mtDNA haplogroup determination as an additional tool for authenticating ancient East Asian mtDNA

Hwan Young Lee, Ji-Eun Yoo,
Myung Jin Park, Ukhee Chung,
Chong-Youl Kim, Kyoung-Jin Shin

Department of Forensic Medicine, Yonsei University College of Medicine, Seoul, Korea
Human Identification Research Center, Yonsei University, Seoul, Korea

Merits and limitations of ancient DNA analysis in human genetics

- The analysis of ancient DNA is an ideal way to get a direct grip on the past
- The vanishingly small traces of DNA fragments left behind in old specimens and the ubiquitous nature of contamination DNA make it very difficult to obtain reliable DNA sequence data from most ancient samples

The Nine Criteria by Cooper and Poinar
: A priori exclusion of the possibility of contamination
A need for the addition of a tenth commandment!

A posteriori test for the authenticity of ancient mtDNA data

- Some indicators can positively exclude or question authenticity of ancient mitochondrial DNA sequencing results
  - ✓ Phylogeographic paradox

    (or the principle of phylogenetic expectation)
  - ✓ Mosaic structure
  - ✓ Abnormal mutation spectrum

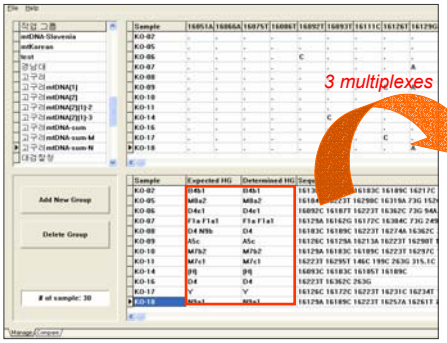
HJ Bandelt ***Eur. J. Hum. Genet.*** *(2005) 13: 1106-1112*

Haplogroup determination as a tool for *a posteriori* authenticity test

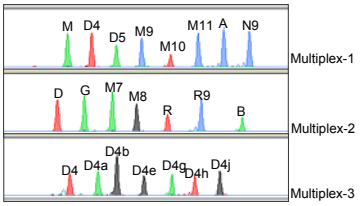
- East Asian haplogroup determination is efficiently carried out through haplogroup-level coding region SNP analysis and subhaplogroup-level control region sequence analysis
- Especially, high incidence of haplogroup-specific mutations in the control region sequence of East Asian mtDNA enables to check the presence of phylogeographic paradox and mosaic structure with ease

East Asian mtDNA haplogroup determination in Koreans

- According to the control region mutation motifs, each obtained haplotype is assigned to appropriate subhaplogroup



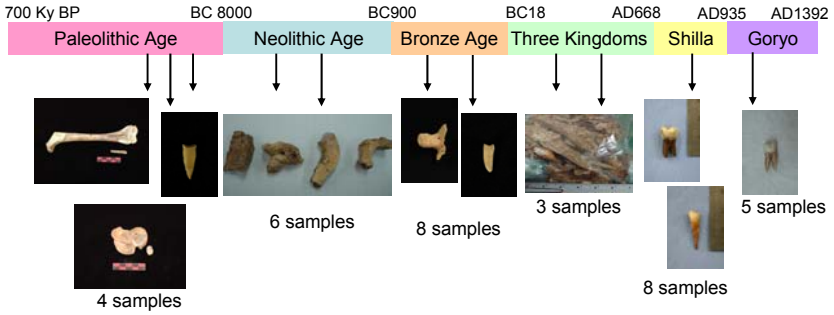
- Depending on the results, diagnostic coding region SNPs are confirmed



(*Lee et al.* ***Electrophoresis*** *in press)*

mtDNA analysis from ancient Korean human remains

- 35 museum samples ranged from the Paleolithic age to Goryo dynasty

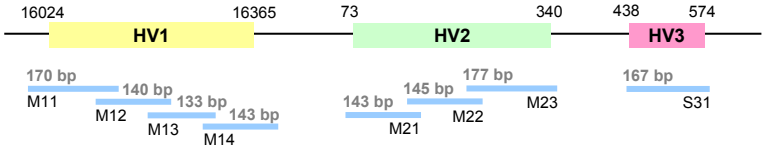


Contamination precautions taken to ensure the reliability of results

- Physical methods that remove the bone surface and UV irradiation that makes DNA unsuitable for PCR
- Isolated laboratory where no post-PCR work has been conducted
- Testing of control extracts in parallel with extracts from old specimens
- Multiple extractions from the same samples at different times
- Quantitation of amplifiable DNA using PCR

Control region sequence analysis using small PCR amplicons

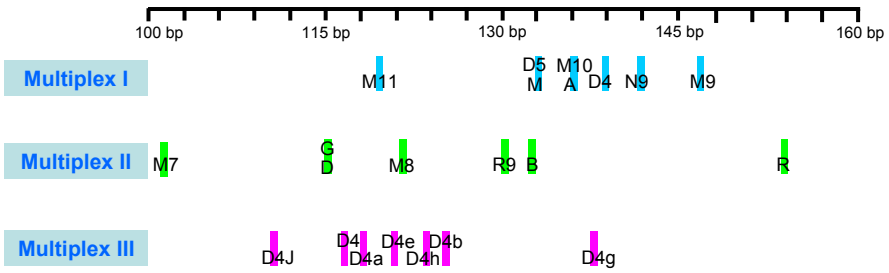
- Mitochondrial DNA control region sequences were obtained from 8 small overlapping PCR fragments (133 – 177 bp)



The screening for cross-contamination or sample mix-up was required for assessing authenticity

Coding region SNP scoring using three PCR multiplexes

- Small amplicon sizes of the three PCR multiplexes enabled SNP score to be successfully analyzed in old skeletal remains



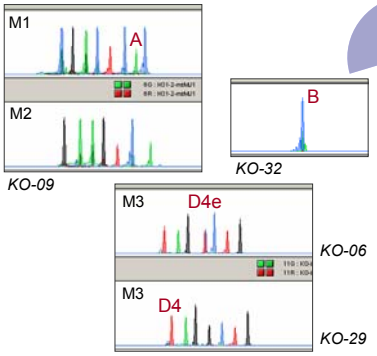
mtDNA haplogroup determination by the control region mutation motifs

Sample	Relative dating	Haplogroup	Control Region Sequence		
			nt16024-nt16365	nt073-nt340	nt438-nt548
KO-02†	Paleolithic Age	B4b1	16136-16182C-16183C-16189-16217-16284-16357	73-199-202-207-263-309.1C-309.2C-315.1C	499
KO-06†	Three Kingdoms	D4e1	16092-16187-16223-16362	73-94-263-315.1C	489
KO-07	Goryo	F1a1	16129-16162-16172-16304	73-249d-263-309.1C-315.1C	523d-524d
KO-08†	Goryo	D6	16183C-16189-16223-16274-16362	73-263-309.1C-309.2C-315.1C	489
KO-09	Goryo	A5c	16126-16129-16213-16223-16290-16319	73-152-235-263-309.1C-315.1C	
KO-18†	Goryo	N9a1	16129-16189-16223-16257A-16261	73-150-263-309.1C-309.2C-315.1C	
KO-28†	Neolithic Age	B4f	16168-16172-16183C-16189-16217-16249-16266-16325	73-200-257-263-309.1C-315.1C	
KO-29	Neolithic Age	D4c	16223-16245-16362	73-263-315.1C	489
KO-30	Paleolithic Age	G3a	16223-16274-16325-16362	73-143-152-263-309.1C-315.1C	489
KO-32†	Neolithic Age	B4b1	16136-16183C-16189-16217-16284N	73-199-202-207-263-309.1C-315.1C	499
KO-34	Bronze Age	D4	16223-16362	73-152-263-315.1C	489-523d-524d
KO-35	Bronze Age	D4c	16223-16224-16245-16292-16362	73-146-263-315.1C	489

*Determined haplogroups and haplogroup-specific control region mutation motifs are indicated in blue and red, respectively
†Haplotypes which have partial but articulate sequences

Confirmation of the haplogroup-specific coding region SNPs

- Diagnostic coding region SNPs were confirmed using monoplex SNaPshot, multiplex SNaPshot or sequencing



Sample	Haplogroup	Coding Region SNP
KO-02	B4b1	9bp del
KO-06	D4e1	3010, 14668, 11215
KO-07	F1a1	3970
KO-08	D6	4883, 12654
KO-09	A5c	8794
KO-18	N9a1	5417
KO-28	B4f	9bp del
KO-29	D4c	3010, 14668
KO-30	G3a	4833
KO-32	B4b1	9bp del
KO-34	D4	3010, 14668
KO-35	D4c	3010, 14668

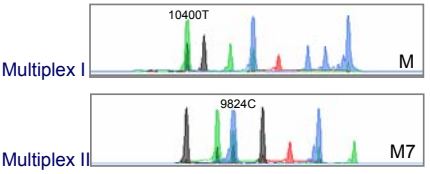
mtDNA haplogroup determination in ancient Korean human remains

- Contamination from the investigators was excluded on the basis of mtDNA sequence comparison results
- 12 of 35 mtDNAs were successfully assigned to appropriate East Asian mtDNA haplogroups or subhaplogroups
  - ✓ No compound haplotype
  - ✓ No mosaic structure
  - ✓ No abnormal mutation

mtDNA analysis in samples from geographically distant region

- A 800 year-old skeletal remain sample was obtained from East Mongolia
- mtDNA haplogroup could be determined by the control region sequence analysis and the coding region SNP analysis
- Haplogroup-directed database comparison was performed in 1192 East Asian mtDNA database

HV1-HV2-HV3 region sequence
16129-16152-16179-16192-**16223**-16362
73-263-315.1C
489



Haplogroup-directed database comparisons to reveal mtDNA origin

- Sequence variations shown in the skeletal remain were detected in the Ulchi of the Lower Amur, not in 1192 East Asian mtDNA database. This supports the sample authenticity

HV1 sequence variation

Skeletal remain found in Mongolia
16129-16152-16179-16192-**16223-16362**
M7 observed in Ulchi
16129-16152-16179-16189-**16223-16362**

Starikovskaya EB et al. ***Ann. Hum. Genet.*** *(2005) 69:67-89*

Concluding remarks

- East Asian haplogroup determination and haplogroup-directed database comparison can be efficiently used for the detection of presence of phylogeographic paradox, mosaic structure and abnormal mutation in mtDNA control region sequences
- We suggest mtDNA haplogroup determination and haplogroup-directed database comparison as an additional tool for authenticating ancient East Asian mtDNA besides rigid adherence to the 9 criteria suggested by Cooper and Poinar.

Acknowledgement

to our lab members and to Koguryo Research Foundation for research fund support