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변사체 신원 확인시 mtDNA HV1과 HV2 영역의 모계내 돌연변이 사례

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Intergenerational substitution in mitochondrial DNA HV1 and HV2 regions observed during the identification of human remains in forensic caseworks

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Abstract - We investigated mitochondrial DNA (mtDNA) sequences for the identification of unknown human remains from February 2002 to September 2005 at our institute and found seven cases of intergenerational differences. The mtDNA sequences of the 342-bp HV1 region and 268-bp HV2 region were determined. Intergenerational mutations in the form of homoplasmy in the mtDNA sequences of human remains and corresponding putative maternal relatives were observed in five cases at positions 16093, 16111, 16126, and 16189 in HV1 and 309 in HV2. Heteroplasmic substitutions were observed in two cases at the same position, i.e., 16093 in HV1.

Keywords : Human remains identification, Mitochondrial DNA typing, Mutation, Heteroplasmy, Casework

I. Introduction

The investigation of mitochondrial DNA (mtDNA) has gained increasing importance for the identification of unknown human skeletal remains[1]. Excluding the presence of any mutation, the mtDNA sequences of all maternally linked relatives are essentially identical. However, mutations can occasionally hinder the identification of human remains[2].

A high copy number in each cell and high mutation rate in the mtDNA control region lead to heteroplasmic states. The heteroplasmic condition is the co-existence of two or more subpopulations of mtDNA. There are two types of heteroplasmies, namely, sequence-based heteroplasmy and length-based heteroplasmy. Sequence heteroplasmy is represented by the presence of two different populations of mtDNA that differ at a given nucleotide position. Length heteroplasmy occurs in HV1 and HV2, and is represented by multiple populations of mtDNA containing polycytosine stretches (C-stretches) of various lengths[3].

Meiotic bottleneck in mtDNA transmission was proposed as the mechanism that restricts the level of mtDNA variation transferred through generations[4]. This creates the potential for different heteroplasmic ratios among different individuals of the same maternal relatives. In extreme cases, the difference could be sufficiently large to appear as a substitution in the homoplasmic state[2]. Frequent transition to heteroplasmy was observed[5-8], along with shifts between generations

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from one apparent homoplasmic state to another, sometimes without a heteroplasmic intermediate[9].

In this paper, we report the intergenerational substitution in mtDNA HV1 and HV2 regions observed during the identification of human remains in forensic caseworks.

II. Material and methods

2.1. DNA extraction

Genomic DNA of unknown human remains was prepared from decalcified bones, teeth, and liver samples by using the QIAamp Mini kit (Qiagen, Germany) or the traditional phenol/chloroform method. Genomic DNA of putative maternal relatives was extracted from blood and buccal cells.

2.2. PCR amplification

MtDNA HV1 was amplified using L15971 (5' - TTAACTCCACCATTAGCACC-3') and H16391 (5' - GAGGATGGTGGTCAAGGGAC-3') as forward and reverse primers, respectively. L15 (5' - CACCCTATTAACCACTCAG-3') and H408 (5' - CTGTTAAAAGTGCATACCGCCA-3') were used as forward and reverse primers, respectively, for the amplification of mtDNA HV2. The PCR reaction mixture (25 μl) contained 0.1-1.0 ng/μl template DNA, 1x AmpliTaq Gold buffer, 2.5 mM $MgCl_2$, 200 μM of each dNTP, 4g bovine serum albumin (BSA), 20 μM of each primer, and 2.5U AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). The PCR conditions were as follow: 95° C for 11 min, 32 cycles of 95° C for 20 s, 56° C for 30 s, 72° C for 45 s, and 72° C for 7 min. Excess nucleotides and primers were eliminated from the PCR products by using the QIAquick PCR product purification kit (Qiagen).

2.3. DNA sequencing

DNA sequencing was carried out using the BigDye version 3.0kit (Applied Biosystems). The sequencing

Table 1. Nucleotide differences observed among the mtDNA sequences from the missing person, putative mother, and additional putative older brother. A single nucleotide difference at position 16093 was observed between the mtDNA sequences from bone sample and putative maternal relatives.

Position (Anderson)	Anderson sequence[10]	Bone sample (Case #1)	Putative mother	Additional putative older brother
(HV1) 16093	T	T	C	C
(HV1) 16111	C	T	T	T
(HV1) 16129	G	A	A	A
(HV1) 16223	C	T	T	T
(HV1) 16257	C	A	A	A
(HV1) 16261	C	T	T	T
(HV1) 16311	T	C	C	C
(HV2) 73	A	G	G	G
(HV2) 150	C	T	T	T
(HV2) 263	A	G	G	G
(HV2) 309	C	1C	1C	1C
(HV2) 315	C	1C	1C	1C

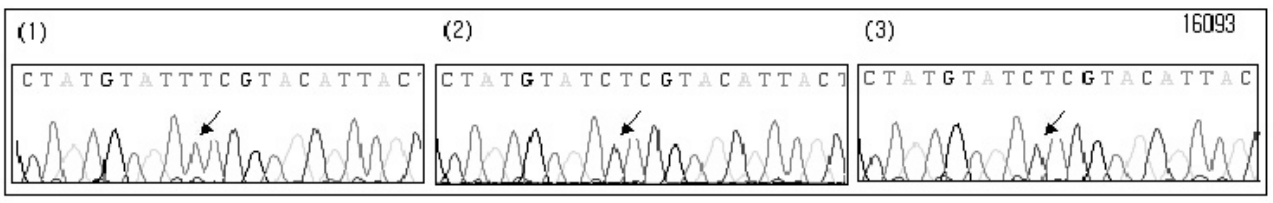

Fig 1. Electropherograms of the mtDNA sequences of the missing person (1), putative mother (2), and additional putative older brother (3) showing homoplasmic substitution at position 16093 in the HV1 region.

product was purified using the DyeEx spin kit (Qiagen). The purified product was electrophoresed using ABI 310 Genetic Analyzer (Applied Biosystems). The nucleotide positions 16024-16365 in HV1 and 73-340 in HV2 were analyzed and compared to the reference sequence[10] and the sequences of corresponding maternal relatives. In cases where the sequence of the missing person and the reference sequence differed by a single nucleotide, additional samples of maternal relatives were run in an attempt to obtain a clear interpretation.

III. Results and discussion

case #1

A decomposed body was found flowing in the sea. Police investigations led to a woman who was missing for two months from a neighboring village. MtDNA data from the missing person and a putative mother were identical, except at position 16093 (Table 1). The sequences were compared to the mtDNA sequences of 513 Korean individuals in the D-loop database[11]. However, no identical sequences were found. At position 16093, nucleotide T was present in the mtDNA sequence of the missing person, while nucleotide C was present in the mtDNA sequence of the putative mother. We then analyzed the mtDNA sequence of an additional putative older brother. However, at position 16093, nucleotide C was present in the mtDNA sequence of the putative older brother; this was identical to the mtDNA sequence of the putative mother (Fig. 1).

Intergenerational substitution in the form of homoplasmy at position 16093 was reported in one case in mtDNA sequencing of close maternal relative pairs[9] and in one pedigree study[12]. Heteroplasmic substitution at position 16093 was found in case #6 and #7.

case #2

The human skeletal remain of a male was found near

Table 2. Nucleotide differences observed among the mtDNA sequences from the missing person, putative older brother, and additional putative older sister. A single nucleotide difference at position 16111 was observed between the mtDNA sequences from the bone sample, additional putative older sister, and putative older brother.

Position (Anderson)	Anderson sequence[10]	Bone sample (Case #2)	Putative first older brother	Additional putative second older sister
(HV1) 16111	C	C	T	C
(HV1) 16207	A	G	G	G
(HV1) 16304	T	C	C	C
(HV1) 16362	T	C	C	C
(HV2) 73	A	G	G	G
(HV2) 146	T	C	C	C
(HV2) 152	T	C	C	C
(HV2) 249	A	d	d	d
(HV2) 263	A	G	G	G
(HV2) 315	C	1C	1C	1C

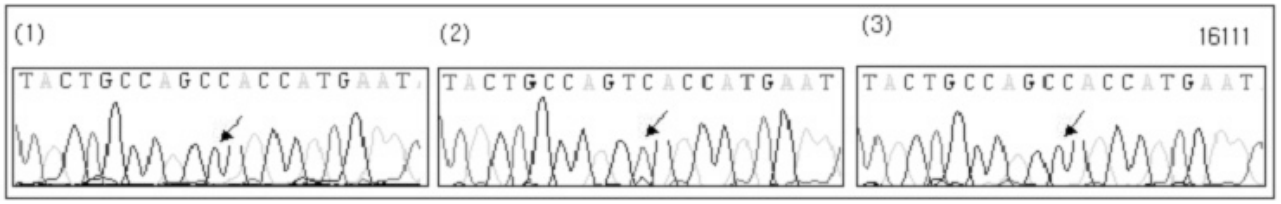

Fig. 2. Electropherograms of the mtDNA sequences of the missing person (1), putative older brother (2), and an additional putative older sister (3) showing homoplasmic substitution at position 16111 in the HV1 region. However, in this case, the distinction of homoplasmic and heteroplasmic mutation at position 16111 is somewhat unclear, because we cannot distinguish low level of heteroplasmy from the noisy background.

the reed riverside of Nack-dong River. A resident identification card was found in his clothes. DNA was prepared from a bone sample from the remains and a blood sample from a putative older brother and sequenced. Both the sequences were identical, except at position 16111 (Table 2). Furthermore, the comparison with the mtDNA sequences of Korean individuals in the D-loop database yielded no identical sequence. We then analyzed the mtDNA sequence of an additional putative older sister. At position 16111, nucleotide C was present in the mtDNA sequences of both the victim and the additional older sister, while nucleotide T was present in the mtDNA sequence of the putative older brother (Fig. 2).

Homoplasmic or heteroplasmic substitution at position 16111 was not reported in the study of the close maternal relatives comparison[6, 8, 9, 12, 13].

case #3

A suicide-prone man was found hanging in a tree in the middle of a cliff. Several materials, including a sack and five bottles of liquor, which were thought to be the personal belongings of this man, were found at the top of the cliff. DNA was prepared from the femur and teeth samples. DNA was also prepared from the blood sample of a putative younger brother of the missing person. The HV1 and HV2 regions of both samples were identical, except at position 16126 (Table 3). The sequence of the missing person with haplotype G3 (i.e., 16223, 16274, 16362, 73, 143, 152, 204, 263, 315.1C) was observed twice in the mtDNA sequences of Korena individuals in the D-loop database. However, the sequence of the putative younger brother was not found in the database. At position 16126, nucleotide T was present in the mtDNA sequence of the missing person, while nucleotide C was present in the mtDNA sequence of the putative younger brother. Therefore, we analyzed the mtDNA sequence of an additional putative mother. At position 16126, nucleotide T was present in the mtDNA sequence of the additional putative mother; this was identical the mtDNA sequence of the missing person

Table 3. Nucleotide differences observed among the mtDNA sequences from the missing person, putative younger brother, and additional putative mother. A single nucleotide difference at position 16126 was observed between the mtDNA sequences from the bone sample, additional putative mother, and putative younger brother.

Position (Anderson)	Anderson sequence[10]	Bone sample (Case #3)	Putative mother	Additional putative older brother
(HV1) 16126	T	T	C	T
(HV1) 16223	C	T	T	T
(HV1) 16274	G	A	A	A
(HV1) 16362	T	C	C	C
(HV2) 73	A	G	G	G
(HV2) 143	G	A	A	A
(HV2) 152	T	C	C	C
(HV2) 204	T	C	C	C
(HV2) 263	A	G	G	G
(HV2) 315	C	1C	1C	1C

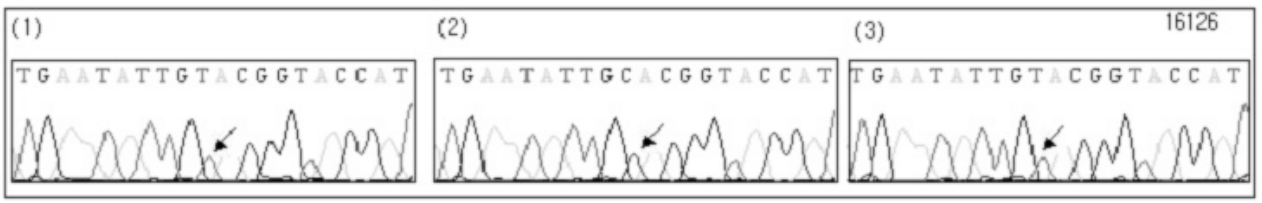

Fig. 3. Electropherograms of the mtDNA sequences of the missing person (1), putative younger brother (2), and additional putative mother (3) showing homoplasmic substitution at position 16126 in the HV1 region.

(Fig. 3).

Homoplasmic or heteroplasmic substitution at position 16126 was not reported in other intergenerational comparison studies previously mentioned in case #2.

case #4

The human skeletal remain was found in a forest. Information from his personal belonging was pointing to the murderer who had killed someone and had disappeared 4 months ago. DNA was prepared from the femur sample and analyzed as described above. DNA was prepared from the blood samples obtained from the two putative siblings of the missing man and subjected to sequencing. The mtDNA sequences from the missing person as well as from the two putative siblings showed identical differences to the Anderson reference sequence, except at position 16189 (Table 4). The comparision with the mtDNA sequences of Korean individuals in the D-loop database yielded no identical sequence. At position 16189, nucleotide T was present in the HV1 sequence of the putative younger brother, while nucleotide C was present in the HV1 sequence of the putative older sister and missing person (Fig. 4).

Homoplasmic substitution at position 16189 was not reported in other intergenerational comparison studies previously mentioned in case #2. However, Huhne has previously reported two heteroplasmies at position 16189 in a comparison of mother and child pairs[8].

Table 4. Nucleotide differences observed between the mtDNA sequences from the bone sample of the missing person and two putative siblings. A single nucleotide difference at position 16189 was observed between the mtDNA sequence from the bone sample, putative older sister and putative younger brother.

Position (Anderson)	Anderson sequence[10]	Bone sample (Case #4)	Putative younger brother	Putative older sister
(HV1) 16129	G	A	A	A
(HV1) 16182	A	C	C	C
(HV1) 16183	A	C	C	C
(HV1) 16189	T	C	T	C
(HV1) 16194	A	C	C	C
(HV1) 16195	T	C	C	C
(HV1) 16232	C	T	T	T
(HV1) 16249	T	C	C	C
(HV1) 16304	T	C	C	C
(HV1) 163111	T	C	C	C
(HV1) 16344	C	T	T	T
(HV2) 73	A	G	G	G
(HV2) 152	T	C	C	C
(HV2) 207	G	A	A	A
(HV2) 249	A	d	d	d
(HV2) 263	A	G	G	G
(HV2) 315	C	1C	1C	1C

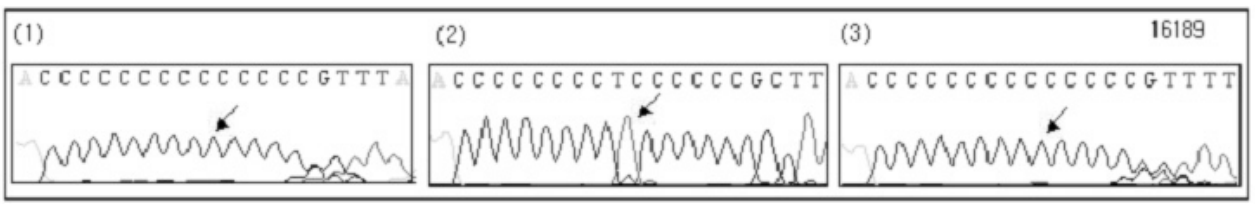

Fig. 4. Electropherograms of the mtDNA sequences of the missing person (1), putative younger brother (2), and putative older sister (3) showing homoplasmic substitution at position 16189 in the HV1 region.

case #5

The remains of burnt skull and femur fragments was found at a waste-burning site. A characteristic of this missing person was the number of artificial joint in the femur. The remains of this person indicated that he was murdered and his body was burnt in order to destroy the evidences of murder. The extracted DNA was subjected to mtDNA sequencing as well as STR typing. The DNAs were prepared from a bone sample from the missing person and a blood sample from a putative mother and sequenced. Both sequences were identical, except at position 309 (Table 5). The comparison with the mtDNA sequences of Korean individuals in the D-Loop database yielded no identical sequence. The mtDNA sequence of the remains and the maternal reference sequence showed a difference in the homopolymeric tract of the HV2 region with regard to the number of Cs inserted. At position 309, the HV2 sequence of the missing person showed no insertion, while that of the putative mother showed a clear 309.1C (Fig. 5). In this case, STR typing was performed successfully, and STR result confirmed their familial relationship (data not shown).

Length homoplasmy at position 309 was reported in one case in Parson' s study[9], and in seven and five cases in two pedigree studies[12 and 13, respectively].

case #6

The burnt body of a male was found in his house. This man had consumed liquor and had gone to sleep without extinguishing a burning candle. DNA was prepared from the victim' s liver sample. DNA was prepared from the blood sample of a putative younger sister was sequenced. The HV1 and HV2 regions of both the sequences were identical, except at position 16093. The

Table 5. Nucleotide differences observed between the mtDNA sequences from the bone sample of the missing person and putative mother. Length variation at position 309 in the HV2 homopolymeric C-stretch region was observed between the mtDNA sequences from the bone sample and putative mother.

Position (Anderson)	Anderson sequence[10]	Bone sample (Case #5)	Putative mother
(HV1) 16126	T	C	C
(HV1) 16223	C	T	T
(HV1) 16290	C	T	T
(HV1) 16319	G	A	A
(HV1) 1632	T	C	C
(HV2) 73	A	G	G
(HV2) 152	T	C	C
(HV2) 200	A	G	G
(HV2) 235	A	G	G
(HV2) 263	A	G	G
(HV2) 309	C	C	1C
(HV2) 315	C	1C	1C

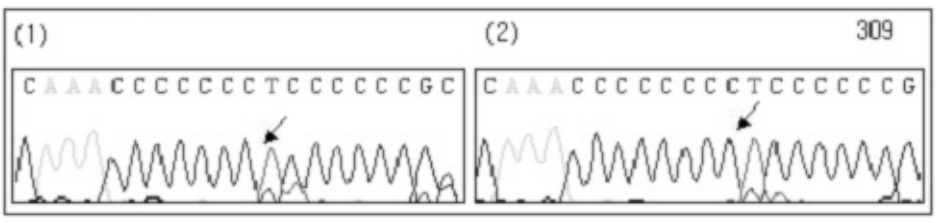

Fig. 5. Electropherograms of the mtDNA sequence of the missing person (1) and putative mother (2) showing homoplasmic length substitution at position 309 in the HV2 region.

comparison with the mtDNA sequences of Korean individuals in the D-Loop database yielded no identical sequence. The nucleotide at position 16093 showed a T/C heteroplasmy in the mtDNA sequence of the victim, while nucleotide C was present at the same position in the mtDNA sequence of a putative younger sister. The sequence of an additional putative maternal aunt was then analyzed. However, at position 16093, nucleotide C was present in the mtDNA sequence of the putative maternal aunt; this was identical to the mtDNA sequence of the putative younger sister.

Intergenerational substitution in the form of T/C heteroplasmy at position 16093 was not reported in other intergenerational comparison studies previously mentioned in case #2. However, heteroplasmy at position 16093 was known as a hot spot in the population study on heteroplasmy that was conducted using the sensitive method of denaturing gradient gel electrophoresis (DGGE)[14].

case #7

A burnt and decomposed body of a male was found in a forest along with a bottle of liquor and a bucket of thinner. This person was identified from his passport and a bank deposit passbook that remained after burning. DNA was prepared from the tissue of the missing person and blood sample from a putative sister and sequenced. Both the sequences were identical, except at position 16093 (Table 7). The comparison with the mtDNA sequences of Korean individuals in the D-Loop database yielded no identical sequence. The nucleotide at position 16093 showed a clear heteroplasmy C/T in the HV1 sequence of the missing person, while nucleotide C was present at the same position in the HV1 sequence of the putative sister (Fig. 7). The heteroplasmic substitution at position 16093 was the same as that observed in case #6.

The cases reported in this study illustrate the evidence of intergenerational mutations in mtDNA sequences, in which a single nucleotide difference or heteroplasmy is involved. Intergenerational mutations in the form of

Table 6. Observed nucleotide differences observed among the mtDNA sequences from tissue sample of the missing person, putative younger sister, and additional putative aunt. A sequence heteroplasmy at position 16093 was observed in the mtDNA sequences from the tissue sample, putative younger sister, and additional putative aunt.

Position (Anderson)	Anderson sequence[10]	Tissue sample (Case #6)	Putative younger sister	Additional putative aunt
(HV1) 16093	C	T/C	C	C
(HV1) 16129	G	A	A	A
(HV1) 16223	C	T	T	T
(HV1) 16249	T	C	C	C
(HV1) 16362	T	C	C	C
(HV1) 73	A	G	G	G
(HV1) 152	T	C	C	C
(HV1) 263	A	G	G	G
(HV2) 309	C	1C	1C	1C
(HV2) 315	C	1C	1C	1C

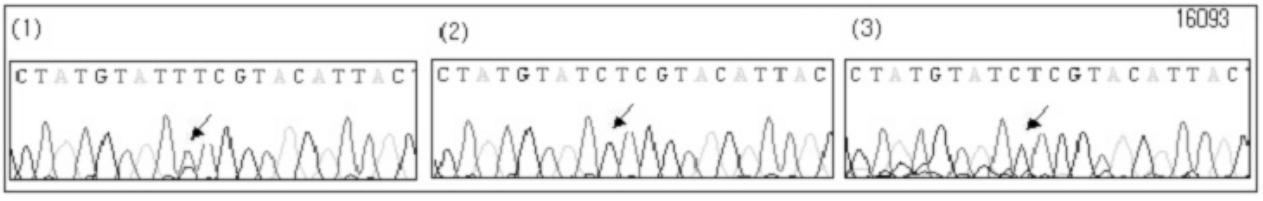

Fig. 6. Electropherograms of the mtDNA sequences of the missing person (1), putative younger sister (2), and additional putative aunt (3) showing heteroplasmic substitution at position 16093 in HV1 region.

Table 7. Nucleotide differences observed between the mtDNA sequences from the tissue sample of the missing person and putative older sister. Sequence heteroplasmy at position 16093 was observed in the mtDNA sequences of the tissue sample and putative older sister.

Position (Anderson)	Anderson sequence[10]	Tissue sample (Case #7)	Putative older sister
(HV1) 16093	T	C/T	C
(HV1) 16129	G	A	A
(HV1) 16213	G	A	A
(HV1) 16223	C	T	T
(HV1) 16298	T	C	C
(HV2) 73	A	G	G
(HV2) 195	T	C	C
(HV2) 249	A	d	d
(HV2) 309	C	1C	1C
(HV2) 315	C	1C	1C

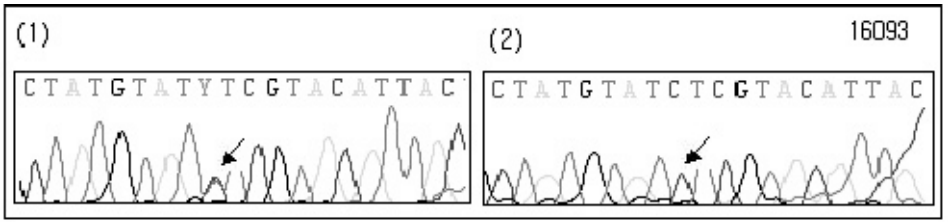

Fig. 7. Electropherograms of the mtDNA sequences of the missing person (1) and putative older sister (2) showing heteroplasmic substitution at position 16093 in the HV1 region.

homoplasmy were observed in five cases at positions 16093, 16111, 16126, and 16189 in HV1 and 309 in HV2. Heteroplasmic substitutions were observed in two cases at the same position, i.e., 16093 in HV1. These mutations complicate the identification of human remains based on mtDNA evidence. The co-occurrence of heteroplasmy in the mtDNA sequences of human remains and corresponding putative maternal relatives was not found.

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