B5 A Student Experience Evaluating the Ability of a Methylation-Based Age Prediction Model to Be Implemented in the Forensic Laboratory as Part of a Collaborative Study

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Learning Overview: After attending this presentation, attendees will better understand the students' experience in practicing a multiplex SNaPshot™ assay and a Methylation-Sensitive Restriction Enzyme (MSRE) Polymerase Chain Reaction (PCR) assay for methylation-based age prediction. Attendees will understand the advantages and the disadvantages of these two methods in forensic applications for methylation analysis.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing the results from practicing and evaluating a multiplex SNaPshot™ assay as well as a preliminary test of an MSRE PCR method for methylation-based age prediction. Both procedures have a strong potential to be implemented in a general forensic laboratory. These DNA methylation-based age prediction methods would provide useful information to narrow the search for suspects when the profile from evidence samples does not match any of the reference samples or any profile in the available database.

DNA methylation analysis is reported to be a promising tool for age prediction for forensic applications. DNA methylation is a form of epigenetic regulation of gene expression that widely occurs on the CpG sites of the genome. As age-associated DNA methylation is tissue specific, most age prediction models developed are specific to the type of body fluid tested. This study focuses on the prediction for blood samples, the most commonly found body fluid at the crime scene. The commonly used methods to detect differential DNA methylation at a CpG site of interest are bisulfite-based approaches and MSRE PCR analysis. Bisulfite conversion-based approaches, including pyrosequencing, SNaPshot™-based Single Base Extension (SBE), and massively parallel sequencing, are the most commonly used in forensic research to date. Age prediction models have been proposed using methylation status obtained from bisulfite-conversion approaches, including a multiplex SNaPshot™ assay for blood samples based on the methylation status of five CpG sites from ELOVL2, FHL2, KLF4, C1orf132, and TRIM59 genes developed by Lee et al.[1] The alternative method for methylation analysis is MSRE PCR analysis. This approach utilizes the differential cleavage ability of MSRE depending on the methylation status of the CpG site of interest. The MSRE approach depends on specific recognition sites that flank the CpG site. Compared to bisulfite conversion, MSRE requires less DNA input, which could be a huge advantage for forensic investigations with a limited amount of sample.

This study aims to evaluate bisulfite-based SNaPshot™ assay and MSRE PCR analysis in successfully estimating the age of an individual. For bisulfite-based approach, the study practiced with the age prediction model developed by Lee et al. as a part of a collaborative study to demonstrate its accuracy in age prediction and ability to be implemented in general forensic laboratories.[1] This method was tested on familial samples of the children versus their corresponding parents. Preliminary results show that this model correctly grouped the samples into the corresponding age group. A sample of a 2.5-year-old was also tested, and the age prediction was 2.28. For MSRE PCR analysis, five age-associate CpG sites that can be recognized and cleaved by the methylation sensitive restriction enzyme HhaI were selected to develop an age prediction model. These sites are reported to have strong correlation between their methylation status and age in blood samples. This study demonstrates an effective procedure for MSRE-based analysis consisting of DNA extraction, quantification, MSRE digestion, PCR amplification, and capillary electrophoresis. The preliminary results show that the methylation status at locus cg23500537 is significantly different between child and adult samples.

Reference(s):

1. Jung S.E., Lim S.M., Hong S.R., Lee E.H., Shin K.J., Lee H.Y. DNA methylation of the ELOVL2, FHL2, KLF14, C1orf132/MIR29B2C, and TRIM59 genes for age prediction from blood, saliva, and buccal swab samples. *Forensic Science International: Genetics* 28 (2019) 1-8.

DNA Methylation, Age Prediction, Bisulfite Conversion and MSRE