CHARACTERIZATION OF HETEROPLASMY ACROSS VARIOUS TISSUE TYPES AND AGE GROUPS

C. Calloway and R. Reynolds

Roche Molecular Systems

Mitochondrial DNA (mtDNA) has proven to be a useful tool for forensics studies because of its high copy number, maternal inheritance, and high degree of sequence variability between individuals. For these reasons, mtDNA analysis is currently used by specialized laboratories for identification of telogen hairs and missing person remains. In addition, this method of analysis has been proposed for the identification of mass disaster remains, which often consist of a variety of small tissue samples from many individuals. While these features of mtDNA make it a particularly useful target for forensic analyses, there are biological aspects of the organelle that need to be considered to ensure that mtDNA typing results are interpreted appropriately.

In particular, the presence of more than one mtDNA sequence within an individual (heteroplasmy) might lead to ambiguous results, which frequently can be resolved with further testing. Since differences in the level of heteroplasmy across tissues have been observed in individuals with mtDNA diseases, heteroplasmy should be characterized across tissues from individuals in the normal population prior to the routine use of mtDNA typing in cases of mass disaster. This study characterizes the level and frequency of heteroplasmy across various tissues and age groups to gain information for the purpose of using mtDNA typing in cases of mass disaster.

An immobilized sequence specific oligonucleotide (SSO) probe system that detects sequence polymorphisms within 5 regions of the HVII region was used to screen for heteroplasmy and type tissue samples (heart, brain, muscle, and blood) from 43 cadavers. During the collection of autopsy samples and extraction of tissues, extensive measures to prevent contamination were carried out. Since both heteroplasmy and contamination may appear as a mixture of sequences, they cannot be differentiated without further tests. Therefore following the collection of the samples, replicate extractions and repeated amplifications and typings were performed to confirm the initial results. Also, sequence and cloning analyses were conducted to confirm that the observed multiple sequences were attributable to heteroplasmy and not contamination and to characterize the observed heteroplasmy. Each available tissue sample was sequenced for the HVII region, and brain and muscle samples were sequenced for the HVI region. Heteroplasmic point mutations were detected in 5 of the 43 individuals (11.6%) by the SSO probe system and in 22 of 43 individuals (51.2%) by sequence analysis.

This difference between the two detection methods is due to the presence of an apparent heteroplasmy “hot spot” that is not detected by the current SSO probe strips. The frequency and level of heteroplasmy differed across tissue types, being higher in muscle. In 3 cases in which heteroplasmy was detected only in the muscle sample, the second sequence was present at a higher level than the sequence observed across all tissues. Amplified mtDNA from several tissue samples was cloned and 30 clones were sequenced for each sample. The number of sequence variants observed by cloning was also greater in muscle tissue. Heteroplasmy was observed at multiple positions within a single individual and multiple individuals were heteroplasmic at identical positions. Heteroplasmy was also observed more frequently in the HVII region than in the HVI region, consistent with previously reported hair studies. The frequency of heteroplasmic point mutations increased with age, while heteroplasmy occurring within the C-stretch was independent of age.

In conclusion, the results from this study provide valuable new information about the frequency and level of heteroplasmy across tissues and age groups that will aid the analysis of remains from mass disaster.