ANALYSIS OF POLYMORPHIC CODING REGIONS OF THE MITOCHONDRIAL GENOME

Martina Nilsson, U. Gyllensten and Marie Allen
Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Sweden

Analysis of mitochondrial DNA has become an important complementary analysis in cases where enough nuclear DNA cannot be obtained. The use of sequence analysis of the mt-DNA control region is associated with the drawback of a relatively high probability of identity by chance.

To evaluate the possibility to increase the discrimination power by analysing mt-DNA-coding regions, sequencing of the entire mitochondrial genome in 26 European individuals was performed. Additional regions, with multiple highly polymorphic positions, could be a useful complement to the information obtained in the analysis of the HVI and HVII regions in the control region. The hypervariable regions, which are analysed in routine forensic casework analysis, consist of less than 5% of the entire mt-genome and more than 60% of the SNP's reported to the MITOMAP database are located in the coding region.

Additional regions, that are found to have a relatively high rate of informative polymorphic positions, have been characterised in this study. Out of the 216 found SNP's, two-thirds were localized in the coding region distributed in protein coding genes, rRNA genes, tRNA genes and in non-coding spacer regions between coding regions. The addition of coding regions in mt-DNA analysis may be useful to distinguish between the most common HVI/HVII types or to search for additional differences in inconclusive tests where a single difference has been found between samples. Moreover, the advantage of using additional polymorphic positions for identification is that it could increase the discrimination power in forensic DNA analysis of materials with limited DNA amounts. The study has clearly showed the potential to provide discrimination between common HVI/HVII types using analysis in the coding region in addition to the hypervariable regions.

The polymorphisms found in this study will be used as essential information for analysis using rapid techniques to detect the most informative SNP's in the coding region. In future analysis of additional regions the number of determined nucleotides in the mitochondrial DNA will increase and a database of the most interesting regions throughout the entire mt-genome could be created relatively quickly and easily. New methods, such as Pyrosequencing and Microarrays, have been evaluated for rapid, large-scale and high throughput analysis and will be useful in forensic analysis. The Pyrosequencing analysis is based on analysis of 11 fragments in the coding region based on the variation determined in this study in addition to parts of the hypervariable regions HVI and HVII. These 11 regions in the coding region cover most of the SNP's found at high frequencies in the mitochondrial genome. Moreover, a micro-array analysis system based on detection of 21 mitochondrial and 12 nuclear markers on the chip has been developed.