Longitudinal Epigenome-Wide Association Study of Pre- and Post-HIV Infected Subjects

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BACKGROUND

- Extensive genetic and genomics association studies have revealed essential host factors for HIV-1 control and disease progression, which notably led to the development of a new class of antiretroviral inhibitors (CCR5 antagonists).
- Overall, these associations account for ~20% of the observed phenotypic variance suggesting that other factors are yet to be discovered.
- Epigenetic mechanisms are key regulators of gene expression that are not coded by DNA primary sequence and can impact complex diseases.

OBJECTIVE

To explore for the first time whether HIV-1 infection impacts the host epigenome DNA methylation patterns.

SUBJECTS AND METHODS

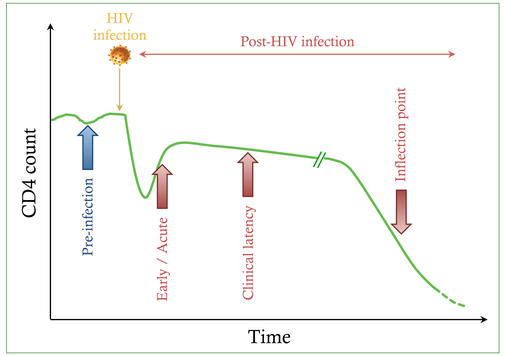


Figure 1: 80 PBMC samples from 19 untreated DC Gay individuals at pre-infection, early post-infection (<12 months), post-infection during clinical latency and post-infection at or near the inflection point.

Quality control and normalization. Using the minfi R package, probes with a detection P-value >0.01, probes failing in >5% samples, SNP probes, non-CpG probes, and sexual chromosomes probes were excluded from analysis. The color balance and background adjustments, and normalization were performed with the lumi R package. Finally, the β and M values were computed and the cell composition was estimated with the minfi R package.

Statistical analysis. Comparison of pre-infection *vs.* post-infection samples adjusting for batch effect, age, stage of infection, progression profile and cell composition using the limma R package. All DNA methylation probes exhibiting a FDR <0.05 were considered significant.

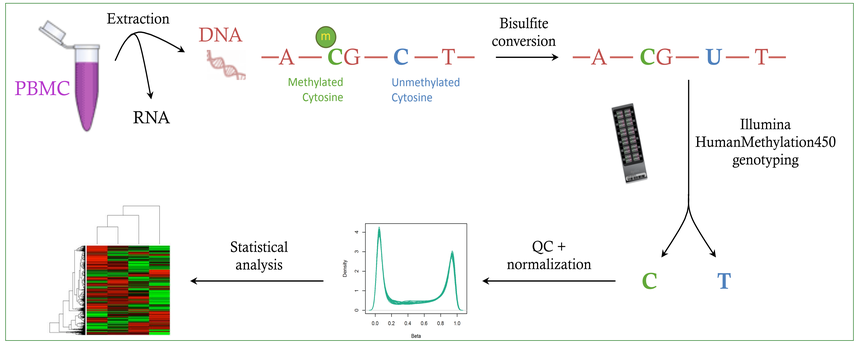


Figure 2: Experimental and analysis pipeline. Following DNA and RNA extraction, bisulfite-converted DNA was genotyped with the Illumina Infinium HumanMethylation450 arrays, covering over 485,000 methylation sites across the genome. After quality control (QC) and normalization, we compared the DNA methylation profiles of pre-infection *vs.* post-infection samples.

RESULTS

Probe ID	Chr.	Coordinates (v.37)	P-value	Gene	Function
cg26312951	21	42,797,847	2.40E-08	MX1	IFN-induced protein involved in cellular antiviral response by antagonizing RNA and DNA viral replication. Expression regulated HIV-1 Tat, Vpr and Vpu proteins.
cg26305729	5	118,608,672	3.79E-08	TNFAIP8	TNFα-induced protein associated with several cancers (including ovarian, endometrical, cervical and lung).
cg10540721	1	200,358,418	4.57E-08	-	-
cg08122652	3	122,281,939	5.89E-08	PARP9	IFNγ-regulated polymerase. Associated with B-cell lymphoma and early HIV-1 replication stages. Interacts with HIV-1 Tat protein.
cg05696877	1	79,088,769	6.65E-08	IFI44L	IFN-induced protein associated with psychiatric disorder and response to HBV vaccine. Over-expressed in HIV-1 gp120-treated vaginal epithelial cells.

Table 1: Top 5 of CpG methylation sites differentially methylated before and after HIV-infection (FDR<0.01).

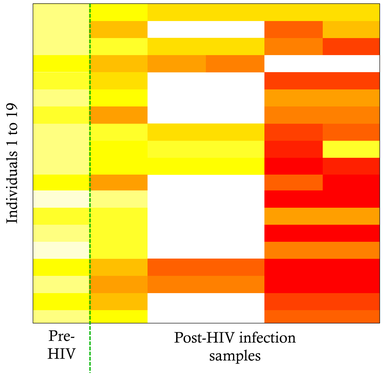


Figure 3: Methylation profile for *MX1* cg26312951 probe.

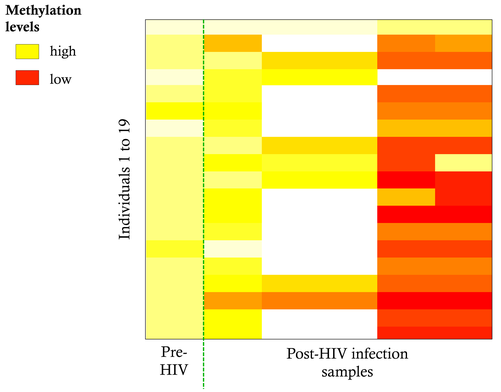


Figure 4: Methylation profile for *PARP9* cg08122652 probe.

CONCLUSIONS

- **Unique sample collection** to study DNA methylation changes within an individual and between individuals following HIV-1 infection and during the HIV-1 time course.
- This **first EWAS** identified immune-related and HIV-associated targets of epigenetic modifications by HIV-1.
- Exploring host epigenetic mechanisms opens a **new promising avenue** for discovery of critical host factors interacting with HIV-1.

Limitations: heterogeneous cell population (PBMC); sample size; preliminary analysis.

Perspectives: improve analysis pipeline; replication and validation of targets; exploration of DNA methylation changes associated with disease progression, and differentiating slow and fast progressors.