

## Sequence Note

# Characterization of the South African HIV Type 1 Subtype C Complete 5' Long Terminal Repeat, *nef*, and Regulatory Genes

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### ABSTRACT

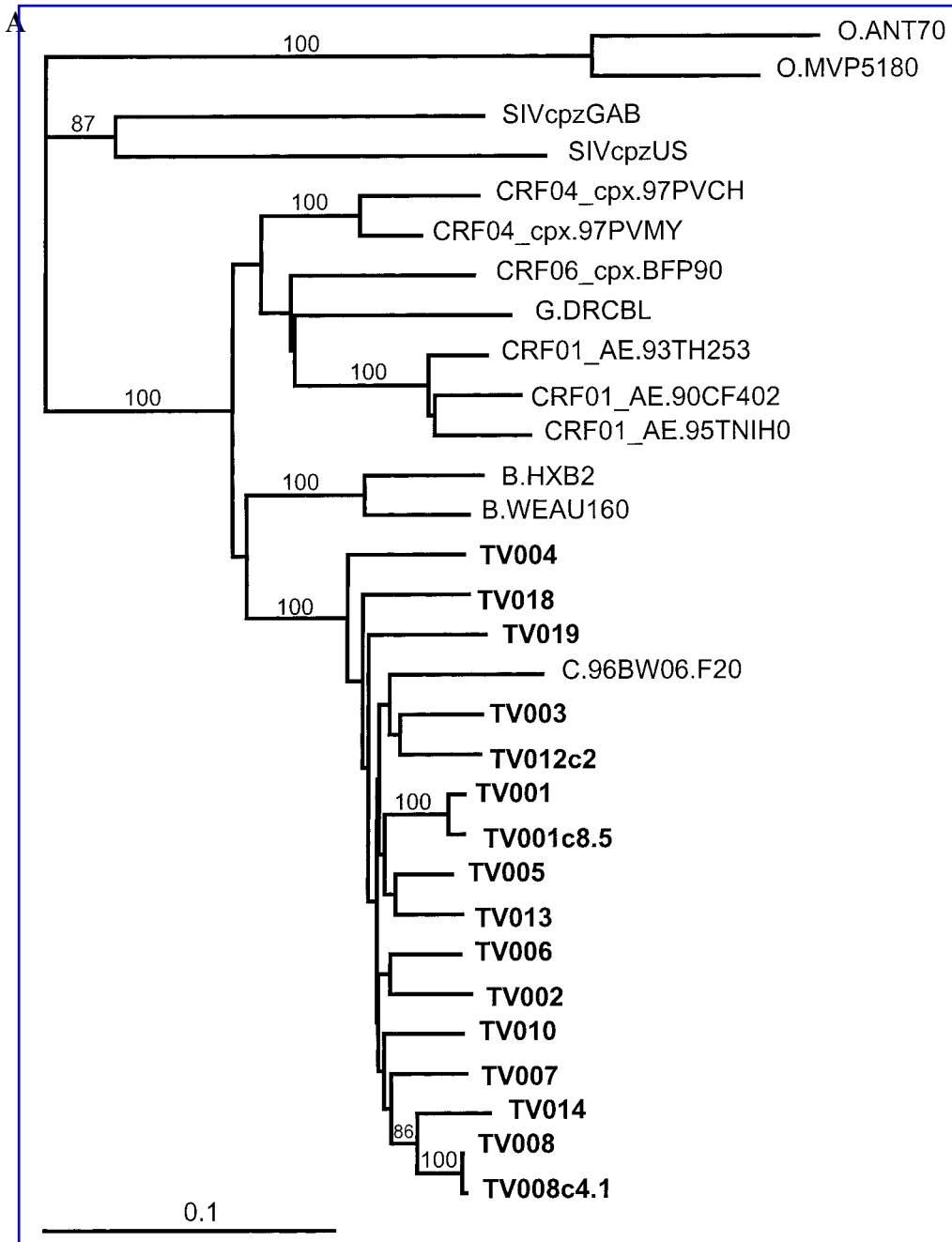
**Human immunodeficiency virus type 1 (HIV-1) subtype C has become the major etiological agent in the global and especially African epidemic. To gain better understanding of the genetic diversity and rapid transmission of HIV-1 subtype C, we have characterized the complete 5' long terminal repeat (LTR) region along with the regulatory genes *tat* and *rev* as well as the accessory gene *nef* of 14 South African HIV-1 subtype C isolates. Phylogenetic analysis revealed a subtype C 5' LTR cluster, as well as subclustering of our *nef* sequences with various subtype C strains separate from the India and China subclusters. At least 3 NF- $\kappa$ B sites were present in the 5' LTR of most isolates and 13 isolates had the subtype C-specific Rev truncation. Some length variation in exon 2 and the absence of a critical cysteine were found in Tat. Residue variation in the myristoylation signal and motifs involved in CD4 and MHC-I downregulation was recorded in our *nef* gene sequences.**

**T**HE HUMAN IMMUNODEFICIENCY VIRUS type 1 (HIV-1) subtype C epidemic is responsible for most HIV-1 infections worldwide and close to half of the HIV-1 infections in sub-Saharan Africa.<sup>1</sup> This epidemic has attained a rapid global distribution and, whereas transmission of other subtypes such as B seems to be on the decline, HIV-1 subtype C is spreading at alarming rates.<sup>1,2</sup> To date, the majority of genetic characterization studies of South African HIV-1 strains have been focused on the *env* and the *gag* genes, and thus little is known about the regulatory regions and genes of local subtype C strains. Specifically, the 5' long terminal repeat (LTR) region as well as the *tat* and *rev* genes of South African subtype C strains remain to be comprehensively characterized. Thus analysis thereof may provide more data about the diversification of this efficiently transmitted subtype. In this study we sequenced and phylogenetically analyzed the *tat*, *rev*, and *nef* genes as well as the 5' LTR of 14 previously described HIV-1 subtype C isolates from South Africa.<sup>3,4</sup>

The three genomic regions containing *tat* and *rev* exons 1 and 2 as well as *nef* were amplified in a nested polymerase chain reaction (PCR) from a 9-kb, nearly full-length fragment as described previously.<sup>3,4</sup> The 5' LTR was amplified by single-step PCR from DNA prepared from primary isolates. Primer pairs NefF (5'-CCTAGAAGAATAAGACAGGGCTT-3') and NefR (5'-CCTGGAACGCCCCAGTGG-3'); NewS1\_1T/NewS1\_1C (5'-GCAGGACGTCAAGCTTGGAAAGGGTTA-ATTTACTC[T/C]AAGAA-3') and S1\_651Not (5'-CCT-TGGCCAGCGCCGCCCTG TTCGGGCGCCACTGCTA-GAGA-3'); TatX1F (5'-AATTGGGTGCCAGCATAGC-3') and NACD (5'-CCATAATAGACTGTGA-CCCACAA-3'); and RevX2F (5'-TGCTGTGCTCTCTATAGTRA-3') and RevX2R (5'-TCCTATCTGTTCCCTCAGCTA-3') were used for the amplification of the ~660-bp fragment containing the *nef* gene, the ~640-bp 5'-LTR region, the ~570-bp fragment containing the *tat/rev* exon 1, and the ~350-bp fragment containing the *tat/rev* exon 2, respectively. 5'-LTR amplicons of

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**FIG. 1.** Rooted trees depicting the evolutionary relationships among the 5' LTR region (**A**) as well as the *nef* (**B**) nucleotide sequences from South African HIV-1 subtype C isolates. The Kimura two-parameter distance matrix and neighbor-joining method were used in the phylogeny calculations. The distance scale indicates 0.1 nucleotide replacements per site. Horizontal branch lengths are drawn to scale and the validity of the branching orders was estimated with 500 bootstrap replicates. The significant (above 85%) bootstrap values are indicated at the respective branch bases.

three isolates (TV001, TV004, and TV012) were cloned into the pGEM-11Zf(+) vector (Promega, Madison, WI) according to the manufacturer protocol and can be identified by their clone numbers. Sequencing reactions were carried out by using the ABI Prism BigDye terminator cycle sequencing protocol and sequencing was performed on a 310 genetic analyzer (PE Applied Biosystems, Foster City, CA). The PCR primers were used as direct sequencing primers with the addition of the LTRFor (5'-ACAAAGACTGCTGACACA-3') and LTRRev (5'-TGT-

GTCAGCAGTCTTTGT-3') primers for the 5' LTR region. Sequences were verified manually and sequence assembly, multiple alignments, and translation into predicted amino acid sequences were performed as described.<sup>3,4</sup> Calculation of distance matrices, phylogeny inference, and construction of trees were performed with the PHYLIP software packages SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE. Rooting and manipulation of the trees were done in TREEVIEW.

The phylogenetic tree constructed with all full-length 5' LTR

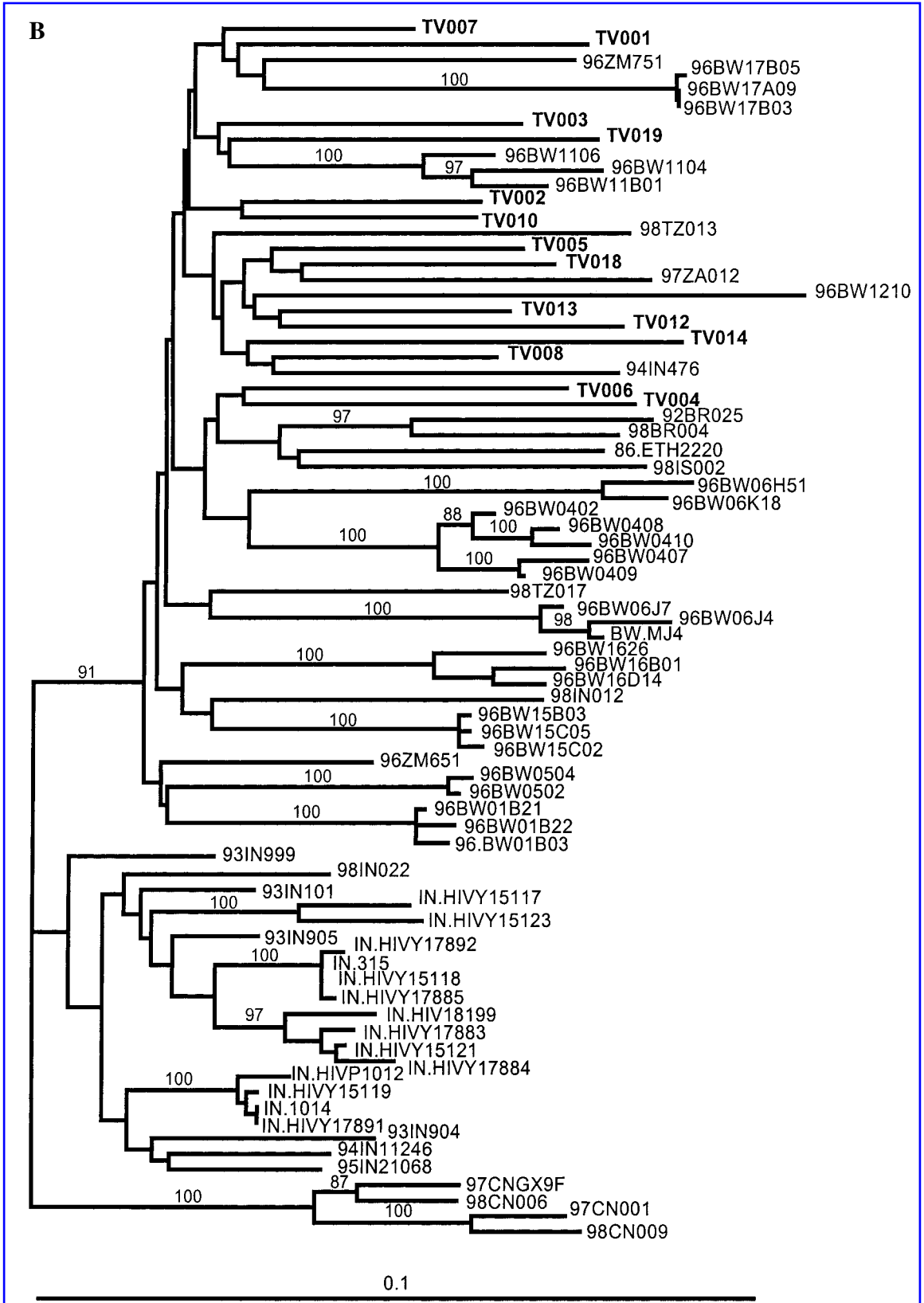


FIG. 1. Continued.

sequences from the Los Alamos database (Fig. 1A) shows a clustering of our isolates with the only available subtype C full-length 5' LTR from Botswana (isolate 96BW06.F20). The tree constructed with all available subtype C *nef* coding sequences (approximately 625 bp in length) (Fig. 1B) shows the South African isolates including the recently described 97ZA012.1 (AF286227) from Durban,<sup>5</sup> subcluster with isolates from Botswana, Ethiopia, Brazil, Tanzania, and Zambia as well as one Israeli and two Indian isolates. This monophyletic subtype C clade is distinctly separate from the Chinese and Indian clades, as supported by statistically significant bootstrap values. These data thus display a lesser degree of diversity for the South African strains than was reported for the *gag* and *env* genes<sup>4</sup> as well as the genomic fragment encompassing the *vif*, *vpr*, and *vpu* genes.<sup>3</sup> In contrast with the *nef* sequence results, these previous studies showed a diversity within the South African isolates that virtually matches the diversity of the global C subtypes studied to date as a whole. However, this finding is expected because *nef* is a smaller, highly conserved coding region. Interestingly, two Indian sequences fall among the main monophyletic clade. Considering the distinct subclustering of the Indian isolates, which indicates that they share a common ancestor, this suggests that these two isolates may have originated in sub-Saharan Africa and migrated to or been transmitted in India through the frequent movement of people.

Nucleotide alignments of the full-length 5' LTR sequences confirm the presence of all potential transcription factor-binding sites, with the third NF- $\kappa$ B site reported for HIV-1 subtype C sequences (Fig. 2<sup>5a</sup>).<sup>6-8</sup> All three sites in most of our isolates conform to the reported NF- $\kappa$ B sequence,<sup>9</sup> while NF- $\kappa$ B-like sites have been identified in TV008 [GGGGCGG**T**CC], TV012c2 [GG**A**ACTTTCC], and TV019 [GGGACTT**T**CT]. The C10T substitution seen in TV019 has previously been identified in another South African isolate, 98ZA445ZA.<sup>8</sup> In their previous report, Rodenburg *et al.*<sup>5</sup> described frequent deletions or mutations in the third NF- $\kappa$ B site, questioning the causal role of the third site in the explosive subtype C epidemic. In contrast, 12 of our isolates have 3 NF- $\kappa$ B sites and only TV008 and TV012 have two sites, with a third NF- $\kappa$ B-like site. Our data thus indicate that in the majority of South African isolates analyzed in this study, the presence of the three NF- $\kappa$ B sites may well confer a stronger promoter activity resulting in more rapid transmission. The Sp1-I, Sp1-II, and Sp1-III sites in the core promoter are conserved, with Sp1-III being the most variable and Sp1-II the most conserved. This contrasts with a report stating that Sp1-I is the more conserved element.<sup>10</sup> The guanine residue at position 4 of the Sp1 sites, imperative for maximizing Sp factor recruitment, is conserved for all our sequences.<sup>10</sup> A fourth Sp1 site (positions -433 to -441) discovered to be essential for negative-sense transcription in the HXB2 sequence (5'-TGGGAGTGA-3') has up to three substitutions in our sequences.<sup>11</sup> The NF-AT sites show little divergence among our isolates, but differ substantially from the HXB2 sequence. A reported subtype C consensus in the core region of the NRE (CGCARACAYVTK) was almost perfectly conserved in all our sequences.<sup>12</sup> Johansson *et al.*<sup>13</sup> reported that the G/A CAGA motif within the above-mentioned sequence is diagnostic for subtype C strains. All but three of our isolates possess this motif: TV003 displays a G4C, TV010 a C2T, and TV019 a G4A substitution. The alanines at positions 5 and 9,

as well as the guanine at position 10, of the TCF-1 $\alpha$  site are conserved across our isolates and the reference strains, with the exception of subtype B and group O. TV019 has a C-to-T substitution in the bulge of the TAR region, which has been identified in subtype G, circulating recombinant forms, as well as SIV strains. The T-to-C nucleotide change at position 2 of the loop structure in the TAR element occurs in eight of our isolates.

The deduced Nef amino acid sequences (Fig. 3A) show previously described length variations, especially in the reported N-terminal variable region. Thirteen South African isolates have seemingly intact Nef open reading frames. The premature stop in the N-terminal of the protein sequence of isolate TV006 suggests that this isolate would start translation at the second initiation site.<sup>14,15</sup> This protein would lack the myristoylation signal and would thus, because of unsuccessful myristoylation, most probably not fold correctly or be directed to the membrane.<sup>16</sup> All isolates with the exception of TV005, TV006, TV012, and TV013 as well as reference strain 96BW12-10 from Botswana have conserved GGXXS motifs in their myristoylation signals. It has been described that GSXXS mutations are possibly associated with slow disease progression<sup>17</sup> and that deletion or substitution of the glycine residues, to which the myristic acid is linked, prohibits myristoylation.<sup>18</sup> Other studies and sequences in the Los Alamos sequence database suggest sequence diversity is not uncommon among myristoylation signals.<sup>19</sup> Residue and length variations observed in our Nef proteins correlate with those in the other subtype C strains, suggesting relatedness. Most of the significant domains such as those involved in CD4 and MHC-I downregulation as well as altering of cell signaling are found to be well conserved in all sequences and variations correlate with results obtained in previous studies.<sup>17,19-22</sup> Furthermore, considering the presence of intact WL<sub>57</sub> motifs, which allow for proteolytic cleavage, post-translational modification of Nef seems to be intact. None of the gene deletions associated with nonprogression or slow progression of HIV-1 disease have been found to be present in our isolates and, in fact, the disease progression of the 14 patients is not in any way abnormal.<sup>3</sup> However, some of the amino acid variations reported to be more frequent in progressors as opposed to nonprogressors<sup>20</sup> were present in our sequences as well as some subtype C reference sequences. Specifically, the A15, C163, and Q170 variations were found in most subtype C sequences. In addition, the N51 amino acid variation found to be more frequent in nonprogressors was predominant in subtype C.<sup>20</sup>

Whereas most South African isolates have Tat amino acid sequences of 101 residues in length, some length variation was found in 5 isolates (Fig. 3B). TV001 lacks the orthodox stop at position 102 and is terminated at the next position, whereas TV004, TV010, TV012, and TV018 terminate prematurely at position 100. Yet, all 14 isolates show intact open reading frames and, interestingly, this two-residue truncation in Tat has been identified in HIV-1 subtypes D, F, H, J, and K. Notably, the basic domain GRKKRRQRR (residues 48-56) as well as the highly conserved ESKKKVE motif are 100% conserved among all subtype C sequences except in TV012, which contains an EPKKKVE motif. The critical cysteine residues at positions 22, 25, 27, 30, 34, and 37 of all sequences are found to be 100% conserved. Yet at position 31, all isolates except

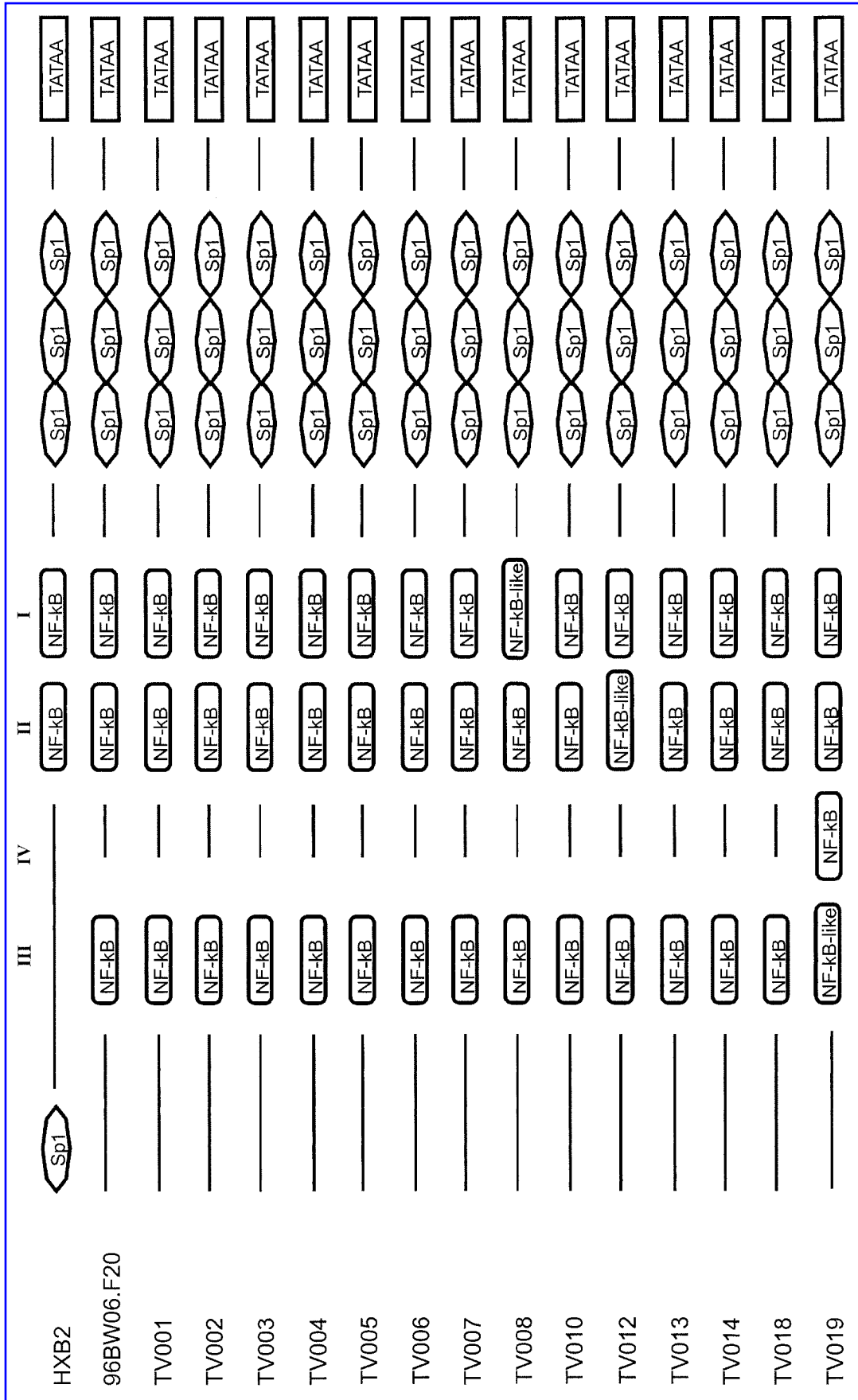


FIG. 2. Graphical representation of the nucleotide sequence alignment of the 5' LTR from South African subtype C isolates along with the available subtype C strain from Botswana and HXB2. Regulatory elements are represented as blocks for clarity. For a detailed review of these elements refer to Pereira *et al.*<sup>5a</sup>



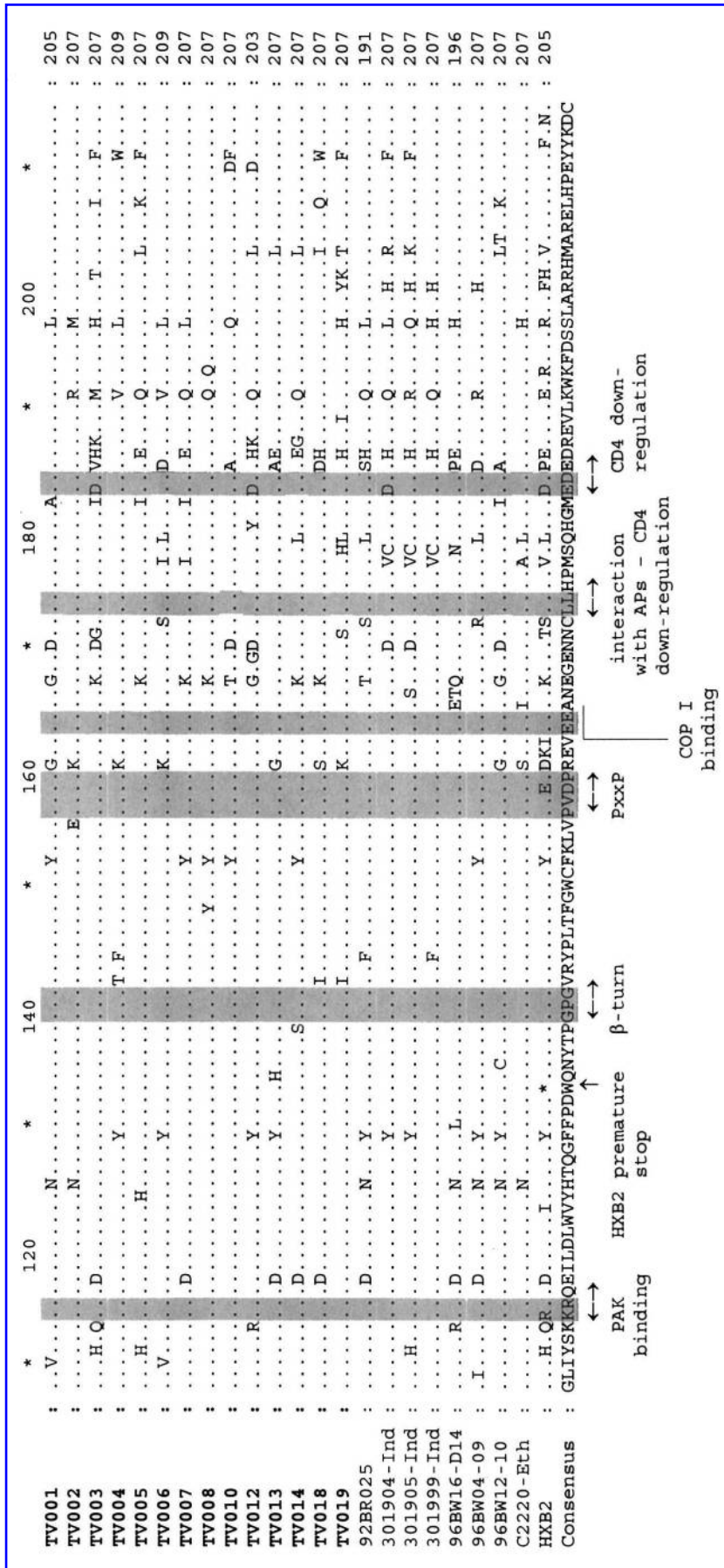


FIG. 3. Continued.



C

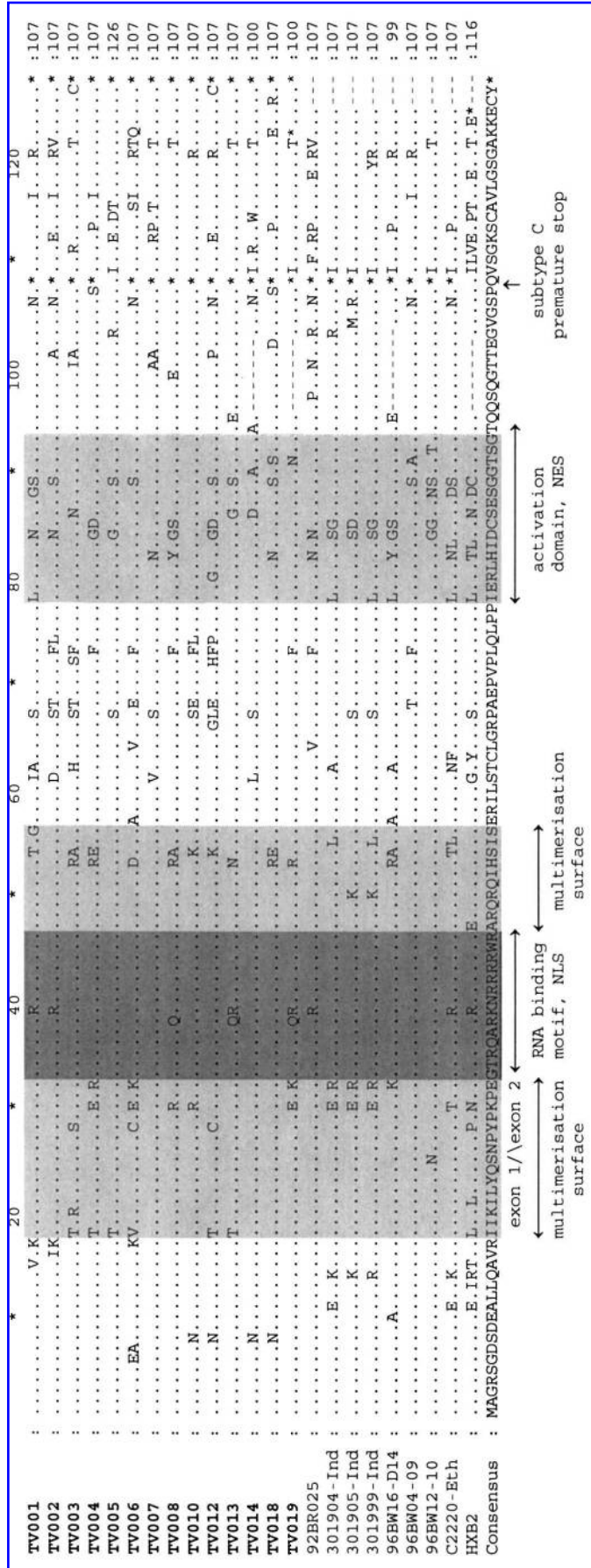


FIG. 3. Continued.

TV004, TV010, and TV014 have a putative serine residue instead of the prevalently conserved cysteine. Similarly to Tat, Rev shows well-conserved amino acid sequences with some variation in the C-terminal region and intact reading frames in all isolates (Fig. 3C). The truncation in exon 2 of Rev at position 108, which seems to be exclusive to HIV-1 subtype C strains, is found in our isolates. Notably, isolate TV005 does not have this subtype C-specific Rev truncation and thus has an Rev protein 126 amino acids in length. Isolates TV014 and TV019 have a seven-residue deletion directly following the nuclear export signal (NES), which is also present in one subtype C reference strain. In correlation with Tat and Nef, residue variations within both exons of Rev in our isolates are similar to those seen in the other subtype C strains.

As reported in the study of the *env* genes of these isolates,<sup>4</sup> no significant or prominent sequence variations in the Rev responsive element that may have implications on interaction with the Rev protein have been detected.

The rapid transmission of HIV-1, and in particular HIV-1 subtype C, has been the topic of extensive research to fathom the mechanisms involved. Yet, limited data concerning the subtype C epidemic in southern Africa make this a daunting task. Reports have suggested that the more rapid transmission of subtype C in relation to the other subtypes could be due to higher levels of LTR promoter activity resulting in upregulation of viral transcription.<sup>6,23</sup> This could be attributed to either the extra NF- $\kappa$ B-binding site and/or the distinctly different NRE sequence in the LTR of subtype C strains. The upregulation of transcription should thus be apparent in the isolates that show a conserved subtype C NRE region, especially TV019, which has three NF- $\kappa$ B sites as well as an additional NF- $\kappa$ B-like site. Gene expression should be efficient in all our isolates, as a result of the TATA box being well conserved. Moreover, stronger C-strain transcription may possibly be the combined effect of these unique features in the LTR along with the differences found in the cysteine-rich domain of subtype C Tat. Being the *trans*-activator of HIV-1 gene expression, mutations in the functional domains of Tat may well have significant effects on interaction with the promoter regions in the LTR. However, in their study, Jeang *et al.*<sup>24</sup> found that although a C31E substitution results in decreased *trans*-activation activity of Tat, a C31S substitution has no significant effect. In addition to the missing critical cysteines, the two-residue truncation in exon 2 of Tat in some isolates could possibly also contribute to variable interaction with the LTR. If the truncation of Rev confers enhancing effects on the efficiency of Rev-dependent transcript export and thus expression of late viral mRNAs and their proteins, this may point to a coevolution of the subtype C LTR, Rev, and Tat to constitute a transcriptional regulation pathway that functions at higher levels. Alternatively, the rapid transmission of subtype C could be a result of nonvirological factors and further studies will have to be performed to investigate the validity of these hypotheses. The local subtype C isolate that did not contain the signature truncation in Rev at position 108 (TV005) was the only isolate that was able to utilize the coreceptors CXCR4, CCR2b, CCR3, and CCR4 in addition to CCR5 in HOS-CD4 coreceptor transfectants. However, it was still nonsyncytium inducing in MT-2 cells and no changes in charge associated with the syncytium-inducing phenotype were present in the V3 loop area of the envelope protein.<sup>25</sup>

When taken alongside the characterization of the *env*, *gag*, and accessory gene sequences, these data show that the 14 isolates from Tygerberg Hospital are nonrecombinant subtype C isolates representative of the South African HIV-1 subtype C epidemic.

## ACKNOWLEDGMENTS

This work was supported by grants from the South African AIDS Vaccine Initiative (SAAVI) and the Poliomyelitis Research Foundation.

## SEQUENCE DATA

GenBank accession numbers are as follows: *rev* exons 1 and 2, AY047230–AY047257; *tat* exons 1 and 2, AY047258–AY047285; *nef*, AY047286–AY047299; LTRs, AY047300–AY047315.

## REFERENCES

1. UNAIDS/WHO: *Report on the Global HIV/AIDS Epidemic, December 2000*. World Health Organization, Geneva, Switzerland, 2000 (<http://www.unaids.org/epidemic-update/report/index.html>).
2. McCutchan FE, Salminen MO, Carr JK, and Burke DS: HIV-1 genetic diversity. *AIDS* 1996;10(Suppl. 3):S13–S20.
3. Scriba TJ, Treurnicht FK, Zeier M, Engelbrecht S, and Janse van Rensburg E: Characterization and phylogenetic analysis of South African HIV-1 subtype C accessory genes. *AIDS Res Hum Retroviruses* 2001;17:775–781.
4. Engelbrecht S, De Villiers T, Sampson CC, zur Megede J, Barnett S, and Janse van Rensburg E: Genetic analysis of the complete *gag* and *env* genes of HIV-1 subtype C primary isolates from South Africa. *AIDS Res Hum Retroviruses* 2001;17:1533–1547.
5. Rodenburg CM, Li Y, Trask SA, *et al.*: Near full-length clones and reference sequences for subtype C isolates of HIV type 1 from three different continents. *AIDS Res Hum Retroviruses* 2001;17:161–168.
- 5a. Pereira LA, Bentley K, Peeters A, Churchill MJ, and Deacon NJ: A compilation of cellular transcription factor interactions with the HIV-1 LTR promoter. *Nucleic Acids Res* 2000;28:663–668.
6. Naghavi MH, Salminen MO, Sönnnerborg A, and Vahlne A: DNA sequence of the long terminal repeat of human immunodeficiency virus type 1 subtype A through G. *AIDS Res Hum Retroviruses* 1999;15:485–488.
7. Salminen MO, Johansson BO, Sönnnerborg A, *et al.*: Full-length sequence of an Ethiopian human immunodeficiency virus type 1 (HIV-1) isolate of genetic subtype C. *AIDS Res Hum Retroviruses* 1996;12:1329–1339.
8. Hunt G and Tiemessen CT: Occurrence of additional NF- $\kappa$ B-binding motifs in the long terminal repeat region of South African HIV type 1 subtype C isolates. *AIDS Res Hum Retroviruses* 2000;16:305–306.
9. Lenardo MJ and Baltimore D: NF- $\kappa$ B: A pleiotropic mediator of inducible and tissue-specific gene control. *Cell* 1989;58:227–229.
10. McAllister JJ, Phillips D, Millhouse S, *et al.*: Analysis of the HIV-1 LTR NF- $\kappa$ B-proximal Sp site III: Evidence for cell type-specific gene regulation and viral replication. *Virology* 2000;274:262–277.
11. Peeters A, Lambert PF, and Deacon NJ: A fourth Sp1 site in the human immunodeficiency virus type 1 long terminal repeat is essential for negative-sense transcription. *J Virol* 1996;70:6665–6672.

12. Naghavi MH, Schwartz S, Sönnnerborg A, and Vahlne A: Long terminal repeat promoter/enhancer activity of different subtypes of HIV type 1. *AIDS Res Hum Retroviruses* 1999;14:1293-1303.
13. Johansson BO, Sherefa K, and Sönnnerborg A: Multiple enhancer motifs in HIV type 1 strains from Ethiopia. *AIDS Res Hum Retroviruses* 1995;11:761-764.
14. Ratner L and Niederman TMJ: Nef. *Curr Topics Immunol Microbiol* 1995;193:169-201.
15. Allan JS, Coligan JE, Lee T, McLane MF, Kanki PJ, Groopman JE, and Essex M: A new HTLV-III/LAV encoded antigen detected by antibodies from AIDS patients. *Science* 1985;230:810-813.
16. Piguet V and Trono D: A structure-function analysis of the Nef protein of primate lentiviruses. In: *Human Retroviruses and AIDS 1999*. Los Alamos National Laboratory, Los Alamos, New Mexico, 1999.
17. Huang Y, Zhang L, and Ho DD: Biological characterization of *nef* in long-term survivors of human immunodeficiency virus type 1 infection. *J Virol* 1995;69:8142-8146.
18. Gordon JI, Duronio RJ, Rudnick DA, Adams SP, and Gokel GW: Protein N-myristoylation. *J Biol Chem* 1991;266:8647-8650.
19. Shugars DC, Smith MS, Glueck DH, Nantermet PV, Seillier-Moisewitsch F, and Swanstrom R: Analysis of human immunodeficiency virus type 1 *nef* gene sequences present in vivo. *J Virol* 1993;67:4639-4650.
20. Kirchhoff F, Easterbrook PJ, Douglas N, Troop M, Greenough TC, Weber J, Carl S, Sullivan JL, and Daniels RS: Sequence variations in human immunodeficiency virus type 1 *nef* are associated with different stages of disease. *J Virol* 1999;73:5497-5508.
21. Mangasarian A, Piguet V, Wang J, Chen Y, and Trono D: Nef-induced CD4 and major histocompatibility complex class I (MHC-I) down-regulation are governed by distinct determinants: N-terminal alpha helix and proline repeat of Nef selectively regulate MHC-I trafficking. *J Virol* 1999;73:1964-1973.
22. Rhodes D, Solomon A, Bolton W, Wood J, Sullivan J, Learmont J, and Deacon N: Identification of a new recipient in the Sydney Blood Bank cohort: A long-term HIV type 1-infected seroindeterminate individual. *AIDS Res Hum Retroviruses* 1999;15:1433-1439.
23. Tong-Starksen SE, Wesch TM, and Peterlin BM: Differences in transcriptional enhancers of HIV-1 and HIV-2 response to T-cell activation signals. *J Immunol* 1990;145:4348-4354.
24. Jeang K, Xiao H, and Rich EA: Multifaceted activities of the HIV-1 transactivator of transcription, Tat. *J Biol Chem* 1999;274:28837-28840.
25. Truernicht FK, Smith T-L, Engelbrecht S, et al. Genotypic and phenotypic analysis of the *env* gene from South Africa: HIV-1 subtype B and C isolates. *J Med Virol* (in press).

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1. Luna Li, Satinder Dahiya, Sandhya Kortagere, Benjamas Aiamkitsumrit, David Cunningham, Vanessa Pirrone, Michael R. Nonnemacher, Brian Wigdahl. 2012. Impact of Tat Genetic Variation on HIV-1 Disease. *Advances in Virology* **2012**, 1-28. [[CrossRef](#)]
2. Yujie Liu, Michael R. Nonnemacher, Devin L. Stauff, Luna Li, Anupam Banerjee, Bryan Irish, Evelyn Kilareski, Nirmala Rajagopalan, Joyce B. Suchitra, Zafar K. Khan. 2010. Structural and functional studies of CCAAT/enhancer binding sites within the human immunodeficiency virus type 1 subtype C LTR. *Biomedicine & Pharmacotherapy* **64**:10, 672-680. [[CrossRef](#)]
3. Pascal Obong Bessong. 2008. Polymorphisms in HIV-1 subtype C proteases and the potential impact on protease inhibitors. *Tropical Medicine & International Health* **13**:2, 144-151. [[CrossRef](#)]
4. S SETIYANINGSIH, M DESPORT, M STEWART, N HARTANINGSIH, G WILCOX. 2007. Sequence analysis of mRNA transcripts encoding Jembrana disease virus Tat-1 in vivo#. *Virus Research* . [[CrossRef](#)]
5. Manoj Kumar, Swatantra Kumar Jain, Syed Tazeen Pasha, Debasish Chattopadhyaya, Shiv Lal, Arvind Rai. 2006. Genomic Diversity in the Regulatory nef Gene Sequences in Indian Isolates of HIV Type 1: Emergence of a Distinct Subclade and Predicted Implications. *AIDS Research and Human Retroviruses* **22**:12, 1206-1219. [[Abstract](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]
6. Thomas J. Scriba, Jan zur Megede, Richard H. Glashoff, Florette K. Treurnicht, Susan W. Barnett, Estrelita Janse van Rensburg. 2005. Functionally-inactive and immunogenic Tat, Rev and Nef DNA vaccines derived from sub-Saharan subtype C human immunodeficiency virus type 1 consensus sequences. *Vaccine* **23**:9, 1158-1169. [[CrossRef](#)]
7. A Jere. 2004. Genetic analysis of Indian HIV-1 nef: subtyping, variability and implications. *Microbes and Infection* **6**:3, 279-289. [[CrossRef](#)]
8. Michel P. de Baar, Almaz Abebe, Aletta Kliphuis, Girma Tesfaye, Jaap Goudsmit, Georgios Pollakis. 2003. HIV Type 1 C and C' Subclusters Based on Long Terminal Repeat Sequences in the Ethiopian HIV Type 1 Subtype C Epidemic. *AIDS Research and Human Retroviruses* **19**:10, 917-922. [[Abstract](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]
9. Estrelita Janse van Rensburg, Tracey-Lee Smith, Michelle Zeier, Brenda Robson, Candice Sampson, Florette Treurnicht, Susan Engelbrecht. 2002. *Aids* **16**:18, 2479-2480. [[CrossRef](#)]
10. Jan zur Megede, Susan Engelbrecht, Tulio de Oliveira, Sharon Cassol, Thomas J. Scriba, Estrelita Janse van Rensburg, Susan W. Barnett. 2002. Novel Evolutionary Analyses of Full-Length HIV Type 1 Subtype C Molecular Clones from Cape Town, South Africa. *AIDS Research and Human Retroviruses* **18**:17, 1327-1332. [[Abstract](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]