A87 Mitochondrial DNA (mtDNA) Mutations as a New Approach for Age-at-Death Estimation

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After attending this presentation, attendees will consider the possibility of using mitochondrial DNA mutations as an alternative to classical anthropological methods for age-at-death estimation.

This presentation will impact the forensic science community by introducing a potential quantitative indicator of age, demonstrating its accuracy of age-at-death estimation in two Spanish populations.

Age-at-death estimation is one of the fundamental parameters in the identification of human remains, particularly in mass disaster scenarios where skeletons are often incomplete, complicating the correct identification of the victims. Teeth are frequently preserved long after all other tissues have disappeared and are often used to estimate characteristics like age-at-death.

There are several approaches to age estimation based on dental development. In forensic anthropology, the Lamendin technique and its variants are non-invasive methods of age-at-death estimation; however, these methods can only be applied to single-rooted teeth and their accuracy is not guaranteed due to differences in population-specific references. In contrast, the new methodologies for age estimation are based on the natural process of aging, which causes alterations of tissues and organs on different biochemical levels. One of these alterations is the increase in the production of free radicals with age, playing a key role in the degenerative processes of senescence. The increase in free radicals, oxidative stress, induces an accumulation of non-repaired lesions in mitochondrial DNA (mtDNA). Some studies have pointed out the relationship between mtDNA mutations and age in different tissues. These studies are potentially interesting in forensic identification because they could help to improve the estimation of age-at-death.

Since teeth are the hardest tissue of the human body and one of the most abundant types of biological remains available in forensic cases, the goal of this study is to evaluate the mutations in mtDNA from dentin and pulp and their relation with the age, and assesses the reliability of this methodology in two Spanish populations.

Thirty healthy erupted third molars from Asturias, NW Spain, and 30 healthy erupted third molars from Cataluña, NE Spain, (aged 20-70 years) were collected from dental clinics. The Smithsonian Institution's ethical committee approved all procedures related to experimentation with human subjects. The teeth were cleaned and the enamel and cementum removed. The dentin was isolated, mechanically ground, and divided in aliquots of 200mg each; pulp also was isolated. The dentin and pulp were submitted for DNA extraction and quantification. As a control of correct human DNA extraction, the Amelogenin gene was amplified. To study the mtDNA mutations, Hypervariable region 2 (HV2) of the mitochondrial D-loop was chosen. This region was analyzed by Real Time Polymerase Chain Reaction (RT-PCR). Each sample was tested in triplicate. The analysis of relative gene expression data was calculated using the $2^{\Delta CT}$ method.

Quantitative-PCR (qPCR) results were similar in the two populations. There was an age-dependent decrease in the amplification of HV2 region in dentin and some variation in pulp. Using a regression analysis, a negative significant strong linear correlation was found between the mtDNA amplification and the age in dentin, with almost the same value in both populations. In contrast, a correlation was not found between mtDNA amplification and the age in pulp. The reason for this variation is the projection of the odontoblastic processes from pulp to dentin, which houses numerous mitochondria. As a result, the majority of the oxidative stress is generated in the dentin, making it possible to relate with age.

The findings from this research provide a new quantitative tool for estimating age-at-death which, in combination with traditional age markers, could improve identification accuracy in forensic cases. Future research may be able to expand on these results, using different types of teeth, analyzing different populations, and extending the age range.

Age-at-Death, Anthropological Methods, Mitochondrial DNA Mutations