Thompson* (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics.* **48,** 361-372.

Kemp, B.M., and *D.G. Smith* (2005). Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. *Forensic Sci Int*. **154**, 53-61.

Lijnen, I., and G. Willems (2001). DNA research in forensic dentistry. *Methods Find Exp Clin Pharmacol.* **23**, 511-517.

Malaver, P.C., and J.J. Yunis (2003). Different dental tissues as source of DNA for human identification in forensic cases. *Croat Med J*. **44**, 306-309.

Marshall, T. (1998). Cervus statistical software, Ver.1.0, University of Edinburgh.

Minaguchi, K. (1999). The DYS19 system in the Japanese population and its detection using teeth as a source of DNA. *Bull Tokyo Dent Coll.* **40**, 21-26.

Mörnstad, H., Pfeiffer, H., Yoon, C., and A. Teivens (1999). Demonstration and semi-quantification of mtDNA from human dentine and its relation to age. *Int J Legal Med.* **112**, 98-100.

Pfeiffer, H., Steighner, R., Fisher, R., Mörnstad, H., Yoon, C.L., and M.M. Holland (1998). Mitochondrial DNA extraction and typing from isolated dentin-experimental evaluation in a Korean population. *Int J Legal Med.* **111**, 309-313.

Puzović, D., Dunjić, D., Popović, B., Stojković, O., Novaković, I., and J. Milasin (2006). Population data on HLA-DQA1, LDLR, GYPA, HBGG, D7S8, and GC PCR-based loci in Serbia. *J Forensic Sci.* **51**, 699.

Puzović, D., Popović, B., Novaković, I., and J. Milasin (2009). Analysis of microsatellite markers D18S70 and D20S116 in DNA isolated from dentin: use in forensic medicine. *Srp Arh Celok Lek*. **137**, 43-46.

Ricaut, F.X., Keyser-Tracqui, C., Crubézy, E., and B. Ludes (2005). STR-genotyping from human medieval tooth and bone samples. *Forensic Sci Int.* **151**, 31-35.

Rowold, D.J., and R.J. Herrera (2005). On human STR sub-population structure. *Forensic Sci Int.* **151**, 59-69.

Rowold, D.J., and R.J. Herrera (2003). Inferring recent human phylogenies using forensic STR technology. *Forensic Sci Int.* **133**, 260-265.

Trivedi, R., Chattopadhyay, P., and V.K. Kashyap (2002). A new improved method for extraction of DNA from teeth for the analysis of hypervariable loci. *Am J Forensic Med Patho.* **23**, 191-196.

Yamamoto, K. (1996). Molecular biological studies on teeth, and inquests. *Forensic Sci Int*. **80**, 79-87.

Weir, B.S. (1990). Multiple tests. In Genetic data analysis. Sinauer Associates, Inc., Sunderland, Massachusetts 109-110.