Massively Parallel Sequencing of The Whole Mitochondrial Genome from Human Hair Shafts

Bo Min Kim · Eun Young Lee · Hwan Young Lee · Kyoung-Jin Shin

Department of Forensic Medicine, Yonsei University College of Medicine, Seoul, Korea

Introduction

Mitochondrial DNA (mtDNA) with a significantly high copy number offers advantages in forensic casework that mainly contains low-quantity or no detectable nuclear DNA, such as hair shed. mtDNA analysis has been focused on the control region, but a recent development of massively parallel sequencing (MPS) has made whole mitochondrial DNA sequencing easily accessible. Here, we analyzed the whole mtDNA sequences of head hair obtained from 20 Korean males using MPS method. The obtained whole mtDNA sequences were aligned to the revised Cambridge Reference Sequence and compared with those obtained from bloods and buccal swabs of the same individual. In addition, we determined the mtDNA haplogroup of each samples and scrutinized the presence of point heteroplasmy among tissues.

Materials and Methods

Blood and buccal swab samples

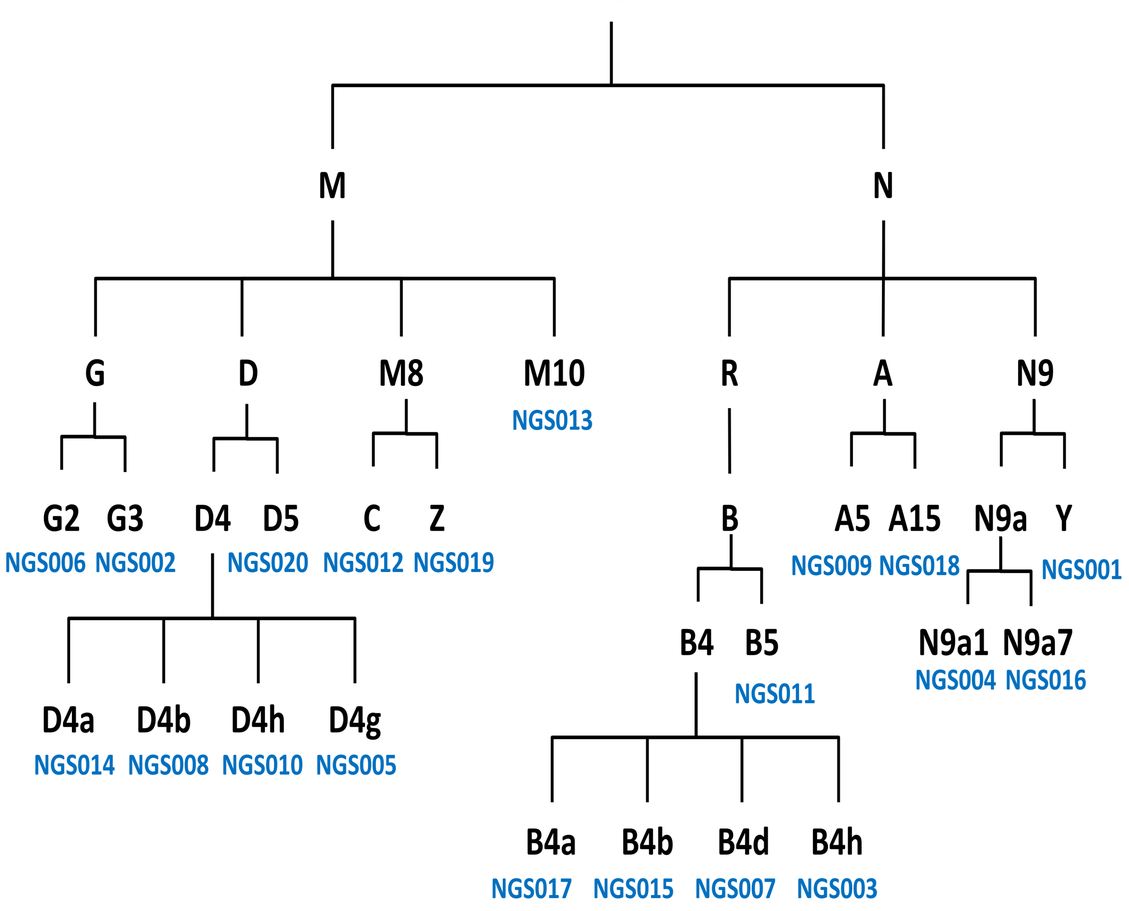
- **Sample collection:** Peripheral blood and buccal swab samples were collected from 20 unrelated Korean males.
- **DNA Extraction:** Genomic DNA from blood and buccal swab was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction.
- **Long range PCR amplification:** Whole mtDNA was amplified with Takara LA Taq HS (Kusatsu, Shiga, Japan) using 1 ng of DNA extracted from blood and buccal swab samples.
- **Massively parallel sequencing:** MPS library preparation using 250 pg of two long range amplicons was performed according to the protocol of Nextera XT DNA Library Prep Kit (Illumina, Inc., San Diego, CA, USA). The quantified and pooled library was sequenced on a MiSeq™ (Illumina) system using a MiSeq Reagent Kit v3 (Illumina).

Hair shaft samples

- **Sample Collection:** More than 5 hair shaft samples were collected from each donors who provided also blood and buccal swab. Hair shaft samples had been stored at a room temperature for more than a year. 2 cm of each hair including the root was removed to exclude nuclear DNA following mtDNA analysis. The second proximal 2 cm fragment of each hairs was prepared in duplicate.
- **Decontamination**: Hair fragments were placed into a tube containing 5% TergAZyme (Alconox White Plains, NY, USA) solution and placed into a sonicating water bath at a room temperature for 15 minutes. Then, briefly the fragments were rinsed with distilled water, 0.85% saline and 100% ethanol sequentially.
- **DNA Extraction:** Genomic DNA from hair shafts was extracted using a QIAamp DNA Investigator Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.
- **Massively parallel sequencing:** Extracted DNA was subjected to PCR amplification of whole mtDNA using the Precision ID mtDNA Whole Genome Panel kit (Thermofisher scientific, Waltham, MA USA). Barcoded MPS libraries were prepared, quantified and enriched on an Ion Chef (Thermofisher scientific) using an Ion 520 chip (Thermofisher scientific). The libraries were sequenced on an Ion S5 System (Thermofisher scientific).

Results

Fig 1. mtDNA haplogroup determined in 20 Korean samples



- mtDNA haplogroup of each samples was determined based on the observed haplogroup specific mutations and referenced to the mtDNA tree Build 17 at the PhyloTree (http://www.phylotree.org/).
- 20 mtDNA haplogroups which were identified from the hair shaft samples were corresponding with mtDNA haplogroups from buccal swabs.

Table 1. Observed mtDNA point heteroplasmy (PHP) among tissues

	Position	Blood	Buccal swab	Hair shaft #1	Hair shaft #2
NGS001	152	C/T	C/T	C	C/T
	8555	C	C	C	C/T
NGS004	709	G	G	G	G/A
	16320	C/T	C/T	C	C
NGS006	41	C	C	C	C/T
	10873	T	T	T	T/C
	11847	G	G	G	G/A
	16610	C	C	C/T	C
NGS013	10873	C	C	C	C/T
NGS015	204	T/C	T/C	T	T
	15034	A/G	A/G	A/G	A/G
	15236	G	G	G/A	G
NGS016	16261	T/C	T	T	T
NGS017	930	A/G	A/G	A/G	A/G
	15279	T	T	T	T/C
	16103	G/A	G/A	A	G/A
NGS019	9947	G	G	G/A	G
	10873	C	C	C	C/T
	10644	G/A	G/A	G/A	G
	16260	T/C	T	T	T
NGS020	10873	C	C	C/T	C
	16162	A	A	A/G	A

- Control region mutations were written in blue, coding region mutations were written in black.
- In the heteroplasmy, major variants were written as big font size, minor variants were written as small font size.
- mtDNA variant calling was following the parameters: 90% variant probability, and a minimum coverage of 100 reads.
- Length heteroplasmy was ignored in this study.

Conclusion

- Whole mtDNA sequence analysis using MPS was possible with hair shafts stored at room temperature for over a year.
- Whole mtDNA sequence of 20 Korean males has been identified and mtDNA haplogroup of each samples was determined. All the mtDNA haplogroups belong to East Asian groups.
- Point heteroplasmy was observed in hair shafts more frequently than the blood and buccal swab samples.
- Even though the hair shafts were collected from the same donor, the distribution of point heteroplasmy could be different. Thus, the special care is required when interpreting mtDNA sequences from hair samples.
- Whole mtDNA sequencing using MPS of hair shaft will be useful tool for increasing discriminant power in forensic caseworks .

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