Combined use of STRs and mtDNA genetic profiles for the identification of Korean War victims

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Introduction

An effort to establish a database of mitochondrial DNA control region sequences (HV1 and HV2) using skeletal remains of war victims killed in the Korean War (1950-1953) and bloods from suspected war-bereaved families has been made since the beginning of the national project, "The Excavation on Casualties from Korean War" in 2000. According to the circumstantial evidences and the matching results of mtDNA genetic profiles, some of missing casualties were identified and returned to their families.

However, relatively high mitochondrial HV1/HV2 haplotype frequencies in particular haplotypes suggest the need for additional genetic analyses to better identify individuals with scientific exactitude. In this regard, additional autosomal STR typing analyses were carried out using PowerPlex® 16 and miniSTRs (by Butler JM). As old skeletal remains contain degraded genetic materials, miniSTR system which was developed to produce PCR fragments with reduced STR amplicon length was employed efficiently.

Here, we report the use of additional STR typings on confirmation of genetic relationship between missing casualties and their bereaved families which was revealed by comparison of mtDNA genetic profiles. The strategy of combined use of STRs information with sequence polymorphisms in mitochondrial HV1 and HV2 region will be greatly helpful to the effort of identifying missing casualties of Korean War.

Materials and Methods

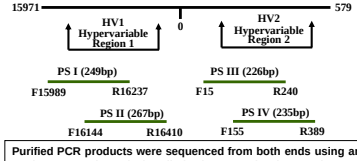
❖ Sample preparation

Femur and/or tooth samples of the recovered skeletal remains were provided by the excavation team of Korean Army Headquarters. Femoral bones were cut into slices of approximately size of 1 x 3 cm using dental bur. The outer surface of the bone and tooth specimen were cleaned and irradiated with UV light. Bone and tooth pieces were frozen in liquid nitrogen and ground in a SPEX Mill (SPEX CertiPrep, Metuchen, NJ). Blood samples of the bereaved families to be used as references were collected.

❖ DNA extraction

DNA from the femur and tooth samples were extracted using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) with slight modifications by Yang *et al.* after incubation with extraction buffer (0.5 M EDTA pH 8.0, 0.5% SDS and 1 mg/ml proteinase K) at 56 °C for 1-2 days. Reference DNA was extracted from blood samples using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations.

❖ Amplification and sequencing of mtDNA HV1 and HV2

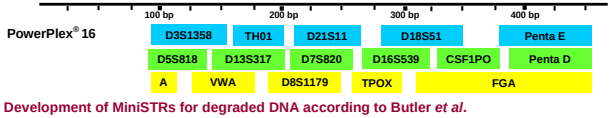


PCR reaction: Total 25 μl reaction contained 2 μl of DNA from the bone/tooth powder, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 ng/μl BSA, 0.4 μM primers, 200 μM dNTP, and 2.5 U of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA).

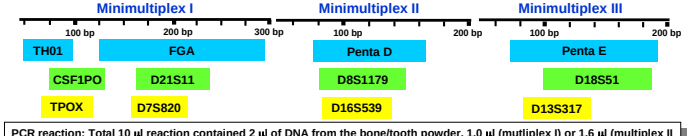
Cycling condition: 95°C 10'; (95°C 20'', 50°C 20'', 72°C 30'') x 45 ~ 50; 72°C 7'

Purified PCR products were sequenced from both ends using an ABI 310 automated sequencer and a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). The results were analyzed using Sequencing Navigator 1.01 (Applied Biosystems).

❖ Amplification and genotyping of STRs in nuclear DNA



Development of MiniSTRs for degraded DNA according to Butler *et al.*



PCR reaction: Total 10 μl reaction contained 2 μl of DNA from the bone/tooth powder, 1.0 μl (mutliplex I) or 1.6 μl (multiplex II and III) of Gold STR buffer (Promega, Madison, WI), 2.0 U of AmpliTaq Gold polymerase (Applied Biosystems), and primers.

Cycling condition: 95°C 11'; (94°C 1', 55°C 1', 72°C 1') x 35 ~ 36; 60°C 45'

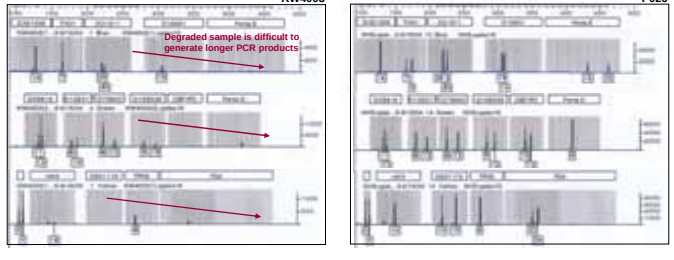
Results

❖ Mitochondrial DNA profiles

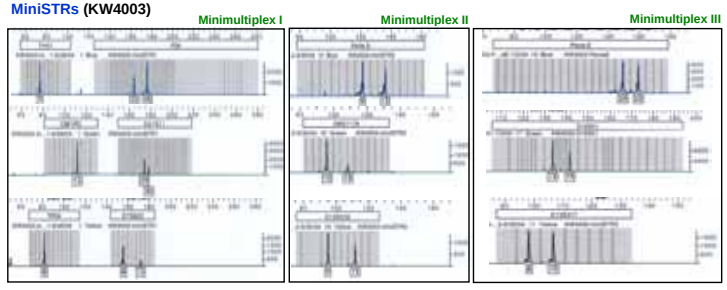
Casualties	Maternal relatives	Sequences of hypervariable regions of matched samples
KW4003	**M4327**	**16189C 16223T 16278T 16362C 73G 260A 263G 309.1C 309.2C 315.1C 16292T**
KW4004	M4267	16223T 16362C 73G 152C 263G 309.1C 309.2C 315.1C
KW4008	M4076 M4231 M4235	16129A 16182C 16183C 16189C 16232A 16249C 16304C 16311C 16344T 73G 152C 249d 263G 315.1C
KW4010	M4032 M4130 M4136	16223T 16319A 16362C 73G 152C 263G 315.1C
KW4017	M4196	16129A 16223T 16362C 73G 152C 263G 309.1C 315.1C
KW4019	M4013	16182C 16183C 16189C 16217C 16295T 73G 150T 195C 263G 309.1C 309.2C 309.pC 315.1C
KW4025	M4069	16187T 16223T 16290T 16319A 73G 195C 235G 263G 309.1C 309.2C 315.1C

❖ Autosomal STR profiles

Powerplex® 16



MiniSTRs (KW4003)

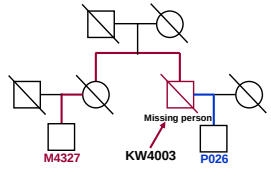


The DNA typing data using the PowerPlex® 16 and the miniSTR systems

	D3S1358	TH01	D21S11	D18S51	PentaE	D5S818	D13S317	D7S820	D16S539	CSF1PO	PentaD	vWA	D8S1179	TPOX	FGA
KW4003	16-16	7-7	29-30	13-15	20-22	11-12	8-10	8-12	9-13	12-12	9-12	16-18	10-13	8-8	22-25
P026	16-16	7-9	28.2-30	13-14	16-22	11-12	8-12	8-12	9-12	10-12	9-9	16-16	10-15	8-8	22-24

Paternity probability: 0.99989612

❖ Pedigree of a confirmed case of war victim identification



The STR typings confirmed the genetic relationship between KW4003 and war-bereaved family member, M4327 and P026, which was presumed by comparison of mtDNA genetic profiles.

Discussion

A identification case of the Korean War victim by mtDNA and nDNA analyses is reported. Due to the relatively low mtDNA power of discrimination and high mtDNA haplotype frequencies in particular haplotypes, true victim-relative pairs are often out-numbered by unrelated pairs who coincidentally share mtDNA haplotypes. Therefore, the STR analyses on nDNA needs to be performed for better identification of victims. Using miniSTR systems, we could generate STR genotyping data from the old skeletal remains of victims, which are directly comparable to the asserted reference samples.

Here, the STR data from KW4003 and P026 confirmed the asserted relationship presumed from mtDNA matching result. As shown in this case, the combined use of STRs and mtDNA genetic profiles will facilitate the identification of Korean War victims.

References

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3. **Yang D. Y., Eng B., Waye J. S., Dudar J. C., Saunders S. R. improved DNA extraction from ancient bones using silica-based spin columns, Am J Phys Anthropol, 105; 539-43, 1998.**
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