B10 Mitochondrial DNA Heteroplasmy Among Hairs in a Single Individual

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The goal of this presentation is to study how frequently a heteroplasmy or a mutated sequence of mitochondrial DNA (mtDNA) was observed among hairs in a single individual.

Mitochondrial DNA (mtDNA) analysis is a useful tool in forensic analysis. The advantages of using mtDNA for human identification are:
(1) The many polymorphisms in the control (non-coding) region can be used to distinguish between non maternally-related individuals; (2) The mtDNA of maternally-related individuals can be used to verify the identifications; and (3) The large numbers of mitochondria per cell permit mtDNA extraction from minute or degraded samples when there is insufficient nuclear DNA. One disadvantage of using mtDNA for human identification purpose is the possibility that a heteroplasmy (different base pairs at the same mtDNA site) may exist. MtDNA analysis often applies hair samples in caseworks. The proportion of heteroplasmy varied among hairs if the person has obvious heteroplasmy in blood or saliva sample, which contains a large amount of mtDNA. Small proportion of heteroplasmy is thought to be contained in most persons, but it is not detected by normal sequencing techniques. At this time, there is no report about the frequency of hairs containing heteroplasmic or mutated sequences within a single individual by examining large number of hairs. In order to know whether heteroplasmic or mutated sequences are found in hairs in a single individual whose blood sample doesn't contain heteroplasmy by an ordinal sequencing method, DNA was extracted from many hairs of a single individual, then analyzed with DGGE method.

A total of 144 hair shafts were collected from 7 head regions (16 hairs each from the left / right frontal, temporal, and occipital regions; and 48 hairs in the parietal region of a male individual). Hair DNA was extracted by using QIAamp DNA mini kit (QIAGEN). For DGGE analysis 3 regions [HV1-B region (15998-16173: 176bp), HV1C region (16209-16400: 192bp) and HV2-E (30-289: 260bp)] were amplified respectively with the primer attached with GC-clamp and the primer labeled with FAM. DGGE electrophoresis was performed with the DCode mutation detection system (Bio-Rad Laboratories). Then DNA were visualized with FluorImager595 (Amersham-Pharmacia). The base positions of heteroplasmies were determined by sequencing with the BigDye Terminator Sequencing kit (Applied Biosystems).

In this study, DNA from 144 hairs could be successfully amplified in all three regions, and 14 contained the heteroplasmies by DGGE analysis. Heteroplasmies were observed in all 3 regions. A hair in a right frontal region contained heteroplasmy in both the HV1-B and the HV1-C regions, and a hair in a parietal region also contained heteroplasmy in both the HV1-C and the HV2-E regions. A total of 9 hairs (4 hairs in right frontal, 1 in left temporal, 2 in right temporal, 1 in right occipital, and 1 in parietal) contained heteroplasmies only in the HV1-C region and the remaining 3 hairs (1 hair in left frontal, 1 in right occipital, and 1 in parietal) contained them only in the HV2-E region. The base position of heteroplasmy observed in thee HV1-C region were in the same area (16291) except 1 out of 11 hairs, but that in HV2-E region was different from each other. The proportions of heteroplasmy in the nucleotide position 16291 varied among hairs. Although the heteroplasmy was not observed in the blood and saliva samples of this person, his mother has the 16291 (C/T) heteroplasmy. Therefore, the heteroplasmy of this position might be derived from his mother. The origins of other heteroplasmies were unknown, but it was probably derived from the mutation during the development of hairs. These results indicate that heteroplasmies are not rare a phenomenon in hairs from a single individual. Therefore, it is sometimes difficult to decide the match of the mtDNA sequence between hairs. For the interpretation of mtDNA analysis of hairs, the existence of heteroplasmies and mutations should be taken into account and the criteria for inclusion and exclusion should be established in each laboratory.

mtDNA, Heteroplasmy, Hair DNA

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