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DNA Methylation for Age Estimation: An Epigenetic Approach in Forensic Anthropology

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Abstract:

In recent years, forensic research has focused on age estimation using DNA methylation pattern analysis. It is still unclear if other specimen types are appropriate for forensic epigenetic age assessment and whether further decomposition may impact the patterns of methylation of CpG sites. Buccal swabs from living individuals are a convenient way to gather DNA for estimating epigenetic age. The age at death-calculation of the unidentified deceased may be another forensic use for epigenetic age estimation. Numerous studies have documented age-related DNA methylation alterations in different tissues and bodily fluids, as well as age-predictive models. Despite the fact that age-related DNA methylation alterations can be tissue-specific, there is a multi-tissue age estimator that has substantial applicability to a variety of tissues and body fluids.

***Keywords**: DNA Methylation, age estimation, buccal swabs, forensic anthropology*

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Introduction

In a similar way to how the term "genome" refers to a cell's genetic makeup, the phrase "epigenome" describes its entire epigenetic state. The collection of chemical alterations to DNA known as the epigenome affects gene expression. Epigenetic modifications influence how and when genes are switched on or off, which controls how much protein is produced in particular cells. chromatic structuring, histone modification, and DNA methylation are examples of epigenetic variation types. One frequent kind of epigenetic change is DNA methylation. Only the essential genes are expressed when the chromatin proteins connected to DNA are activated or silenced, such as when producing specific proteins **(Bird, 2007; Vidaki *et al.*, 2013).** In a manner similar to how the DNA sequence is passed down from each generation to the next, the epigenetic sequence is maintained during cell division. However, during the course of a person's lifetime, they can evolve.

A methyl group (-CH3) is added to the 5′ carbon (C5) of cytosines and guanines in the DNA double helix in mammalian cells. This chemical alteration of DNA methylation occurs in a 5′-3′ orientation. The majority of the human genome's "CpG" dinucleotides are methylated **(Ehrlich *et al.*, 1982)** which are 5′-3′ CG methylation regions in DNA. Unmethylated CpGs, also known as "CpG islands," is most frequently seen in 300–3000 bp–long clusters that have a large CG density (>55% CG material), and are typically found at the promoter of keeping genes **(Antequera and Bird, 1993; Espada and Esteller, 2010)**. Recent research has demonstrated that as a person ages, specific CpG sites frequently become either hypermethylated or hypomethylated **(Zhang *et al.*, 2011).**

The development of a biological profile is crucial for searching missing person reports in forensic investigations involving skeletal remains in order to identify probable matches and aid in victim identification **(Cunha *et al.*, 2009)** Height, ancestry, sex, and age are frequently found in biological profiles. Thus, one of the crucial elements used for the recognition of human remains is age estimation.

A potential answer to this issue would be the use of biological methods that are based on the aging process. It used to be thought that the most reliable method for determining an adult's age was to racemize the aspartic acid in dentin. However, it has been demonstrated that a study of transmissible alterations in gene expression known as epigenetics **(Egger *et al.*, 2004)** can be a useful and more precise technique for age prediction. It has been demonstrated that the process of epigenetic DNA methylation, which entails the inclusion of a methyl group to a cytosine nucleotide in CpG islands, is particularly beneficial **(Declerck and Berghe, 2018).**

This approach has its origins in earlier research that involved building "epigenetic clocks" based on the association between patterns of methylation and age. This assumption, together with the requirement to broaden the use of DNA methylation in the field of forensic anthropology, serves as the foundation for the review's objective, which is to examine the patterns of methylation in buccal swabs tissue from humans and link these patterns with age in order to improve existing age-at-death estimates **(Horvath, 2013).**

Literature Review

DNA Methylation marker for Age Estimation of Individual

Human genome regions were found to be age-dependent DNA methylation patterns (Yi et al., 2015). Predict biological age within 5 years of chronological age. CpG methylation pattern The promoter regions of the three genes, namely EDARADD, TOM1L1, and NPTX2 have been shown to vary linearly (EDARADD and NPTX2 are hypomethylated, whereas TOM1L1 is hypomethylated). hypermethylation with increasing age from 18 to 70 years) Individuals (Bocklandt et al., 2011). 5 In further studies Gene (NPTX2, TRIM58, GRIA2, KCNQ1DN, BIRC4BP) have been shown to continuously donate methyl groups With increasing age (hypomethylation) in various human tissues (Koch & Wagner, 2011), the gene ELOVL2 showed that the degree of methylation increases with age (Garagnani et al., 2012). ELOVL2 methylation marker in human blood The sample is very stable and does not change significantly It is maintained in 1 month and up to 70% is retained even after 10 years. **(Rana, 2018).**

Techniques and methods for the analysis of DNA methylation

A highly sensitive and specific method for detecting 5mC methylation patterns on DNA recovered from samples needs to be developed, which can be routinely performed in forensic laboratories. One of the easiest ways to detect and identify DNA methylation patterns is to use Illumina Infinium BeadChip arrays (the latest technology being the BeadChip (Moran et al., 2016)), which remains routine. It is a very expensive method that is commonly used. However, there are other molecular approaches and techniques that have been known for decades, some of which have recently been developed for the detection and analysis of 5mC

methylation patterns. Note that all of these methods and analyzes are based on either bisulfite treatment of DNA, the use of methylation-sensitive restriction enzymes, or use of antibodies against methylated bases, or a combination thereof. These methods and techniques allow for the detection of a specific fraction expressed in picograms, nanograms, or micrograms. Amounts of DNA are required, and the amounts vary. Resolving methylation status for bp can be very expensive or relatively cheap. Some of the techniques and methods are: **(Rana, 2018).**

- Bisulfite Sequencing and Methylation-Specific PCR (MSP)
- The COBRA Methylation Assay
- Methylation-sensitive restriction enzyme-based microarray (MRE- microarray)
- Methylation analysis through Single Base Extension
- Methylated DNA Immunoprecipitation PCR/Sequencing (MeDIP-PCR/seq) **(Rana, 2018).**

Tissue Samples are taken for DNA Methylation Analysis-Based Age Estimation

Along with dental samples, buccal epithelial tissue can also be used as a sampling site in the mouth cavity. Using a swab is the method for removing this tissue **(Horvath and Raj, 2018).** Mayer *et al*., 2016 carried out this study. The buccal mucosa of a patient was swabbed in order to collect samples. Because DNA can be cultivated rather simply and the procedure is non-invasive, buccal epithelial cells from a patient are sampled. The buccal mucosal swab results, however, show a large number of combined buccal cells and white blood cells with various epigenetic configurations. As a result, we require a unique gene indicator that is particular to DNA methylation. The genes, CD6, PDE4, CSERPINB5, ASPA, and ITGA2B were chosen as particular markers in this investigation **(Soedarsono *et al.*, 2021).**

Buccal Swab Cytology

Smears were created on specimen slides using buccal swabs from bodies that were 1, 5, and 7 stages of decomposition. The smears were stained using the Pappenheim procedure, which involves drying the slides, dipping them in undiluted May-Grünwald stain for five minutes, rinsing them in distilled water, dipping them in Giemsa stain diluted in distilled water in a ratio of 1:9 for fifteen minutes, and then rinsing them once more in distilled water. The Nikon C-TEP3 equipment was used to capture photographs of the smears after staining them at a 100-fold resolution **(Koop *et al.*, 2021).**

DNA Methylation for Age Estimation in Deceased and living persons

For age prediction through DNA methylation to be applied to postmortem samples, it is necessary to address the posed question of postmortem variations in the DNA methylation pattern. There have not yet been any comprehensive studies on how decomposition affects the calculation of epigenetic age. However, (**Koop *et al.*, 2021)** presented their study which focuses on determining whether advanced postmortem decomposition makes it possible to estimate epigenetic age by the study of buccal swabs in certain circumstances. They collected buccal swab samples of living individuals and decedents with different stages of decomposition.

In their study, they found that the regression of data within each decomposition stage was displayed as the level of PDE4C CpG1 methylation vs. chronologi cal age in order to analyze how decomposition affected the estimation of methylation-dependent age. There is no evidence that the state of disintegration has a significant impact on the dispersion of data.

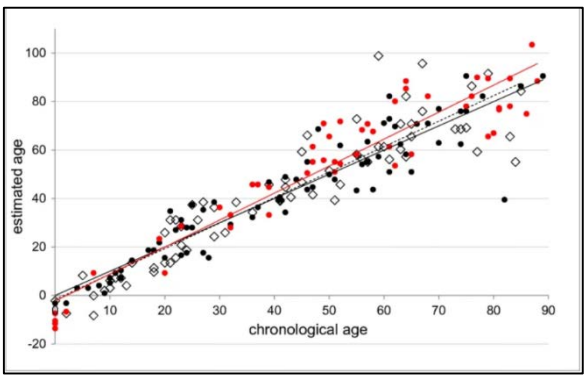

Figure No. 1: Living individual chronological age vs. estimated age (black dots and black regression line and rhombi white and dashed regression line for living individuals and red points and red regression line are for corpses).

The regression of data within each decomposition stage was displayed as the level of PDE4C CpG - 1 methylation vs. Surprisingly substantial amounts of DNA were extracted from corpses in numerous cases with just mild symptoms of decomposition. This might be because buccal mucosa stability is impacted by the degradation processes. In comparison to swabs collected from intact buccal tissue of living people, deceased persons showing indications of

decomposition may have more cells on the swab. The study of buccal swabs from bodies at various stages of decomposition is used to support this theory. **(Koop *et al.*,2021).**

Result and Conclusion

This paper discusses the fundamental ideas surrounding age estimation with DNA methylation and its approach in forensic anthropology. Due to the abundance of DNA methylation sites, the relationship between DNA methylation and age is a solid method for determining an individual's age. Since genes that are methylated have a lot of CpG sites that exhibit a linear association with aging, several studies have used DNA methylation as an essential marker with a low rate of error. In forensics, methylation of DNA is a promising possibility for determining the age of death. The molecular structure of tissues and organs changes as we age. These molecular changes may make it easier for forensic investigators to determine the age of a dead corpse or a living person.

The genetic aspects of aging were first the focus, but more recently, epigenetic processes have come to light as the primary causes of the changes in genome structure as well as function that accompany aging. The required age markers are frequently absent in forensic anthropological practice cases, necessitating the use of additional methods. This is the situation, among others, with some charred remains, mutilated bodies, and incomplete bodies. However, age continues to be a necessary characteristic when a victim's body is still fresh and when there is no suspicion as to who the victim is. An alternative is likewise necessary in certain cases.

The genetic strategy using methylation of DNA is a reliable option. Many studies discussed the DNA methylation pattern in blood, bone, tissues, buccal swab, and teeth to identify the age of the person however, if the buccal epithelial cell of the decomposed body has a sufficient amount of DNA present then it may be possible to estimate the age of the individual. This approach can be an aid for the forensic anthropologist to find the age of the unknown that leads to further investigation.

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