B53 DNA Methylation-Based Assay for the Identification of Smoking Status

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Learning Overview: The goal of this presentation is to demonstrate an effective method to determine the smoking status of individuals based on DNA samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by proposing a new method using a pyrosequencing-based technique to predict smoking status of a suspect from the blood or saliva samples recovered from the crime scene. This presentation will illustrate a 4-CpGs assay used to differentiate between current, former, or never smokers.

DNA methylation signatures of candidate sites have been shown to serve as useful biomarkers for various traits. Interest in such applications has resulted in several genome wide association studies using large scale epigenetic arrays. However, because DNA methylation analysis is mainly performed by array studies which require laborious bioinformatic analysis, applying DNA methylation is still difficult in the clinical and forensic regimes due to the complexity of the instrumentation and the need for relatively large sample quantities. The authors decided to examine and sequence some of these locations using bisulfite modified PCR followed by pyrosequencing. Pyrosequencing is a technique that can measure the relative methylation level at each CpG site at high accuracy (the p indicates that C and G are connected by a phosphodiester bond).

In this study, pyrosequencing techniques were utilized to identify CpG sites indicative of tobacco smoking by investigating DNA sequences surrounding ten frequently reported smoking-related CpGs at six genetic loci. The authors examined a total of 88 CpG sites located at the six genetic loci to check their association with tobacco smoking. Blood and saliva (buccal swab) samples (n=161 each) were collected from volunteers and were categorized into three groups based on their self-reported smoking history: never smokers, former smokers, and current smokers. The entire samples were randomly divided into three sets: discovery, training, and validation. DNA samples were extracted, and bisulfite modified to convert the unmethylated cytosines to uracil while maintaining the methylated ones as cytosine. Next, the DNA was PCR amplified, and the methylation level at each CpG site was quantified by pyrosequencing.

In this study, the authors identified novel smoking-specific CpG sites in various genes. Overall, 15 CpGs in blood and 10 CpGs in saliva showed a significant decrease in methylation level with current smokers. Second, a quick and inexpensive assay was developed consisting of four consecutive CpG sites at Aryl-Hydrocarbon Receptor Repressor (*AHRR*). This assay can be used to measure the DNA methylation patterns in blood and saliva. Using a multinomial regression model, the assay was utilized to differentiate the smoking status of various individuals.

Tobacco Smoking, DNA Methylation, Pyrosequencing