B192 Epigenetic-Aging-Signature — The Future?

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After attending this presentation, attendees will better understand the potential of applying epigenetic markers in forensic age prediction.

This presentation will impact the forensic science community by proposing a quantitative Next Generation Sequencing (NGS) approach in estimating chronological age and by demonstrating this approach's accuracy, reproducibility, and applicability in a set of whole blood samples and stains.

Estimating an individual's biological age can be of great use when studying ageing or predicting disease susceptibility; however, estimating someone's chronological age would also be of significant value in criminal investigations. In cases in which there are no suspects or eyewitnesses available to provide investigative leads, predicting a bloodstain donor's age could eliminate potential suspects. There are various chemical or biological methods that have been proposed for age estimation in bones, teeth, or other tissues, such as lead accumulation, protein modifications, telomere length, and mitochondrial DNA (mtDNA) deletions; however, they all suffer from limitations including poor accuracy. Most of these methods are more likely to suggest an age group (generation) rather than accurately predicting age, which restricts their applicability in forensic casework.

Epigenetic analysis has been reported in the literature as an alternative or supplementary method for age prediction since DNA methylation of cytosines followed by guanines (known as CpG sites) is known to be one of the mechanisms responsible for cell differentiation and the cellular response to aging. Various genome-wide DNA methylation analyses investigating thousands of CpG sites at the same time have revealed a substantial decrease in global DNA methylation levels with advancing age. As a result, various age prediction models using a subset of these sites have recently been proposed for demonstrating good accuracy (average prediction error <5 years); however, due to the potentially low quantity and quality of crime scene samples, genome-wide approaches are not applicable in forensic genetics. Therefore, there is a need to develop methodologies that analyze only a few CpG sites without compromising accuracy and sensitivity.

In this study, hundreds of previously reported CpG sites were carefully selected from the literature as potential age-associated markers, and a dataset comprised of ~2000 whole blood samples was created using publicly available DNA methylation data. In an attempt to identify a subset of these markers to be included in the epigenetic-aging-signature, linear regression analysis was applied. Utilizing only the CpG sites showing the strongest association with age, an age-prediction model was generated using Artificial Neural Networks (ANN). The potential of ANN models in predicting complex characteristics has been previously explored showing great accuracy and reproducibility. Predictions were highly accurate for both the verification and blind tests. To apply this epigenetic aging signature in forensic casework, a protocol based on bisulfite conversion and sequencing using Illumina's® MiSeq® platform was developed and optimized using artificially made DNA standards of known methylation levels. Validation experiments revealed that the method is highly sensitive, accurate, and reproducible; therefore, the model's prediction accuracy was further investigated by analyzing a set of whole blood samples and mock casework samples. Following treatment with sodium bisulfite and amplification of the fragments containing the proposed CpG sites in multiplex bisulfite PCR reactions, libraries were prepared using an optimized NGS protocol. The resultant prediction accuracy was not as high as in the ANN model; however, it is believed that further optimization of the method could reduce prediction errors.

These findings provide a new quantitative tool for estimating chronological age in crime scene bloodstains, which, together with current methods, could provide new investigative leads in criminal cases. Future research will be able to expand on these results by identifying new markers, investigating population differences, or extending to different tissues.

Age Prediction, DNA Methylation, Next Generation Sequencing

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