A novel analysis of mitochondrial DNA length heteroplasmy

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Introduction

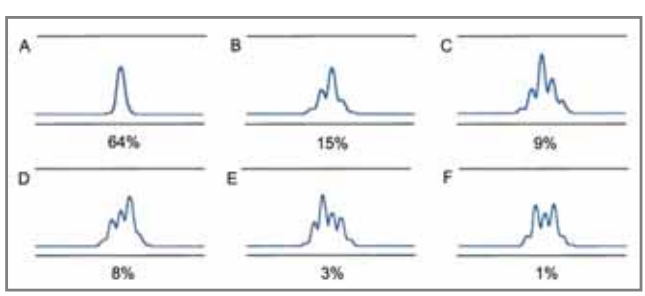
The properties of mtDNA that make it valuable for evolutionary and human identification studies include its high copy number, maternal inheritance and its rapid rate of evolution. The two non-coding HV1 and HV2 fragments of the control region are the most polymorphic regions in mtDNA, and have been analyzed for forensic applications. Although mtDNA is homoplasmic in the majority of cases, heteroplasmy may be observed. There are two type of heteroplasmy, length heteroplamsy and point heteroplamsy, of which length heteroplasmy has been the subject of the genetic investigation and several common diseases, such as, diabetes mellitus and some cancers. However, no guiding criteria for the interpretation have been established due to the sequencing method limitations. Therefore, in an attempt to investigate mtDNA length heteroplasmy, it is prerequisite to develop a new method capable of complementing sequencing analysis.

Materials and Methods

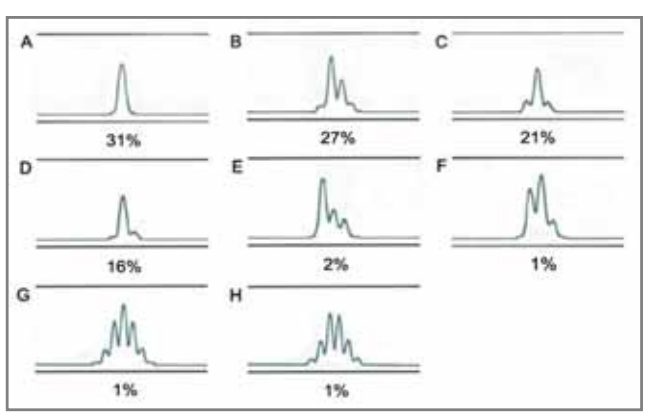
One hundred unrelated Korean DNAs were extracted from buccal swabs using QIAamp DNA Mini Kit (Qiagen). Amplification of two hypervariable regions of mitochondrial DNA (HV1 and HV2) was performed in a PCR mixture of total volume 10.0ul containing 0.1ng of DNA template and two sets of fluorescent primers. Thermal cycling was conducted under the conditions of 25 cycles of amplification with 56°C annealing temperature. The resultant PCR products were separated by capillary electrophoresis using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Results

In the electropherograms, homoplasmic mtDNAs showed a single peak representing only one type of mtDNA, and heteroplasmc mtDNAs produced multiple peaks of different sizes and heights.



The mtDNA peak patterns of the HV1 region in GeneScan electropherograms **According to the analysis, 36% of samples showed length heteroplasmy in the HV1 region.**



The mtDNA peak patterns of the HV2 region in GeneScan electropherograms **According to the analysis, 69% of samples showed length heteroplasmy in the HV2 region.**

Sequencing and GeneScan electropherograms of HV2 length heteroplasmy **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, which enables both the identification of all length variants in a mixture and the confirmation of the existence of a length heteroplasmy. The increased knowledge concerning mtDNA obtained in this study is believed to offer a useful means of determining genetic identity due to increased mitochondrial DNA haplotype diversity, by allowing mtDNAs to be classified into several types of peak patterns. Also, the developed method will present a promising tool for the diagnosis of several common diseases which are etiologically or prognostically associated with mtDNA polymorphisms.