

DNA methylation related to HIV-infected smoking associated with mortality in HIV-infected individuals

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The epigenetic effects of tobacco smoking in non-HIV-infected populations are well-established. Recent epigenome-wide association studies (EWAS) have reported more than one hundred DNA methylated CpG sites (DNAm) associated with smoking. Thus, smoking's effect on DNAm in the genome could lead to cytopathic processes in HIV-1 infected cells. However, smoking modification of the epigenome in HIV-infected individuals has not been studied. Our **goal** is to identify differential DNAm sites between HIV-infected smokers as compared to non-smokers, and to link smoking-associated DNAm with mortality in HIV-infected individuals.

We profiled 480K CpG sites using the Illumina Infinium HumanMethylation 450 Beadchip in 711 HIV-infected African American men (current smokers=414; never smokers=297). An EWAS for smoking was conducted using linear regression model adjusted for confounders (i.e. age, cell types, antiviral treatment). We identified 65 CpGs differentially methylated, 51 hypomethylated and 14 hypermethylated, between smokers and non-smokers. The main findings were: 1) replication of previously reported smoking-associated CpG sites (i.e., *AHRR* cg05575921: $t=-17.1$, nominal $p=3.92 \times 10^{-54}$; *F2RL3* cg03636183: $t=-10.3$, nominal $p=5.37 \times 10^{-23}$); 2) observation of a greater effect of smoking on DNAm in HIV-infected smokers compared to HIV-uninfected smokers; 3) identified 25 unique DNAm sites for HIV-infected smokers (e.g., *CXCR5* cg12678834 was hypomethylated in smokers compared to non-smokers ($t=-5.56$, $p=4.03 \times 10^{-8}$)); 4) a smoking-associated DNAm risk score constructed by summarizing component CpG methylation β 's weighted by the coefficient at each site differentiated smokers and non-smokers, suggesting its potential utility as a biomarker for smoking; and 5) a significantly lower survival rate among the DNAm-defined smokers as compared to non-smokers (hazard ratio 1.45; 95%CI: 1.06 -1.97; $p=0.019$).

These findings suggest that smoking in the setting of HIV-infection has a significant impact on DNAm in the HIV-infected epigenome and demonstrate the translational potential of smoking-related DNAm to the clinical care of patients infected with HIV.