AN INTERNATIONAL STUDY ON THE DETECTION OF HETEROPLASMY IN MITOCHONDRIAL DNA

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Mitochondrial DNA (mtDNA) has a higher error rate associated with replication than does chromosomal DNA, allowing for a more rapid rate of evolution of the mtDNA genome (reported to be 5-10 times faster than chromosomal DNA). While there are mechanisms that clearly exist that restrict the level of mtDNA variation that is passed between generations (e.g., the "bottleneck theory"), it is known that mixtures of two or more populations of mtDNA can exist within an individual. This phenomenon is known as heteroplasmy. The bottleneck theory proposes that at some stage of oogenesis (at the primordial germ cell stage in mice) the number of copies of mtDNA in each reproductive cell (i.e., genomes) is reduced to a relatively small number (some estimates have ranged from 2-200 copies). The subset of the mtDNA population that is transmitted through the bottleneck becomes the founder population for the offspring's mtDNA. This subset could contain a homogeneous population of mtDNA, or a heterogeneous mixture of multiple populations. In addition, the transmitted mixture can manifest itself in varying ratios of the major and minor components when transmitted across multiple offspring, and/or across multiple generations. As a result, many of these heteroplasmic conditions produce apparent substitution events, some within a single transmission (the empirical rate has been reported as one apparent nucleotide substitution every 33 generational events, or meioses).

Through extensive studies performed in this laboratory and others, it has become quite evident that heteroplasmy occurs at a higher rate than originally inferred by phylogenetic studies, and that all humans are heteroplasmic to some degree, the level of which is generally below the detection limits of forensic mtDNA assays. There are two types of heteroplasmy, length-based heteroplasmy and sequence-based heteroplasmy. Length heteroplasmy is seen in both of the hypervariable regions, HV1 and HV2, and is generally represented by multiple populations of mtDNA with varying lengths of polycytosine stretches (commonly referred to as " C-stretches"), but can also exist as deletion events. While C-stretch variants are frequently observed in all of the major racial groups, in most cases length-based variation provides little discrimination potential. Sequence heteroplasmy is represented by the presence of two different populations of mtDNA varying from each other at a given nucleotide position. While data has yet to be generated which allows for the measurement of the rate of heteroplasmy at each nucleotide position in the mtDNA control region, two common hot spots have been identified at positions 16093 and 16129; T/C and G/A heteroplasmy, respectively. Nevertheless, the presence of sequence-based heteroplasmy will generally enhance the discrimination power of mtDNA analysis (e.g., the case of Nicholas Romanov II, the last Russian Tsar, which included heteroplasmy at nucleotide position 16169).

Regardless of the kind of heteroplasmy observed (i.e., length or sequence-based), interpretation can be subtle. Therefore, the goal of this study was to determine how forensic laboratories around the world detect heteroplasmy (19 laboratories from 11 countries participated in this project). Differences in the sequencing chemistries and/or instruments employed were not standardized, as each laboratory used their own methodologies. The first phase of the study assessed a laboratory's ability to detect heteroplasmic positions with a minor component concentration of 20% or greater, i.e., clear positions of heteroplasmy; a total of ten heteroplasmic samples were provided. The second phase of the study assessed the laboratory's level of sensitivity for detecting heteroplasmy. Samples of varying mixtures and/or ratios of major and minor components were provided. The results of this study will be discussed.