

MEETING ABSTRACTS

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Cytosine methylation in the HPV16 3' L1/ 5'LCR region characterized from anal epithelia of HPV-HIV coinfected men

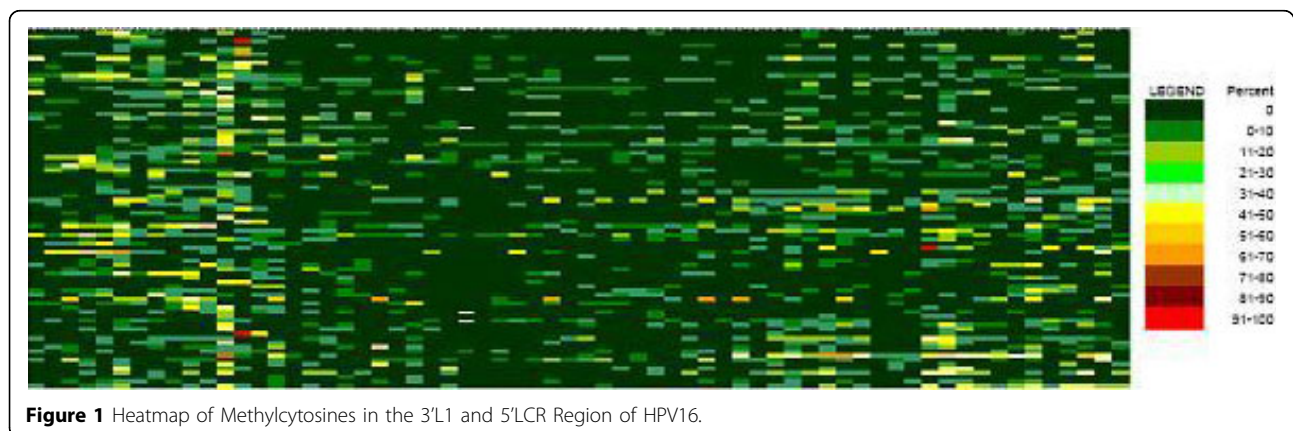
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Anal specimens derived from 83 HIV-HPV16 co-infected men were analyzed for cytosine methylation using bisulfite modification, PCR amplification, cloning, and sequencing. Data were processed using the ClustalW alignment algorithm within BiQ Analyzer Software and input into SAS 9.2 to generate heat maps and histograms (Figure 1). Approximately 10 clones were characterized for each specimen across 81 CpA, CpT, CpC (denovo methylation) and 10 CpG sites within the 3'-L1 and 5'LCR region of HPV16 genomes. Analyses were confined to 64 denovo cytosine residues and 10 CpGs outside the primer annealing regions. Clinical outcome evaluation by a single examiner showed seven men with no anal intraepithelial neoplasias (AIN), 26 with AIN-1, and 50 with AIN-2. Data showed the average prevalence (standard deviation) of

methylcytosines was 11% (0.3) across 7,553 bases/residues and did not vary across maintained or denovo cytosine groups, i.e., 6,723 denovo sites and 830 CpGs. Evaluation by a single examiner using cytology and histology showed 7 with no anal intraepithelial neoplasias (AIN), 26 showed AIN-1, and 50 showed AIN-2. Mean and median prevalence of denovo and CpG methylation were closely approximated across AIN groups. Maxima were observed in two regions: 7111 to 7119, and 7505 to 7514 that showed a methylcytosine prevalence of 24% (0.4), and 18% (0.4), respectively. Logistic regression suggests that with each year of age, risk for moderate-severe methylation $\geq 30\%$ at each site decreases; however, among smokers risk for $\geq 30\%$ methylation increases. Specifically, moderate cytosine-methylation decreased by 5% (OR=0.95, 95% CI,



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(0.92, 0.99) for each year of age, centered around the mean of the sample. Smokers were nearly twice as likely to show moderate cytosine methylation overall (OR=1.9, (1.1, 3.3) and these estimates did not vary across CpG-denovo sites. Although not statistically significant, moderate cytosine methylation was inversely associated with grade of AIN in these data (when compared to high-grade, low-grade and no AIN showed OR=1.5 (0.8, 2.7) and 2.0 (0.9, 4.5), respectively, $p=0.2$).

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