Quantitative and Qualitative Profiling of Mitochondrial DNA Length Heteroplasmy

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Mitochondrial DNA

- The properties of mtDNA that make it valuable for evolutionary and human identification studies include its high copy number, maternal inheritance and its rapid rate of evolution.
- The two non-coding hypervariable regions (HV1,HV2) of the control region are the most polymorphic regions in mtDNA, have been analised in several kinds of biological evidence and validated for forensic application .
- There are two types of heteroplasmy, length and point heteroplasmy.

Subject of Investigation

- The genetic characteristics of length heteroplasmy have been the subject of the investigation in mtDNA.
- No guiding criteria for the interpretation of mtDNA length heteroplasmy have been established due to sequencing method limitation.
- Therefore, In an attempt to investigate mtDNA length heteroplasmy, it is prerequisite to develop a new method capable of complementing sequencing analysis.

Samples

- One hundred unrelated Korean DNAs were extracted from buccal swabs using QIAamp DNA Mini Kit.
- The two hypervariable regions of mitochondrial DNA were amplified in a PCR mixture of total volume 10.0ul containing 0.05~0.1ng of DNA template.

Amplification

- Primer Set

L16144	5'-TGA CCA CCT GTA GTA CAT AA
H16410*	5'-**FAM**-GAG GAT GGT GGT CAA GGG AC
L155	5'-TAT TTA TCG CAC CTA CGT TC
H389 *	5'-**HEX**-CTG GTT AGG CTG GTG TTA GG

- Theraml Cycling

Initial denaturation	95 °C for 11min	
Denaturation	94 °C for 1min	X 25 cycles
Annealing	56 °C for 1min	
Extension	72 °C for 1min	
Final extension	60 °C for 45min	

Size-Based Separation

- The PCR products were separated by capillary electrophoresis using an ABI PRISM 310 genetic analyzer (Applied Biosystems).
- **POP 6** was utilized to the resolution of the separation and **GS STR A module** was adapted as a run module.
- The resulting data were analyzed using GeneScan software 3.1 (Applied Biosystems) and none of smooth option as an analysis parameter.

Confirmation of Length Heteroplasmy

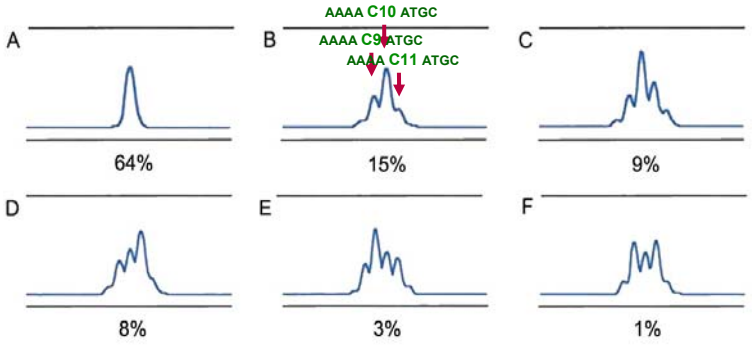
- To analyze sequence encompassing the polymorphic tracts in the HV1 and HV2 regions, PCR products were using a BigDye Terminator Cycle Sequencing v2.0 Ready Reaction Kit (Applied Biosystems).
- In order to identify each mtDNA length variant within the heteroplasmic mtDNA mixture, cloning and sequencing were carried out using pGEM-T Easy Vector (Promega).

Proportion of Length Variation

HV1 Size[a] (bp)	Proportions of length variants	Length homo-plasmy	Length hetero-plasmy	**HV2** Size[a)] (bp)	Proportions of length variants	Length homo-plasmy	Length hetero-plasmy
				234	2	0	2
266	2	1	1	235	3	3	0
267	77	63	14	**236**	34	28	6
268	14	0	14	237	41	0	41
269	7	0	7	238	19	0	19
				239	1	0	1
Sum	100	64	**36**	Sum	100	31	**69**

The study demonstrated **36**% and **69**% of Koreans show length heteroplamy in the HV1 and HV2 regions.

The Peak Pattern of HV1



According to the GeneScan electropherograms, all heteroplasmic mtDNAs were classified into **5 major peak patterns**.

Sequences of HV1

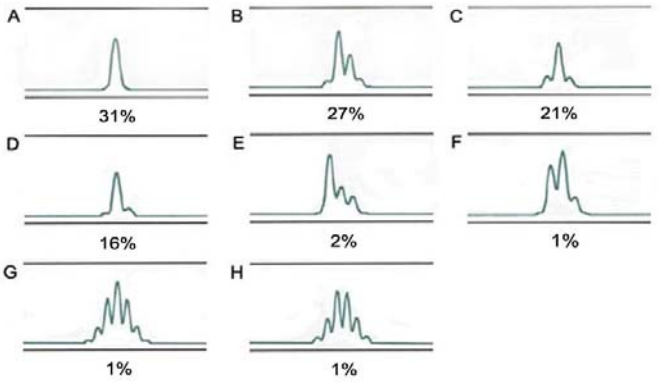
Sequences	A	B	C	D	E	F	Sum
AAAA**CCCCCTCCCC**ATGC	50						50
AAAACCCCCCCCCCATGC					2		2
AAAACCCCCCCCCC**G**TGC		1					1
AAAACCCCCCCCCCCATGC				6		1	7
AAA**C**CCCCCC**C**CCCCATGC			1				1
AAA**C**CCCCCC**C**CCCC**CC**GC		1					1
AAA**C**CCCCCC**C**CCCC**C**ATGC		13		1			14
AA**CC**CCCCCC**C**CCCCATGC			8				8
AA**CC**CCCCCC**C**CCCC**CC**GC				1			1
AA**TC**CCCCC-CCCCATGC					1		1
Sum	0	15	9	8	3	1	**36**

The mtDNA Peak Patterns of HV1

Type	N	%[a)]	The most prevalent length variant	N	%[b)]
A	64		_	63	98.4
			16185T, 16189d	1	1.6
B	15	41.7	16189C	2	13.3
			16189C, 16193.1C	13	86.7
C	9	25.0	16189C	9	100.0
D	8	22.2	16189C	1	12.5
			16189C, 16193.1C	1	12.5
			16189C, 16193.1C, 16193.2C	6	75.0
E	3	8.3	16189C, 16193d	1	33.3
			16189C	2	66.7
F	1	2.8	16189C, 16193.1C, 16193.2C	1	100.0
Sum	100			100	

a) Proportions of the total number of heteroplasic mtDNA
b) Proportions of the most prevalent length variants of each peak pattern

The Peak Pattern of HV2



According to the GeneScan electropherograms, all heteroplasmic mtDNAs were classified into **7 major peak patterns**.

Sequences of HV2

Sequences	A	B	C	D	F	G	H	I	Sum
AA**CCCCCCCTCCCCCC**GC	31	1	1		2				35
AACCCCCCC**C**T**C**CCCCCGC		15	12	16					43
AACCCCCC**CC**T**C**CCCCCGC		11	8						19
AACCCCCC**CCC**T**C**CCCCCGC						1			1
AACCCCCCC-CCCCC-GC							1	1	2
Sum	31	27	21	16	2	1	1	1	100

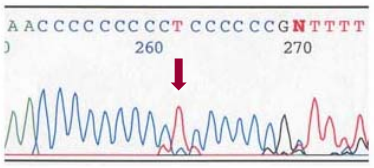
The mtDNA peak patterns of HV2

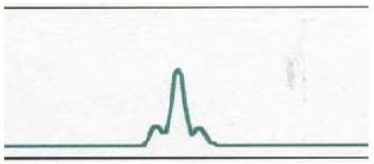
Type	N	%[a)]	The most prevalent length variant	N	%[b)]
A	31		315.1C [c)]	28	90.3
			249d, 315.1C	3	9.7
B	27	39.1	249d, 309.1C, 315.1C	1	3.7
			315.1C	1	3.7
			309.1C, 315.1C	14	51.9
			309.1C, 309.2C, 315.1C	11	40.7
C	21	30.4	315.1C	1	4.8
			309.1C, 315.1C	12	57.1
			309.1C, 309.2C, 315.1C	8	38.1
D	16	23.2	249d, 309.1C, 315.1C	1	6.2
			309.1C, 315.1C	15	93.8
E	2	2.9	315.1C	2	100.0
F	1	1.4	309.1C, 309.2C, 309.3C, 315.1C	1	100.0
G	1	1.4	310d	1	100.0
H	1	1.4	310d	1	100.0
Sum	100			100	

a) Proportions of the total number of heteroplasic mtDNA
b) Proportions of the most prevalent length variants of each peak pattern

Electropherogram of HV2

- The HV2 heteroplasmic peak patterns in GeneScan analysis were very similar to multiple T peaks shown in the middle of homopolymeric C-stretch in sequencing electropherograms.





Conclusions

- We established a new strategy for profiling length heteroplasmies.
- Classification of mtDNAs into several types of peak patterns is believed to offer a useful means of determining genetic identity by increasing mitochondrial DNA haplotype diversity.
- The developed method will present a promising tool for the diagnosis of several common diseases which are etiologically or prognostically associated with mtDNA polymorphisms.