B122 The Development of Epigenetic DNA Methylation Markers to Predict Tobacco Smoking of Unknown Suspects

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The goal of this presentation is to demonstrate to attendees a new method to predict tobacco smoking behavior based on DNA samples recovered from crime scenes.

This presentation will impact the forensic science community by proposing a novel and effective pyrosequencing-based technique able to determine smoking habits of a suspect based on the blood or saliva samples left behind at a crime scene. This presentation will illustrate the model used to distinguish tobacco smokers from non-smokers.

Recent developments in the analysis of epigenetic DNA methylation patterns have demonstrated that certain genetic loci show correlation with the tobacco smoking.[1,2] It is the goal of this study to identify a set of epigenetic methylation markers that exhibit variations in DNA methylation with tobacco smoking and can be used in forensic laboratories. In forensics, finding a suitable biomarker for tobacco smoking could be a very useful tool for predicting the individual's lifestyle. These results may prove beneficial in determining the potential identity of a suspect by narrowing the range of those who could have been the source of the recovered samples.

Different epigenome association studies have reported various genetic loci in which methylation levels were associated with tobacco smoking. From those previous studies, five genetic loci (AHRR, ALPP, IER3, GFI, and F2RL3) that contained smoking-related CpG sites have been identified; however, those results were based on chip arrays that only provide information on single CpG sites.[1,2] Initially, a total of 52 novel CpG sites located on the five genetic loci were examined to check their correlation with tobacco smoking. Among those tested, CpG sites at the AHRR gene were found to have the strongest correlation with tobacco smoking. Biological samples of blood and saliva were collected from volunteers based on smoking status: current smoker and non-smoker. DNA samples were extracted and bisulfite was modified in order to convert the unmethylated cytosines to uracil while maintaining the methylated ones as cytosine. Next, the DNA was Polymerase Chain Reaction (PCR) amplified, and the methylation level at each CpG site was quantified by pyrosequencing.

A prediction model for tobacco smoking was constructed using a set of CpG sites at the AHRR gene. This DNA methylation-based model was very effective in predicting a history of smoking habits using blood samples and was less informative when tested using saliva. Methylation patterns for all CpG sites used in the model exhibited a statistically significant decrease in average methylation levels for smokers when compared to non-smokers. The results indicate that specific CpG sites in AHRR could be used as potential epigenetic markers to predict specific individual lifestyle (tobacco smoking) using blood and saliva specimens. As a result, these epigenetic markers could be used to provide investigative leads in cases with unknown perpetrators.

Reference(s):

1. Breitling LP, Yang R, Korn B, Burwinkel B, Brenner H. Tobacco-smoking-related differential DNA methylation: 27K discovery and replication. *The American J. of Human Genetics.* 2011;88(4):450-7.

2. Monick MM, Beach SR, Plume J, Sears R, Gerrard M, Brody GH, et al. Coordinated changes in AHRR methylation in lymphoblasts and pulmonary macrophages from smokers. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics.* 2012;159(2):141-51.

Tobacco Smoking, DNA Methylation, Pyrosequencing