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Mitochondrial DNA extraction and typing from isolated dentin-experimental evaluation in a Korean population

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Abstract This study reports mtDNA polymorphisms in both hypervariable segments HV1 and HV2 of the non coding D-loop region from 60 unrelated Koreans. In contrast to two previous Korean data base studies, mtDNA was extracted separately from pulp tissue and root dentin of teeth obtained from dentists. Dentin turned out to be a reliable source of mitochondrial DNA. This can be of practical importance in forensic identification case work after a long post-mortem interval since pulp decomposes rapidly. The extraction method is explained in detail. The mtDNA polymorphisms obtained from 60 teeth of unrelated Koreans were compared with the already existing Korean data base.

Key words mtDNA · Korean data base · DNA extraction · Dentin

Introduction

Numerous population data of the hypervariable region 1 and 2 of the mitochondrial DNA control region have been published [1–6] since Anderson described the complete sequence of the 16 569 base pair human mitochondrial genome in 1981 [7]. Mitochondrial DNA has been shown to evolve rapidly and polymorphisms occur 5–10 times faster than in nuclear DNA [8–10]. Therefore the sequence polymorphisms of the control region are of interest for human genomic evolution and population studies. The fact that human cells contain hundreds to thousands of copies of the mtDNA genome [11] and the high individual polymorphism of the control region make mtDNA a powerful tool for forensic purposes [12]. Sequence data bases of the mtDNA control region for various populations are required not only for evolutionary genetic studies but also for validation of mtDNA control region polymorphism in forensic case work [13]. The intention of this study was to extract and to amplify mtDNA from tooth pulp and dentin separately. This method was chosen because pulp decomposes rapidly even though protected by hard tissues [14]. Therefore dentin can be expected to constitute the major source of mtDNA after a long post-mortem interval.

Furthermore, mtDNA was sequenced from both dentin and pulp in selected cases so that sequences could be confirmed by two types of tissue. Finally, the sequencing results were compared with the already existing Korean mtDNA database [1, 2].

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Materials and methods

Samples and sample preparation

A total of 60 teeth from unrelated Koreans were obtained from dentists after extraction and stored at –20°C until use. After mechanical cleaning of the surfaces the teeth were washed in 25 ml of sterile, distilled water followed by 10% commercial bleach and 95% ethanol. Using a diamond cutting disc the crowns were separated from the roots. The roots were then cut through the midline and the pulp tissue was removed using a spoon excavator and placed into a clean labelled microcentrifuge tube. Cementum and pulp chamber layers were removed using a drill. The isolated root dentin was washed in 10% commercial bleach, dried and crushed into a powder.

DNA extraction

For extraction 700 µl extraction buffer (10 m*M* Tris, pH 8.0; 100 m*M* NaCl; 50 m*M* EDTA, pH 8.0; 0.5% SDS) and 20 µl proteinase K

Table 1 Sequence polymorphisms in HV1 of 60 Koreans

Position	16037	16066	16075	16086	16093	16108	16111	16126	16129	16134	16136	16140	16148	16154	16162	16172	16174	16182	16183	16184	16187	16189	16192	16193	16194	16195	16209	16213	16214	16217	16223	16224	16227	16231	16232	16234	16235	16243	16245	16249	16256	16257	16261	16263	16265	16266	16270	16274	16278	16284	16290	16291	16292	16295	16297	16298	16304	16311	16316	16319	16320	16324	16325	16344	16357	16362
	A	A	T	T	T	C	C	T	G	C	T	T	C	T	A	T	C	A	A	C	C	T	C	C	A	T	T	G	C	T	C	T	A	T	C	C	A	T	C	T	C	C	C	C	T	A	C	C	G	C	A	C	C	C	C	T	T	T	T	A	G	C	T	T	C	T	T
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Table 1 (continued)

Position	16037	16066	16075	16086	16093	16108	16111	16126	16129	16134	16136	16140	16148	16154	16162	16172	16174	16182	16183	16184	16187	16189	16192	16193	16194	16195	16209	16213	16214	16217	16223	16224	16227	16231	16232	16234	16235	16243	16245	16249	16256	16257	16261	16263	16265	16266	16270	16274	16278	16284	16290	16291	16292	16295	16297	16298	16304	16311	16316	16319	16320	16324	16325	16344	16357	16362
	A	A	T	T	T	C	C	T	G	C	T	C	T	A	T	T	C	C	A	A	C	C	T	C	C	A	T	T	G	C	T	C	T	A	T	C	C	A	T	C	T	C	C	C	T	A	C	C	G	C	A	C	C	C	C	C	T	T	T	T	A	G	C	T	T	C	T	T	T
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(20 mg/ml) were added to each dentin and pulp sample and to the reagent blank, vortexed and incubated at 56° C overnight. To the extract volume 720 µl phenol/chloroform/isoamyl alcohol (25 : 24 : 1) was added, vortexed and centrifuged for 2 min at 10 000–15 000 rpm in a microcentrifuge. The upper aqueous layer was transferred to a sterile labelled microcentrifuge tube and extraction was repeated twice, then 720 µl 1-butanol was added, vortexed and centrifuged for 2 min at 10 000–15 000 rpm. The lower aqueous layer was transferred to the sample reservoir of a Centricon-100 concentrator (Amicon), 1 ml TE buffer was added and the columns were centrifuged at 1000 × g for 20 min or until the sample had spun through. The washing step with TE buffer was repeated twice. The retentate was transferred to a sterile, labelled microcentrifuge tube and stored at –20° C.

mtDNA amplification and sequencing

The amplification for both hypervariable regions was performed in a Perkin Elmer 9600 Thermal Cycler. The following primer pairs were used:

HV1: F15 971 (5′ TTA ACT CCA CCA TTA GCA CC)
R16 410 (5′ GAG GAT GGT GGT CAA GGG AC)

HV2: F15 (5′ CAC CCT ATT AAC CAC TCA CG)
R448 (5′ TGA GAT TAG TAG TAT GGG AG).

The PCR master mix for a 25 µl reaction consisted of 2.5 µl 10 × Perkin-Elmer PCR buffer (1 × 10 m*M* Tris-HCL, pH 8.3, 50 ml KCl, and 1.5 m*M* $MgCl_2$), 2 µl 2.5 m*M* dNTP mix, 1 µl 10 µ*M* forward amplification primer, 1 µl 10 µ*M* reverse amplification primer, 0.25 µl of AmpliTaq DNA polymerase and 2.5 µl of the DNA extract. The PCR conditions for both HV1 and HV2 were 94° C for 30 s followed by 32 cycles at 94° C for 20 s, 56° C for 10 s, 72° C for 30 s followed by 5° C soak. Of the PCR product 5 µl was added to 1 µl 6 × agarose gel loading buffer, loaded on a 2% agarose gel, electrophoresed at constant voltage (90 V) for approximately 30 min and visualized on a trans-illuminator. A sizing ladder was included on each gel (1 µl of 123 base pair ladder, 4 µl dH_2O and 1 µl of 6 × loading buffer). The gels were photographed with a Polaroid camera fitted with an orange filter. Prior to sequencing the PCR product was purified using Centricon 100 spin dialysis columns (Amicon).

Cycle sequencing was performed using 8.0 µl ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq FS DNA polyermase, 1 µl sequencing primer, 1–11.0 µl purified PCR product and sterile distilled water q.s. to 20 µl for each sample. The sequencing primers were as follows:

HV1: F15 989 5′ CCC AAA GCT AAG ATT CTA AT
R16 380 5′ GAG GAT GGT GGT CAA GGG AC

HV2: F29 5′ CTC ACG GGA GCT CTC CAT GC
R381 5′ GCT GGT GTT AGG GTT CTT TG.

A Perkin Elmer 9600 thermal cycler was used under the following conditions: 25 cycles at 96° C for 15 s, 50° C for 5 s, 60° C for 2 min. After sequencing the samples were passed through Centriflex gel filtration cartridges (Advanced Genetic Technologies Corp.) and dried in a vacuum centrifuge. Automated DNA sequencing was performed on an AB 373 DNA sequencer and 4 µl loading buffer (50 m*M* EDTA/deionized formamide 1 : 5) was added to the dry samples. The samples were vortexed, denatured at 96° C for 2 min and loaded on a polyacrylamide gel. Electrophoresis was run at 18–21 mA, 980–1600 V and a temperature approaching 40° C for 12 h. Analysis of mitochondrial DNA sequencing data was performed on a Macintosh computer using the Sequence Navigator DNA and Protein Sequence Comparison software (Version 2.0, ABI), HV1 was analysed between the positions 16 024 and 16 365, HV2 between the positions 73 and 340 according to the Anderson sequence.

Table 2 Sequence polymorphisms in HV2 of 60 Koreans

Position	73	88	93	94	131	143	146	150	151	152	153	182	183	194	195	199	200	202	204	205	207	210	214	228	235	237	246	249	257	263	282	309.1	309.2	315.1	320	334
	A	T	A	G	T	G	T	C	C	T	A	C	A	C	T	T	A	A	T	G	G	A	A	G	A	A	T	A	A	A	T	I	I	I	T	T
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Table 3 Distribution of sequence polymorphisms in a Korean population (n = 60)

	HV1	HV2
Transitions	251	216
Transversions	23	1
Insertions	13*	103
Deletions	0	4
Total	282	324

*Cases with length heteroplasmy in the homopolymeric C-tract

Results and discussion

Mitochondrial DNA was extracted and amplified from all 60 dentin and pulp samples separately. Amplification products of both dentin and pulp from the same individual were sequenced in 17 cases for HV1 and 30 cases for HV2 and confirmed each other. The processes of the odontoblasts pass the dentin tubes and contain numerous mitochondria [15]. Therefore tooth dentin can be used as a source of mitochondrial DNA in the absence of pulp in forensic cases when decomposed bodies, skeletons or even isolated teeth need to be identified. From 60 unrelated Koreans 57 mitchondrial lineages were created by 102 variable positions, 66 in HV1 and 36 in HV2 (Tables 1 and 2). Three pairs of individuals shared the same haplotype in both HV1 and HV2 (Tables 1 and 2; samples 9/25, 38/39, 51/54). Furthermore, three pairs of individuals (21/40, 11/45, 8/26) and three more individuals (44/47/55) shared the same haplotype in HV1 and 11 pairs of individuals in HV2. This finding of a higher degree of polymorphism in HV1 is in accordance with previous studies [1, 2]. Compared with the Anderson sequence, the most frequent polymorphisms were found at the positions 16223 (C to T transition), and 16362 (T to C transition) in HV1 (Table 1), and 73, 263 (A to G transition), and 315.1 (C insertion) in HV2 (Table 2). Transitions were more prevalent than transversions (Table 3) and C insertions were found with a high frequency in HV2 (Table 3). T to C transitions at position 16189 in HV1 with a characteristic blurred sequence in nucleotides beyond the tract was found in 13 cases (21.7%). The phenomenon of length heteroplasmy has been described to occur frequently, for example in 15% of Europeans [16]. The present sequencing technique is inadequate for determining the real number of C-insertions in this homopolymeric C-tract. Therefore the C-insertions are not mentioned in Table 1, but the number of cases with length heteroplasmy in the C-tract of HV1 is shown in Table 3. Our results concerning the sequence structure in HV1 and HV2 of the mitochondrial genome correspond to previous studies [1, 2] and can be combined in a large Korean data base for further population studies.

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