B4 An Investigation of Forensic DNA Methylation Profiling as a Method of Age Estimation Using High Resolution Melting (HRM)

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Learning Overview: After attending this presentation, attendees will understand how epigenetic modification of specified regions of DNA are correlated with chronological age in human blood samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by contributing to the growing body of knowledge concerning the genome-phenome relationship between methylation and human aging to provide a more accurate rendering for forensic phenotypic prediction, enhancing phenotypic interpretations, ultimately assisting law enforcement to generate better investigative leads and identify missing persons.

Broadly, the results presented here will also more clearly define the relationship between these epigenetic modifications and the illusive and continuous subject of aging and could even present implications for areas of inquiry beyond the scope of forensic science.

Forensic DNA phenotyping investigations have revealed positive correlations between select DNA candidate loci and human appearance.[3] Thus far, only those association studies focused on externally visible characteristics (EVCs) involving pigmentation have been successful isolating informative regions of corresponding genetic instructions and developing predictive assays.[3,4,7]. The construction of a molecular test for chronological age would not only contribute to the creation of a profile to serve as a biological witness, but could also inform other phenotypic variants, such as hair color, hair loss, stature, and general appearance. Numerous studies have established a linear correlation between the degree of methylation at several CpG sites within the human genome and chronological age.[1,2,6, 8-10] It should be noted that methylation generally is a complex phenomenon which is influenced not only by genetic instruction, but also through environmental influences.[5] Thus, it may be more accurate to refer to age predicted via methylation patterns as *biological age* rather than *chronological age* until methylated age data has been thoroughly explored and compared to the age of the donor.

The aim of this study is to examine the utility of methylation status as an informative property for age prediction at five candidate loci in human blood samples. Locus selection was based on a predictive model proposed by Zbieć-Piekarska et. al. in a 2015 study and includes a total of 32 CpG sites at five genetic loci, ELOVL2, C1orf132, KLF14, TRIM59, and FHL2, examined to define the utility of their methylation status as a predictor for age.[9] The sites were evaluated in a total of 96 human blood samples ranging from 1-day-old to 94 years of age from the University of Maryland Hospital. DNA was isolated using a total of 25µl of whole blood from each sample and extracted via the organic phenol chloroform method to ensure a high yield. Resulting extracts were bisulfite treated with the EZ DNA Methylation-Gold™ Kit, Zymo Research, according to their standard protocol, and temperature cycling was accomplished with the GeneAmp PCR System 9700, Thermo Fisher Scientific. Only those bisulfites modified DNA samples yielding a minimum of 5ng/µl of DNA were used for subsequent testing. The methylation status of samples for each locus was captured via high resolution melting (HRM) by the Rotor-Gene Q, Qiagen, and data from each locus was binned into three age categories ranging from ages 1-30, 31-60, and 61-100.

The pre-experimental hypothesis posited a linear correlation between hypermethylation with increased age at CpG sites from C1orf132, TRIM59, KLF14, and FHL2, and hypomethylation with increased age at ELOVL2. Preliminary statistical results of binned age categories indicate moderate concordance between hypermethylation and advancing chronological age within the C1orf132 and FHL2 loci, while methylation detected within the remaining three gene regions was inconsistent with the pre-experimental hypothesis. Additional statistical analysis will be completed with the Rotor-Gene Q ScreenClust HRM Software, Qiagen, to normalize and cluster the multivariate data from each locus.

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Methylation, Phenotyping, Aging