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HIV subtype is not associated with dementia among individuals with moderate and advanced immunosuppression in Kampala, Uganda

Ned Sacktor[1], **Noeline Nakasujja**[4], **Andrew D. Redd**[6], **Jordyn Manucci**[3,6], **Oliver Laeyendecker**[3,6], **Sarah K. Wendel**[6], **Stephen F Porcella**[7], **Craig Martens**[7], **Daniel Bruno**[7], **Richard L. Skolasky**[2], **Ozioma C. Okonkwo**[8], **Kevin Robertson**[9], **Seggane Musisi**[4], **Elly Katabira**[5], and **Thomas C. Quinn**[3,6]

[1]Departments of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD [2]Orthopedic Surgery, Johns Hopkins University School of Medicine, Baltimore, MD [3]Medicine, Johns Hopkins University School of Medicine, Baltimore, MD [4]Department of Psychiatry, Makerere University, Kampala, Uganda [5]Medicine, Makerere University, Kampala, Uganda [6]Laboratory of Immunoregulation, Division of Intramural Research, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD [7]Genomics Unit, Research Technologies Branch, Rocky Mountain Laboratories, Division of Intramural Research, NIAID, NIH, Hamilton MT [8]Department of Medicine, University of Wisconsin, Madison, WI [9]Department of Neurology, University of North Carolina, Chapel Hill, NC

Abstract

Background—HIV-associated neurocognitive disorders (HAND) are a common neurological manifestation of HIV infection. A previous study suggested that HIV dementia may be more common among patients with subtype D virus than among those with subtype A virus among HIV + individuals with advanced immunosuppression. We conducted a study to evaluate the frequency of HIV dementia, and the association of HIV dementia with HIV subtype and compartmentalization among HIV+ individuals with moderate and advanced immunosuppression (CD4 lymphocyte count >150 cells/μL and < 250 cells/μL).

Methods—The study enrolled 117 antiretroviral naïve HIV+ individuals in Kampala, Uganda. HIV+ individuals received neurological, neuropsychological testing, and functional assessments, and gag and gp41 regions were subtyped. Subjects were considered infected with a specific subtype if both regions analyzed were from the same subtype.

Results—41% of the HIV+ individuals had HIV dementia (mean CD4 lymphocyte count= 233 cells/μL). 67 individuals had subtype A, 25 individuals had subtype D, 24 individuals were classified as A/D recombinants, and one individual had subtype C. There was no difference in the

Address correspondence to Dr. Ned Sacktor, Department of Neurology, Johns Hopkins Bayview Medical Center, 301 Building, Suite 2100, 4940 Eastern Ave., Baltimore, MD 21224 Tel: 410-550-1045, Fax: 410-550-0539 sacktor@jhmi.edu.

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frequency of HIV dementia when stratified by HIV subtype A and D and no association with compartmentalization between the cerebrospinal fluid and peripheral blood.

Conclusions—These results suggest that HIV dementia is common in HIV+ individuals in Uganda. There was no association between HIV subtype and dementia among HIV+ individuals with moderate and advanced immunosuppression. Future studies should be performed to confirm these results.

Keywords

HIV; dementia; subtype; Uganda; clade

Human immunodeficiency virus (HIV)-associated neurocognitive disorders (HAND) are characterized by cognitive, behavioral, and motor dysfunction and are a common neurological manifestation of advanced HIV infection. The prevalence of HIV dementia, the most severe form of HAND, is largely unknown in sub-Saharan Africa where more than 60% of the world's HIV infected population resides. One study suggests that the prevalence in Uganda may be as high as 31% in HIV infected individuals with advanced infection (Sacktor et al. 2009). If this proportion were present throughout sub-Saharan Africa, then HIV dementia would be among the top causes of dementia worldwide along with Alzheimer's disease and vascular dementia.

HIV is characterized by extensive genetic diversity. In the US, subtype B is predominant but in sub-Saharan Africa, subtypes A, C, D, and recombinants predominate (Sacktor et al. 2007). Uganda is a unique area in which both HIV subtypes A and D co-circulate (Conroy et al. 2010). Previous studies suggested that HIV subtype impacts HIV disease progression with subtype D having a faster progression to AIDS and a higher mortality rate than individuals infected with subtype A (Kiwanuka et al. 2008, Kaleebu et al. 2002, Baeten et al. 2007). A previous study suggested that HIV dementia may be more common among patients infected with subtype D than among those with A in individuals with advanced immunosuppression [mean CD4 lymphocyte count =127 cells microliters /(μL)] (Sacktor et al. 2009). However, it is unknown whether HIV subtype impacts the risk for dementia among individuals with more moderate immunosuppression (CD4 lymphocyte count > 150 cells/μL and <350 cells/μL).

Compartmentalization of viral strains between the periphery and the central nervous system (CNS) due to specific differences in the HIV envelope (env) gene has been reported and may play an important role in the development of HIV dementia (Harrington et al. 2009).

We conducted a study to evaluate the frequency of HIV dementia and the association of HIV dementia with HIV subtype among HIV+ individuals with moderate and advanced immunosuppression in Uganda. In addition, we sought to characterize the HIV subtype among HIV+ individuals with moderate and advanced immunosuppression and to evaluate whether there was evidence for compartmentalization of HIV subtype between the cerebrospinal fluid (CSF) and blood. We also examined whether the immunological and neurocognitive response to combination antiretroviral therapy (ART) differed by HIV subtype.

METHODS

Participants

The study enrolled 117 HIV+ individuals attending an Infectious Disease Clinic in Kampala, Uganda from August 2009 to October 2010. Inclusion criteria included HIV infection as documented by enzyme-linked immunosorbent assay (ELISA) and confirmed by Western blot or a detectable plasma HIV ribonucleic acid (RNA)) level, a CD4 lymphocyte count <500 cells/μL at the previous clinical visit, and history of no antiretroviral drug use. Exclusion criteria included age <18 years, an active or known past opportunistic infection of the CNS, fever (temperature > 37.5°c), and a history of a chronic neurologic disorder, active psychiatric disorder, alcoholism (defined as >30 standard drinks/week), physical deficit (*e.g.*, amputation), or severe medical illness or functional impairment (Karnofsky functional performance scale <50 that would interfere with the ability to perform the study evaluations (Karnofsky et al. 1948). The evaluations were translated into the local language, Luganda. All patients provided informed consent prior to their inclusion in the study, and the protocol was approved by the Johns Hopkins and Makerere University ethical review boards.

Clinical assessments

HIV-infected individuals received standardized questionnaires for assessment of demographic information and medical, psychiatric, and neurologic history, and underwent a complete neurologic examination (Sacktor et al. 2005, Wong et al. 2007). Patients were also evaluated for fever, headache, neck stiffness, and focal abnormalities. HIV+ patients with a suspected CNS opportunistic infection or neoplasm were excluded.

The neurocognitive assessment included a screening test, the International HIV Dementia Scale (IHDS) (Sacktor et al. 2005), the World Health Organization–University of California–Los Angeles Auditory Verbal Learning test to assess verbal memory (Maj et al. 1994), the Finger Tapping test to assess motor performance, the Symbol Digit modalities test (Smith 1982) and Color Trails test (Maj et al. 1994) to assess executive function, the Digit Span Forward and Backward to assess attention, the Grooved Pegboard test to assess psychomotor speed performance, and the Category Naming test to assess verbal fluency. The functional assessments included the Karnofsky performance scale (Karnofsky et al. 1948) and Instrumental Activities of Daily Living (IADL) (Wong et al. 2007). These assessments were used to assign a neurocognitive stage of normal neurocognitive function or HAND defined as asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), or HIV dementia (Antinori et al. 2007). A diagnosis of ANI required a >1 standard deviation (SD) abnormality but <2 SD abnormality in at least 2 unrelated neurocognitive domains and normal functional performance. A diagnosis of MND required a >1 SD abnormality but < 2 SD abnormality in at least 2 unrelated neurocognitive domains and mild functional impairment (subject not working full time but > ½ time, or Karnofsky score =80). A diagnosis of HIV dementia required impairment in ≥ 3 unrelated neurocognitive domains in which the subject scored <2.0 standard deviations below the locally determined mean for his/her normative age and education group and deterioration in an IADL from baseline, Karnofsky score in the 60-70 range, or work performance <1/2 time (Wong et al. 2007). The demographic characteristics of the HIV- normative control

population were similar to the demographics of the current study except for the absence of HIV infection.

CD4 lymphocyte counts and plasma HIV loads were determined for all HIV-infected subjects on the same day as the neurocognitive assessment. Analysis of CSF specimens (an optional component to facilitate recruitment into the study) was performed in 24 (22%) individuals at the baseline visit. Clinical assessments for establishing HAND stage and CD4 lymphocyte counts were also performed at 6 and 12 months after the baseline visit.

RNA extraction and amplification of gag and gp41 fragments

Viral sequences were obtained for two separate genomic regions with HIV-1–specific primers in the *gag (HXB2 nucleotide [nt] 1240-1907)* and *gp41 (HXB2 nt 7867-8283)* regions as described previously (Conroy et al. 2010, Yang et al. 2000, Yang et al. 2001). These sequences, along with reference sequences from the HIV sequence database, were aligned using the CLUSTALW a cluster analysis multiple-sequence alignment program (Hall 1999) and were optimized by hand using the biological sequences alignment editor BIOEdit), version 5.09 (Felsentein J. 1985).

Phylogenetic analysis and subtype assignment

Phylogenetic trees were generated using Nimble Tree http://sray.med.som.jhmi.edu/SCRoftware/), which incorporates the phylogeny inference package PHYLIP, version 3.572c (Hall 1999). DNADIST was the program used to calculate the genetic distance matrix using a maximum likelihood model with a transition-to-transversion ratio of 2.0 (Carr et al. 1996). Trees were generated from the distance matrix using the neighbor-joining algorithm in NEIGHBOR (Hall 1999). Bootstrap confidence intervals were calculated by randomly permuting the sequence alignment 100 times with the bootstrapping tool, SEQBOOT (Hall 1999). Consensus topology was derived by the use of CONSENSE (Felsentein J. 1985). Bootstrap values >75% were considered significant. Nucleotide positions in relation to HXB2 were determined using the HIV numbering engine and reference sequences for different HIV-1 group M subtypes obtained from Los Alamos (http://web.lanl.gov/seq-db.html). Reference sequences used included fragments of the full-length sequences previously generated from this region (accession numbers AF-484502 to AF-484520) and HXB2 (accession number K03455). Sequences generated for this study have been submitted to the NIH genetics sequence database, GenBank (accession numbers EU841418–EU841480 and EU850351).

Subjects were considered infected with a specific subtype if both regions analyzed were from the same subtype. If there was discordance between the group specific antigen (*gag)* and glycoprotein 41 (*gp41)* subtype assignments, the subject was considered to be infected with a recombinant strain. Subtype assignments were confirmed by using the Web-based REGA subtyping tool. Sequence fragments that demonstrated any evidence of incorporating a recombinant breakpoint based on their position on the tree or from the REGA subtyping tool were further analyzed using SimPlot (Lole et al. 1999). Potential break points were confirmed by generating a phylogenetic tree on each portion on both sides of the putative

break point. If the two portions clustered significantly to two different subtypes, the sequence fragment was considered to have incorporated a recombination break point.

Next-generation sequencing methods

We examined a subset of the study population (n=23) for presence of compartmentalization in the CSF using next-generation sequencing (NGS) (Redd et al. 2011, Redd et al. 2012). Briefly, amplicons of p24 (~390 bp) were generated using a fusion amplicon approach and sequenced as previously described (Redd et al. 2011, Redd et al. 2012). Library pools were normalized by quantitative PCR using a Library Quantification Kit (Kapa Biosciences, Woburn, MA) and diluted to 1×10^5 molecules/μL for a target of 0.175 copies per bead to the deoxyribonucleic acid (DNA) Capture Beads. Preparation of templated beads for NGS followed the emulsion-based polymerase chain reaction (emPCR) Method Manual-Lib-LMV (Roche Branford, CT) with a 75% reduction in amplification primer amount. Enriched DNA Capture Beads were sequenced on a 454 FLX (Roche, Branford, CT) with Titanium chemistry per the manufacturer's instructions using a 2-region gasket (Redd et al. 2011). Sequencing results were analyzed using the GS Amplicon Variant Analyzer version 2.5 (Roche, Branford, CT) with a 75% reduction in amplification primer amount. All sequence reads were compared, and similar sequences were combined into a single consensus sequence. Generated consensus sequences that were within 10 bases from both ends of the amplicon and comprised of a cluster of 10 individual, near-identical sequences or more were determined using the Roche Amplicon software and were classified as being consensus sequences of HIV variants. These consensus sequences were used for subsequent phylogenetic analysis (Redd et al. 2011). Individuals whose plasma and CSF formed distinct phylogenetic clusters, and were at least 1% genetic distance apart were considered to have compartmentalization.

Data analysis

For each neuropsychological test, a *Z* score was calculated using age- and education-adjusted normative data obtained from 100 HIV-uninfected individuals in Uganda (Robertson et al. 2007). Distributional tests have confirmed that the resultant *Z* scores follow a normal distribution, and scores are summarized as mean ± standard deviation (SD). Baseline differences in demographics were examined using the *t* test [or analysis of variance (ANOVA) for multiple group comparison] and the χ^2 test among HIV-infected individuals stratified by both HAND stage and HIV subtypes A and D, the 2 predominant subtypes. A logistic regression model was used to examine for a difference between the frequency of HIV dementia among HIV-infected individuals infected with subtypes D and A. The association between subtype and HAND stage was tested using a χ^2 test of association.

RESULTS

Frequency of HAND and demographic characteristics

For HIV+ individuals receiving phylogenetic analysis of HIV subtype, 8% had normal cognition, 19% had ANI, 32% had MND, and 41% had HIV dementia. Tests of attention and executive function (Digit Span, Color Trails test) were the most likely neuropsychological tests to be abnormal compared to the HIV- normative control

population. The demographic characteristics of the HIV+ individuals stratified by HAND stage are summarized in Table 1. Individuals with HIV dementia were older (p=0.003) and had less education (p=0.01) than less impaired subgroups. There were no differences in gender, CD4 lymphocyte count or log plasma or CSF HIV RNA levels among the 4 HAND stages. There was a trend for increased log plasma HIV RNA level among individuals with ANI, MND, and dementia, compared to HIV+ individuals with normal cognition.

Frequency of HAND stratified by HIV subtype in the blood

Among the 117 HIV+ individuals with phylogenetic analysis for HIV subtype, 67 individuals had subtype A, 25 individuals had subtype D, 24 individuals were classified as A/D recombinants, and 1 individual was classified as subtype C. HIV subtype D-infected individuals had lower mean (SD) years of education [6.7(3.5) years] compared to HIV subtype A-infected individuals [8.4(3.6) years], ($p < 0.02$). There were no differences in age, gender, CD4 lymphocyte count, log plasma HIV RNA level, or log CSF HIV RNA level, when stratified by HIV subtype. Among the 67 individuals with HIV subtype A, 90.9% were classified as having HAND with 15.2% ANI, 28.8% MND and 47.0% dementia. Among the 25 individuals with HIV subtype D, 96.0% were classified as having HAND with 24.0% ANI, 32.0% MND, and 40.0% dementia. Among the 24 individuals with A/D recombinants, 91.7% were classified as having HAND with 25.0% ANI, 50.0% MND, and 16.7% dementia. The one individual with HIV subtype C was classified as having dementia. There was no difference in the frequency of HIV dementia when stratified by subtypes A and D. There was no difference in the performance on any of the individual neuropsychological tests when stratified by HIV subtype.

In addition, we evaluated the frequency of HIV dementia in 2 subgroups: HIV+ individuals with a CD4 count ≤200 cells/μL and HIV+ individuals with a CD4 count >200 cells/μL and <350 cells/μL. There was no difference in the frequency of HIV dementia when stratified by subtypes A and D in either of these 2 subgroups.

Phylogenetic analysis for HIV subtype in the CSF and blood: evaluation for compartmentalization of HIV subtype

Although CSF specimens were obtained in 24 HIV+ individuals in-depth phylogenetic analysis of NGS could only be performed on 8 HIV+ individuals because some specimens had undetectable CSF HIV RNA levels. The number of total reads was significantly higher in the plasma (p=0.007), but this did not result in a significant difference in the number of consensus sequences analyzed (p=0.11, Mann-Whitney Rank Sum test) (Table 2). Only one subject (MY02-085/6465) had evidence of complete compartmentalization, but this subject had relatively low levels of sequence depth in both plasma and CSF (Figure 1a). In the other seven subjects extensive mixing was observed between the two sample types with some minor species that were only found in one sample type (Figure 1b).

Immunological, virological, and neurocognitive response to initiation of ART stratified by HIV subtype

The number of HIV+ individuals receiving ART is small, but preliminary results for the immunological, virological, and neurocognitive response to initiation of ART stratified by

HIV subtype are provided below. During the 12 month period of follow-up, 22 HIV+ individuals initiated ART. The baseline CD4 counts [mean (SD)] for the HIV+ individuals with subtype A (n=12), D (n=7), and A/D recombinants (n=3), were 161(95), 188(68), and 200(52) cells/μL respectively, with no differences at baseline among the three groups. All three groups had an increase in CD4 count after ART initiation [12 month CD4 count [mean (SD)] for the HIV+ individuals with subtype A, D, and A/D recombinants were 335(163), 244(99), and 335(27) cells/μL respectively] with no difference in CD4 count increase when stratified by subtype (p=0.073).

The baseline log plasma HIV RNA [mean (SD)] for the HIV+ individuals with subtype A, D, and A/D recombinant were 4.9(0.6), 4.7(0.8), and 4.8(0.5) respectively. All these groups had a decrease in log plasma HIV RNA after ART initiation [12 month log plasma HIV RNA [mean (SD)] for the HIV+ individuals with subtype A, D, and A/D recombinants were 2.8(0.8), 2.6(0.1), and 2.6(0.0) respectively], with no difference in log plasma HIV RNA decrease when stratified by subtype (p=0.95).

Among the 12 HIV+ individuals with subtype A at baseline who initiated ART 1 had normal cognition, 3 MND, and 8 with dementia. After 12 months of ART, 2 were classified as normal cognition, 3 ANI, 3 MND, and 4 with dementia. Among the 7 individuals with subtype D who initiated ART, 3 had ANI, 3 MND, and 1 with dementia at baseline, and 2 were classified as normal cognition, 2 ANI, and 3 with MND at 12 months. Among the 3 individuals with an A/D recombinant subtype who initiated ART, 1 had ANI, and 2 MND at baseline, and after 12 months, 1 had ANI,and 2 had MND. There were no differences in improvement in HAND stage after ART initiation when stratified by subtype [A versus D, (p=0.10); A versus recombinant, (p=0.38); and D versus recombinant, (p=0.28)].

DISCUSSION

These results suggest that HAND is common among antiretroviral drug naïve HIV+ individuals with moderate and advanced immunosuppression in Uganda. The prevalence rate of HIV dementia in our previous study was 31%, which is similar to the prevalence rate of 41% in our current study (Wong et al. 2007). Advanced age and low education were risk factors for HIV dementia in both studies. Consecutive HIV+ individuals presenting to an Infectious Disease clinic who met the inclusion/exclusion criteria for the study were enrolled. Neurocognitive complaints were not used as a criterion for enrollment. Thus, the frequency of HAND is quite high among antiretroviral drug naïve HIV+ individuals.

In contrast to previous results (Sacktor et al. 2009) we failed to identify an association between HIV subtype and HIV dementia. Thus, one possibility is that HIV subtype is not associated with risk of dementia. However, there was an important difference in the level of immunosuppression between the previous study and the current study. The previous study recruited HIV+ individuals with advanced immunosuppression with a mean CD4 lymphocyte count of 127 cells/μL, who were about to start antiretroviral therapy. In contrast, our current study recruited HIV+ individuals with moderate and advanced immunosuppression with a mean CD4 lymphocyte count of 254 cells/μL for all study participants. These results and the results from the prior study (Sacktor et al. 2009) suggest

that HIV subtype may be a risk factor for dementia only among HIV+ individuals with advanced immunosuppression, but not in HIV+ individuals with less immunosuppression.

It is not known whether the difference in risk of HIV dementia between HIV+ individuals with subtype D and those with subtype A and advanced immunosuppression is due to direct effects from the virus itself or due to the consequences of the immunodeficiency stage induced by the virus. Our results that no subtype-related difference in the risk of dementia for HIV+ individuals with less immunosuppression suggest that it is the more advanced immunodeficiency stage rather than the HIV virus itself which is contributing to the increased risk for dementia among individuals with HIV subtype D compared to subtype A.

Of note, another study evaluating neurocognitive impairment among HIV+ children in Uganda showed that HIV subtype A-infected children were more likely to have neurocognitive impairment than HIV subtype D-infected children (Boivin et al. 2010). The children in this study though were at an earlier stage of immunodeficiency with a mean CD4 lymphocyte count of 675 cells/μL among the 37 subtype A cases and 709 cells/μL among the 16 subtype D cases. There are no other studies evaluating the association between HIV subtypes A and D and risk of dementia. Further studies are needed among a larger number of subjects to evaluate the association between HIV subtype and HIV dementia at specific levels of immunosuppression.

The neuropathogenesis of HAND may be linked to co-receptor usage by the HIV virus. Macrophage-tropic HIV viruses use c-c chemokine receptor type 5 (CCR5) as a co-receptor with CD4 whereas T cell line tropic viruses use the C-X-C chemokine receptor type 4 (CXCR4) co-receptor (Vallat et al. 1998). CXCR4 viruses arise with more advanced immunodeficiency in 40-50% of HIV infected adults, and are associated with rapid HIV disease progression including neurological symptoms such as dementia (Gabuzda & Wang 2000). Several recent studies from Uganda (Kaleebu et al. 2007) and Kenya (Wambui et al. 2012) demonstrate that the probability of having a CXCR4 virus was higher in HIV subtype D infections compared to subtype A infections. For example, 60% of HIV subtype D cases had CXCR4 co-receptor usage compared to 22% of HIV subtype A cases with CXCR4 co-receptor usage. R5 viruses were associated with higher median CD4 cell counts than CXCR4 viruses (Kaleebu et al. 2007). Thus, with advanced immunodeficiency, HIV subtype D may be more likely to use the CXCR4 co-receptor, and thus more likely to develop dementia. Tropism data was not available for the individuals in this study.

Previous studies have reported that compartmentalization with differences in the HIV env gene sequences between the periphery and CNS may play an important role in the development of HIV dementia (Harrington et al. 2009). The current findings do not provide evidence for compartmentalization by HIV subtype among HIV+ individuals with moderate and advanced immunosuppression. It is important to highlight that the methods used in these two studies differed in a few significant ways. NGS significantly increases the depth of the sequencing data as compared to the bulk sequencing used in the previous analysis. Harrington et al also analyzed the envelope gene as opposed to the p24 gene used here. It is possible that compartmentalization between blood and CSF is controlled exclusively by

evolutionary changes in the viral envelope, and therefore would not be observed as readily in the p24 region.

Previous studies of HIV+ individuals with HAND in Uganda have demonstrated improvement in neurocognitive performance after initiation of ART (Sacktor et al. 2006, Sacktor et al. 2013) but the impact of HIV subtype was not examined. Our results suggest that both subtypes A and D show a similar therapeutic benefit for HAND, although there was a trend for less CD4 count improvement in those with D, suggesting that D may be less responsive to treatment. However, only a subset of individuals received ART, so subject numbers were also low to compare the neurocognitive response to ART when stratified by HIV subtype. In addition, in order to evaluate differences in neurocognitive improvement after ART initiation stratified by subtypes, subjects in each subtype should have a similar severity of dementia frequency at baseline, which was not the case in this study.

Several additional limitations should be noted. The assessments for functional status to differentiate between stages of HAND in Uganda may not be ideal in this population. Informant interviews for functional status could not be performed in this study. Only a subset of individuals agreed to the optional lumbar puncture, and CSF studies of subtype could only be performed among specimens with detectable CSF virus, so subject numbers for the compartmentalization studies were low.

Future studies should examine both HIV+ individuals with moderate and advanced immunosuppression simultaneously with a larger number of subjects, and examine mechanisms of neuropathogenesis of HAND in HIV subtypes A and D.

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List of abbreviations

HIV	human immunodeficiency virus
HAND-HIV	associated neurocognitive disorders
CSF	cerebrospinal fluid
AIDS	acquired immunodeficiency syndrome
CNS	central nervous system
env	envelope
ART	antiretroviral therapy
ELISA	enzyme-linked immunosorbent assay
RNA	ribonucleic acid
ANI	asymptomatic neurocognitive impairment

MND	mild neurocognitive disorder
SD	standard deviation
nt	nucleotide
CLUSTALW	cluster analysis program-W
BIO edit	biological sequence alignment editor
PHYLIP	phylogeny inference package
GenBank	NIH genetic sequence database bank
gag	group specific antigen
gp41	glycoprotein 41
NGS	next generation sequencing
emPCR	emulsion based polymerase chain reaction
DNA	deoxyribonucleic acid
ANOVA	analysis of variance
μL	microliter
CCR-5	cc chemokine receptor type 5
CXCR4	C-X-C chemokine receptor type 4

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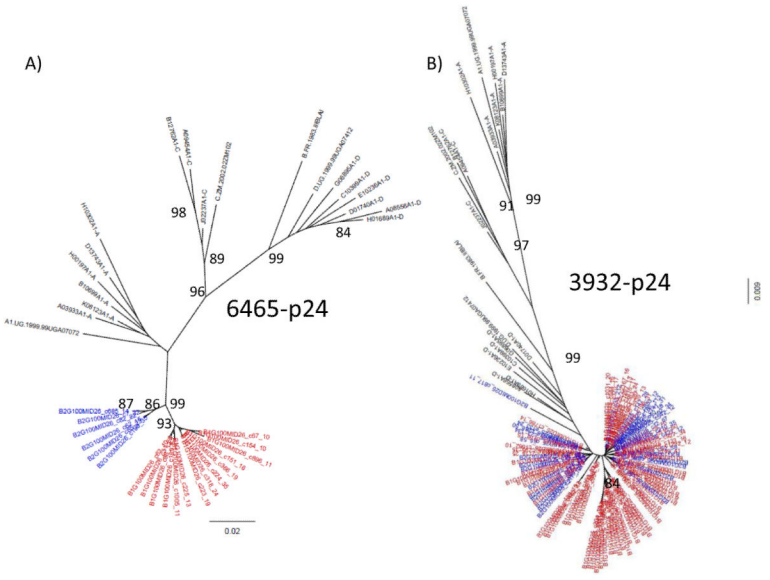

Figure 1.

Phylogenetic trees of consensus p24 viral sequences (≥10 reads) derived from 454 pyrosequencing of plasma (red) and central nervous system (CNS) (blue) for subjects that presented with compartmentalization (A subtype A) and without (B subtype D). All consensus sequences were combined in a joint tree for presence of compartmentalization. Number of repeated sequences represented by each consensus sequence is shown at the end of the consensus identifier. Distance is indicated for the trees by the scale, and samples are grouped with a selection of subtype reference sequences and random sequences from individuals in Rakai district, Uganda (black). Bootstrap values greater than 80 percent are indicated.

Table 1

Demographic characteristics of HIV infected individuals stratified by HIV-associated neurocognitive disorder (HAND) stage

Characteristic	Normal (n=9)	ANI (n=22)	MND (n=38)	Dementia (n=48)
Age, years [Mean (SD)]	35.5 (7.0)	33.6 (10.0)	36.7 (5.3)	39.7 (8.0)*
Education, years	9.5 (3.5)	10.0 (3.8)	8.1 (4.1)	7.0 (3.6)**
Gender, % male	54.6	40.0	39.5	20.4
CD4 lymphocyte count, cells/μL [Mean (SD)]	253.8 (80.3)	259.5 (105.3)	239.3 (118.1)	232.8 (133.9)
Log plasma HIV RNA level, copies/mL [Mean (SD)]	4.2 (1.0)	4.7 (0.7)	4.8 (0.8)	4.9 (0.8)
Log CSF HIV RNA level, copies/mL [Mean (SD)]	0.5 (0.7)	0.0 (0.0)	0.4 (0.5)	0.3 (0.5)

* p=0.003

** p=0.01

ANI=Asymptomatic neurocognitive impairment

MND=mild neurocognitive disorder

CSF=cerebrospinal fluid

CNS=Central nervous system

Table 2

Sequence read totals and consensus distribution for plasma and CSF

STUDY _ID	Plasma		CSF		
	Total # of p24 reads	No. of p24 consensus sequences	Total # of p24 reads	No. of p24 consensus sequences	Subtype by p24
MY02-031/22623	19858	118	8137	39	D
MY02-059/23259	14567	97	5763	35	D
MY02-062/23535	6579	58	3093	8	A
MY02-075/15332	5172	2	2720	7	A
MY02-076/21654	8593	25	4044	23	A
MY02-077/13264	11685	36	2780	10	D
MY02-083/3932	16584	74	6492	31	D
MY02-085/6465	5078	14	3305	6	A
Mean (±SD)	11015 (±5211)	53 (±38)	4542 (±1888)	20 (±13)	