NFκB Binding Motif Genotypes in Proviral Promoters Across HIV-1 Subtypes

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Background

- NFκB binds to the HIV promoter and is one of the major classes of human transcription factors that activates HIV transcription.
- HIV also has a high mutation rate, which can result in the gain/loss of binding sites.

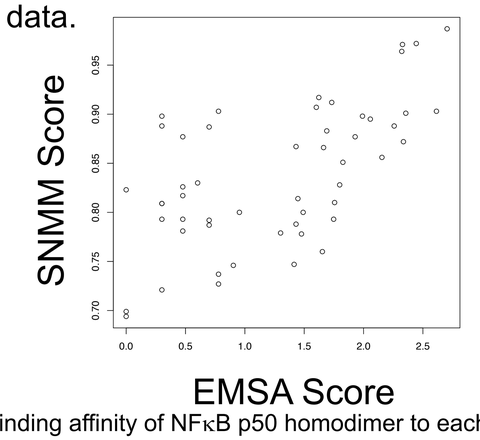
Hypothesis

- We hypothesize that five distinct HIV-1 strains: subtype A1, B, C and D and AE, in genome-intact versus defect proviruses within-hosts, differ in NFκB binding site genotypes, which could imply different likelihoods of viral transcriptional activation.

Methods

- Viral promoter sequences were retrieved from the Los Alamos HIV Sequence database (A1 n=25, B n=247, C n=82, D n=15, AE n=130)
- We also sequenced proviruses from chronically HIV-infected >1-year virologically-suppressed donors (subtype B 615 genomes 15 donors, D 113 genomes 9 donors, AE 89 genomes 2 donors)
- We developed an in-house software NFcount to screen for NFκB motifs that include canonical H, C, and F sites (GGGACTTTCC, GGGGCGTTCC, GGGACTTTCT) and non-canonical/putative motifs (Figure 1).

Figure 1. In additional to the canonical motifs, non-canonical motifs were predicted via a single nucleotide mutation matrix (SNMM) model (Du 2014). Each putative motif was assigned a SNMM score, which positively correlated with electrophoresis mobility shift assay (EMSA) functional data.



Results

Figure 2. HIV-1 subtypes differed in types and counts of canonical NFκB binding motifs (H, C, F).

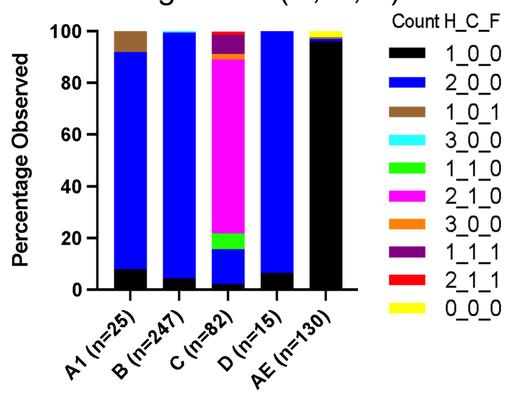


Figure 3. Each HIV-infected donor has a specific profile of canonical NFκB binding motifs H, C, F. (A) All HIV-1 DNA, inclusive of defective genomes. (B) Intact genomes only.

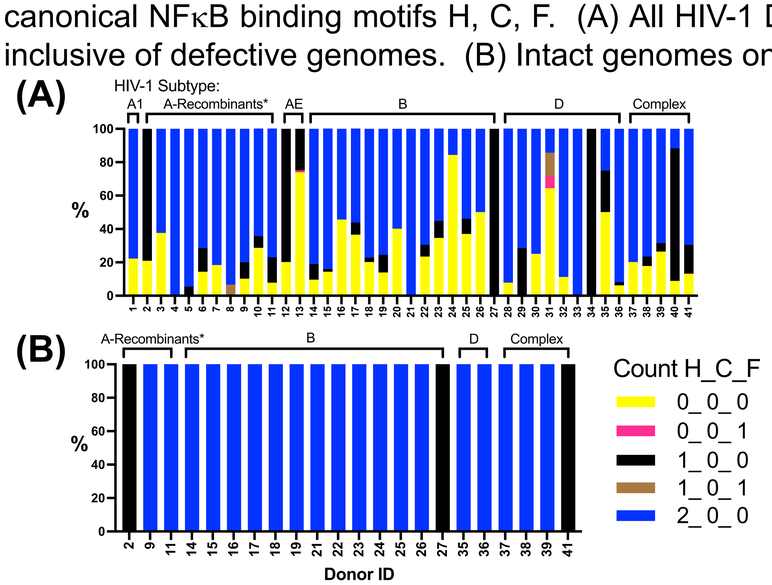


Figure 4. (A) Subtype A1, C, and AE had significantly higher raw counts of canonical and putative NFκB binding motifs relative to subtype B and D, while (B) subtype B had the highest median per genome weighted-SNMM score.

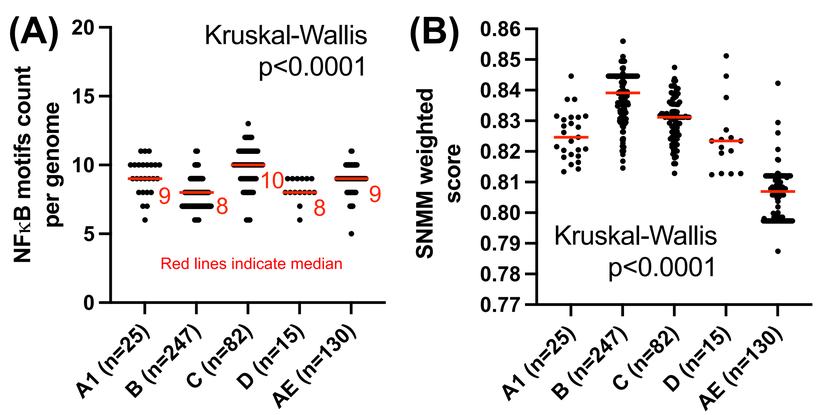


Figure 5. Within each infected individual, NFκB motif profiles were not different between intact versus defective viral genomes except for hypermutated genomes. APOBEC-3G/F associated hypermutation did not eliminate but resulted in 33-50% reduction of median site counts. Results from a representative individual is shown below.

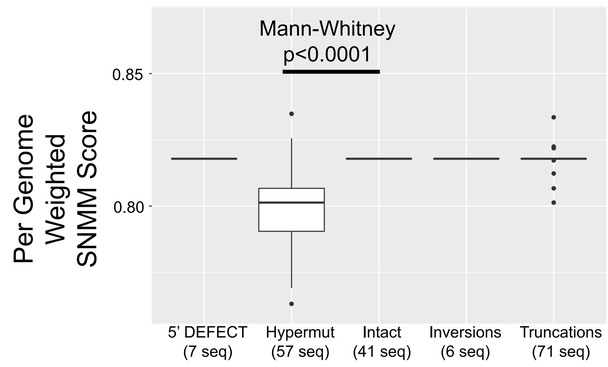
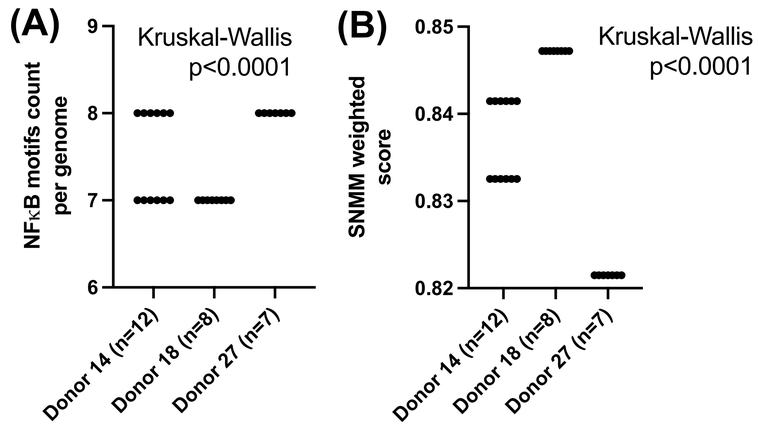


Figure 6. Even within a given viral subtype, (A) motifs count and (B) weighted-SNMM scores were significantly different across donors. Only subtype B genome-intact proviruses are shown below.



Conclusions

- NFκB motif counts significantly differed across HIV-1 subtypes and across infected individuals within a subtype.
- Along with other factors such as chromosome accessibility, NFκB motif counts and genotypes can contribute to varying likelihoods of viral transcriptional activation, which may impact time-to-virologic-rebound in the absence of treatment and should be explored in future studies.

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