Different distribution of mitochondrial DNA length heteroplasmy across human blood, tissue and hairs

Hwan Young Lee[1], Ukhee Chung[1,2], Myung Jin Park[1], Ji-Eun Yoo[1], Gil-Ro Han[3,4] and Kyoung-Jin Shin[1,2,3]

[1]Department of Forensic Medicine and Brain Korea 21 Project for Medical Science, College of Medicine, Yonsei University, [2]Biometrics Engineering Research Center, Yonsei University, [3]Human Identification Research Institute, Yonsei University, [4]Department of Forensic Medicine, National Institute of Scientific Investigation

Introduction

Mitochondrial DNA (mtDNA) has been widely used for forensic identification and evolutionary studies due to its abundance, inherent variability, and ability to survive in extreme environmental conditions. Mitochondrial heteroplasmy is defined as the occurrence of two or more populations of mtDNA in the same individual, the same tissue, the same cell or the same mitochondrion. The occurrence of heteroplasmy can improve the value of mtDNA analyses for forensic purposes. There are two types of heteroplasmy, length-based and sequence-based heteroplasmy. As compared with point heteroplasmy, length heteroplasmy has been considered to be somewhat general and to have less population specificity. Length heteroplasmy within the homopolymeric cytosine tract of the mitochondrial HV2 region is one of the most often observed neutral polymorphisms in normal populations. However, there are a few limitations to analyze length heteroplasmy, which are detection methods and intra-individual differences of mtDNA sequence. Therefore, in an attempt to investigate length heteroplasmy in mitochondrial HV2 region in various tissue samples, we designed a mutagenic primer to minimize stutter production for homopolymeric cytosine tract of HV2 and carried out the size-based separation of PCR products.

Materials and Methods

Sampling and DNA extraction

Postmortem tissue (blood, brain, liver, heart, skeletal muscle) and hair shafts samples were collected from 25 individuals at the morgue of the Korean National Institute of Scientific Investigation(NISI). Prior to DNA extraction, each hair shaft sample was decontaminated according to the previous report. DNA was extracted using the DNA IQ™ system (Promega) and a Tissue and Hair Extraction Kit (Promega)

PCR amplification and the size-based separation of PCR products

We designed a mutagenic PCR primer to minimize stutter production for the C-tract in the HV2 region. The primer was constructed to produce the amplification products containing the C-tract with less than 8 consecutive Cs by inserting the nucleotide T in the middle of the homopolymeric C-tract located in the 3'-terminus of the primer (**F291**: 5'-ATTTCCACCAAACCCCC**T**CC). PCR amplification was carried out in a total volume of 10.0 ul using F291 and fluorescence-labelled **R389** (5'-**HEX**-CTGGTTAGGCTGGTGTTAGG) primers. Thermal cycling was conducted under the conditions with 56°C annealing temperature and 25 cycles of amplification for 1.0 ng of blood and 4 tissues DNA, and 32 cycles of amplification for 0.1 ng of hair shafts mtDNA.

Nuclear DNA genotyping and mtDNA sequencing

To confirm sample sources and to examine the possibility of sample switching or contamination, blood and the 4 tissue samples, were genotyped using PowerPlex16 (Promega). In addition, we sequenced the HV1 and HV2 regions and analyzed CA dinucleotide repeat length polymorphism in the HV3 region of hair shafts.

Results

Using the mutagenic primer of the present study, the PCR amplification of clones with 7-10 consecutive Cs hardly ever produced stutter products (Fig. 1). In 15 heteroplasmic individuals, qualitative and/or quantitative mtDNA peak pattern differences were observed between blood, the various tissues and hair shafts. In tissue samples, 3 individuals showed the qualitative/quantitative peak pattern differences (Fig. 2A), and 5 showed the same C-tract genotypes, but in varying proportions (Fig. 2B). In most cases, hair shaft mtDNA with HV2 region length heteroplasmy displayed highly varying proportions of length variant (Fig. 3A). In 3 homoplasmic individuals, the appearance of heteroplasmic mtDNA peak patterns were observed in hair shafts (Fig. 3B).

Figure 1 Electropherograms of PCR products amplified either with primer of the present study (F291) or with that described previously (F155)

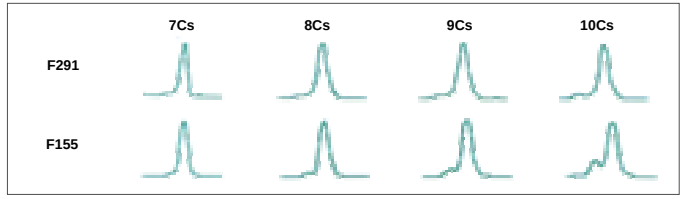


Figure 2 Electropherograms obtained from fragment length analysis of the C-tract of the mitochondrial HV2 region in blood and in the four other tissues

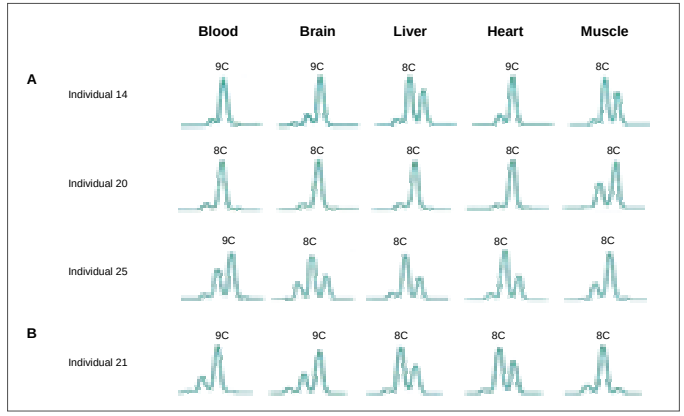
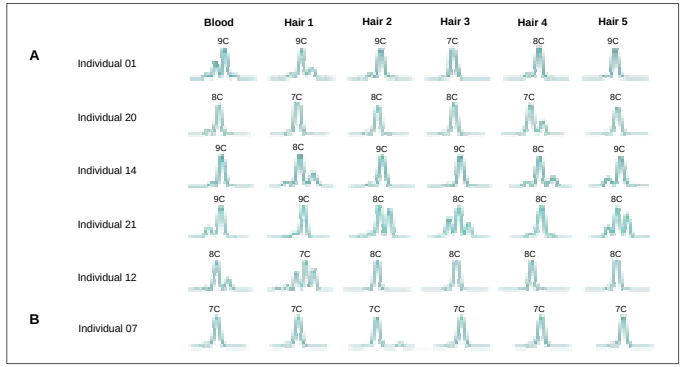


Figure 3 Electropherograms obtained from fragment length analysis of the C-tract of the mitochondrial HV2 region in hair shafts



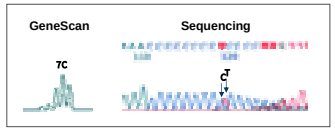


Figure 4 Electropherograms obtained from direct sequencing and fragment length analysis of the C-tract of the mitochondrial HV2 region in individual 12

Conclusion

We analyzed length heteroplasmy in a homopolymeric C-tract of the HV2 region using primer designed to minimize stutter production. We found quantitatively different distribution patterns of length heteroplasmy mostly in blood and 4 organ tissues, and significant variation in qualitative/quantitative peak pattern in hair shaft mtDNA profiles. Therefore, information of length heteroplasmy in a homopolymeric cytosine tract of the mtDNA HV2 region cannot be used alone to support an interpretation of exclusion.

References

1. Hauswirth WW, Clayton DA. Length heterogeneity of a conserved displacement-loop sequence in human mitochondrial DNA. Nucleic Acids Res 1985; 13:8093-8104
2. Pfeiffer H, Lutz-Bonengel S, Pollak S, Fimmers R, Baur MP, Brinkmann B. Mitochondrial DNA control region diversity in hairs and body fluids of monozygotic triplets. Int J Legal Med 2004; 118: 71-74
3. Shinde D, Lai Y, Sun F, Arnheim N. Taq DNA polymerase slippage mutation rates measured by PCR and quasi-likelihood analysis: (CA/GT)n and (A/T)n microsatellites. Nucleic Acids Res 2003; 31: 974-980
4. Jehaes E, Gilissen A, Cassiman JJ, Decorte R. Evaluation of a decontamination protocol for hair shafts before mtDNA sequencing. Forensic Sci Int 1998; 94: 65-71
5. Lee HY, Chung U, Yoo JE, Park MJ, Shin KJ. Quantitative and qualitative profiling of mitochondrial DNA length heteroplasmy. Electrophoresis 2004; 25: 28–34