A85 DNA Methylation Markers as a Novel Tool for Age-at-Death Estimation in Teeth

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

This presentation will impact the forensic science community by introducing a novel, innovative, and accurate approach for age-at-death estimation based on the current state-of-the-art research on aging.

Age estimation represents one of the fundamental parameters in forensic anthropology in creating the biological profile toward the correct identification of an individual. This parameter is particularly important in mass disaster scenarios where skeletons are often incomplete, which makes the correct identification of the victims difficult. 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. New methodologies for age estimation are based on the natural process of aging, which causes alterations of tissues and organs on different biochemical levels. Recently, it has been discovered that one of these alterations are changes in DNA methylation patterns. In fact, some studies identified and correlated DNA methylation biomarkers with age in blood samples. Although these studies were mainly developed in blood samples, these are potentially interesting in forensic identification because they could help to improve the estimation of age at death.

Since teeth are the hardest tissues 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 potential usefulness of DNA methylation biomarkers for age-at-death estimation in dentin and assess the reliability and accuracy of this methodology in this tissue.

Twenty-nine healthy erupted third molars were collected from dental clinics in Spain (aged 19 years to 70 years). 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. Then dentin was submitted for DNA extraction and quantification; 200ng of genomic DNA was bisulfite converted and later amplified by Polymerase Chain Reaction (PCR) for the following genes: ASPA, PDE4C, EDARADD, and ELOVL2. To analyze DNA methylation levels of five CpG sites in these genes, pyrosequencing was performed.

After analyzing pyrosequencing results, a multivariate linear regression model was selected from all methylation sites present in the pyrosequencing assays of ASPA, PDE4C, ELOVL4, and EDARADD by using the step function in R, which selects the model that explains most of the observed variance, predicting age with an adjusted R2 of 0.74 and a Mean Absolute Deviation (MAD) of 4.84 years (p-value <0.001).

This research is the first to explore age-associated methylation in teeth. The findings from this study 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, identifying new markers through whole genome CpG studies, using different types of teeth and extending the age range.

Age-at-Death, DNA Methylation, CpG Marker