DNA Methylation Signatures of Inflammation in Youth Living With Perinatally-Acquired HIV

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BACKGROUND

- Youth living with perinatally-acquired HIV (YPHIV) show early indicators of comorbidities, including metabolic complications such as dyslipidemia and low bone mass.
- Recognized pathways involve chronic immune activation and inflammation leading to cellular senescence and increased vulnerability for HIV-associated comorbidities/complications.
- Epigenetic modifications may highlight mechanisms through which HIV and antiretroviral therapy (ART) exposure during critical developmental periods affect biological pathways and disease risk.
- We examined if perinatally-acquired HIV is associated with genome-wide alterations in the DNA methylome by comparing YPHIV and youth who are perinatally HIV-exposed uninfected (YPHEU) at two timepoints.
- Among YPHIV, we examined associations between viral load (VL), CD4 count, and markers of inflammation with genome-wide alterations in the DNA methylome.

METHODS

Study Population

- 32 YPHIV and 7 YPHEU with peripheral blood mononuclear cell samples collected at two timepoints ≥3 years apart were selected from the US-based PHACS Adolescent Master Protocol.
- All YPHIV had HIV RNA VL indicators within 1 year of birth, and within 1 year of each of the sample time points.
- Exclusion criteria included concurrent Hepatitis B or C, and those who reported any smoking.

Measurements

- Demographic and clinical information including history of ART, HIV RNA VL, and CD4+ T-cell measurements. Cumulative VL and CD4 were calculated by area under the curve (AUC) using the trapezoidal rule.
- Inflammation-related markers included C-reactive protein (CRP), fibrinogen, interleukin-6 (IL-6), soluble vascular cell adhesion molecule-1 (sVCAM-1), adiponectin, E-selectin (sE-selectin), monocyte chemoattractant protein (sMCP-1), intercellular adhesion molecule-1 (sICAM-1), and P-selectin (sP-selectin)
- DNA methylation was assayed using the Illumina MethylationEPIC (850K) array and data processed via a pipeline implemented by the *minfi* package in R.

Statistical Methods

- Using the *limma* package, we tested for differentially methylated CpG sites genome-wide (FDR p-value <0.05 and ≥5% difference in methylation) between groups at each timepoint.
- 15 targeted genes identified from prior work (PMID: 31324826) were also assessed for differentially methylated CpG sites.
- Among YPHIV, genome-wide associations of cumulative VL, cumulative CD4, and inflammation-related markers with DNA methylation were adjusted for ART type and age at ART initiation.

Among youth living with perinatally-acquired HIV, markers of inflammation were associated with epigenetic changes at genes involved in blood pressure regulation, chronic kidney disease, and fatty acid metabolism.

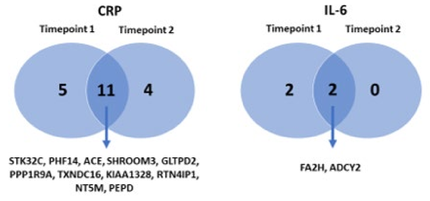
RESULTS

- Overall, median age was 11 and 17 years at timepoints 1 and 2, respectively. Groups were balanced by sex (51% male) and race/ethnicity (64% non-Hispanic Black) (**Table 1**).
- Genome-wide, there were no statistically significant differentially methylated sites comparing YPHIV to YPHEU at either timepoint.
- For targeted genes, 1 CpG site on SPERT and 6 CpG sites on PSMB9 were differentially methylated at timepoint 1 comparing YPHIV to YPHEU. At timepoint 2, 10 CpG sites on PSMB9 and 4 CpG sites on EBF4 were differentially methylated comparing YPHIV to YPHEU.
- Among YPHIV, cumulative VL or CD4 count were not strongly associated with methylation at any CpG sites.
- CRP was statistically associated with methylation at 27 CpG sites (16 genes) at timepoint 1 and 19 CpG sites (15 genes) at timepoint 2, with 11 genes overlapping timepoints (**Figure 1**).
- IL-6 was statistically associated with methylation at 6 CpG sites (4 genes) at timepoint 1 and 2 CpG sites (2 genes) at timepoint 2, with 2 genes overlapping timepoints (**Figure 1**).

Table 1: Characteristics of participants from PHACS

Characteristic; Median (IQR)		YPHIV (N=32)	YPHEU (N=7)
Age at Time 1 (years)		11.4 (9.9, 12.8)	8.1 (7.4, 9.0)
Age at Time 2 (years)		17.0 (16.2, 17.6)	16.0 (15.1, 17.0)
Time between Time 1 and Time 2 (years)		5.2 (3.9, 7.1)	8.0 (7.6, 8.1)
Sex	Male	16 (50.0%)	4 (57.1%)
	Female	16 (50.0%)	3 (42.9%)
Race	White	10 (31.3%)	2 (28.6%)
	Black	21 (65.6%)	5 (71.4%)
	Other	1 (3.1%)	0 (0.0%)
Site Region	Northeast	9 (28.1%)	0 (0.0%)
	South	3 (9.4%)	0 (0.0%)
	West	6 (18.8%)	3 (42.9%)
	Midwest	14 (43.8%)	4 (57.1%)
Age at First ARV (years)		0.23 (0.153, 1.99)	
Age at First HAART (years)		0.32 (0.153, 1.22)	
Cumulative CD4 count at Timepoint 1 (cells/mm^3)		1228.1 (1001.2, 1523.1)	
Cumulative CD4 count at Timepoint 2 (cells/mm^3)		1140.3 (881.3, 1338.3)	
Cumulative VL at Timepoint 1 (log10 copies/mL)		2.515 (2.125, 3.170)	
Cumulative VL at Timepoint 2 (log10 copies/mL)		2.284 (2.017, 2.906)	

Figure 1: Genes mapped to CpG sites significantly associated with CRP and IL-6 at two time points



RESULTS

Table 2: Functions of the 13 genes mapped to CpG sites significantly associated with CRP and IL-6

FA2H	Essential for the proper **functioning of the nervous system**
ADCY2	Polymorphisms of the ADCY2 gene have been associated with **COPD and lung function**
STK32C	Overexpressed in bladder cancer and contributes to tumor progression
PHF14	Involved in **lung development**
ACE	Part of the renin-angiotensin system, which **regulates blood pressure** and the balance of fluids and salts in the body
SHROOM3	Associated with **chronic kidney disease**
GLTPD2	Involved in ceramide transport and intermembrane lipid transfer.
PPP1R9A	Expressed mainly in embryonic skeletal muscle tissues
TXNDC16	Expression in prostate, brain, and other tissues
KIAA1328	Binding partner of SMC3; affects ability of SMC3 to engage in formation of protein complexes
RTN4IP1	Encodes a mitochondrial protein that interacts with reticulon 4, which is a potent inhibitor of regeneration following spinal cord injury
NT5M	Encodes a 5' nucleotidase that localizes to the mitochondrial matrix
PEPD	Involved in protein production and breakdown

CONCLUSIONS

- EWAS did not identify any CpG sites that were strongly associated with HIV in young adults.
- Targeted analysis identified sites on three genes statistically associated with HIV – SPERT, EBF4, PSMB9.
- EWAS did not identify any CpG sites strongly associated with HIV RNA or CD4 in YPHIV.
- Associations between markers of inflammation and epigenetic signatures among genes involved in blood pressure regulation, chronic kidney disease, and fatty acid metabolism were detected in YPHIV at two timepoints in childhood/adolescence.
- The small sample size should be taken into consideration in the interpretation of these findings.
- These genes may provide insight into inflammatory pathways contributing to HIV-associated chronic comorbidities among YPHIV.

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