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HIV-subtype A is associated with poorer neuropsychological performance compared to subtype D in ART-naïve Ugandan children

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DISCLOSURES

None of the authors have any conflicts of interest to disclose with respect to this study. All of the authors have contributed substantially to the present study and merit inclusion as co-authors.

Michael J. Boivin designed the assessment portion of the study, completed the analyses, and wrote the paper.

Theodore D. Ruel helped organize the subtype and clinical lab tests and data management of these assessments, and contributed to the writing of the paper.

Hannah E. Boal helped supervise study recruitment, consent, neuropsychological assessment, data management, and contributed to the writing of the paper.

Paul Bangirana supervised study consent, neuropsychological assessment, data management, data analysis, formulation of tables, and contributed to the writing of the paper.

Huyen Cao directed immunophenotyping assays for T cell activation and laboratory interpretation.

Leigh Anne Eller assisted in the immunological and clinical assays for T cell activation and laboratory interpretation.

Edwin Charlebois helped with patient selection and recruitment, provided statistical and data management support and contributed to the writing of the paper.

Diane Havlir assisted in patient selection and recruitment, clinical coordination and contributed to the conceptualization of the study.

Moses Kanya contributed to study design, patient selection and recruitment, clinical coordination and contributed to the writing of the paper.

Jane Achan contributed to patient selection and recruitment and clinical coordination.

Carolyn Akello contributed to patient selection and recruitment and clinical coordination.

Joseph K Wong developed the overall design of the study, helped organize and interpret the subtype and clinical lab tests, contributed to the interpretation of the study findings, helped write the manuscript, and was PI on the NIMH R21 grant that provided the principal support for this work.

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Abstract

Background—HIV-subtype D is associated with more rapid disease progression and higher rates of dementia in Ugandan adults compared to HIV-subtype A. There are no data comparing neuropsychological function by HIV-subtype in Ugandan children.

Design—102 HIV-infected antiretroviral-therapy-naïve Ugandan children 6–12 years old (mean 8.9) completed the Kaufman Assessment Battery for Children, 2nd edition (KABC-2), the Test of Variables of Attention (TOVA), and the Bruininks/Oseretsky Test for Motor Proficiency, 2nd edition (BOT-2). Using a PCR-based multi-region assay with probe hybridization in 5 different regions (gag, pol, vif, env, gp-41), HIV-subtype was defined by hybridization in *env* and by *total* using ≥ 2 regions. ANCOVA was used for multivariate comparison.

Results—*Env* subtype was determined in 54 (37 A, 16 D, 1 C) children. Subtype A and D groups were comparable by demographics, CD4 status, and WHO Stage. Subtype A infections had higher log viral loads (median 5.0 vs 4.6, $P = 0.02$). Children with A performed more poorly than those with D on all measures, especially on KABC-2 Sequential Processing (memory) ($P = 0.01$), Simultaneous Processing (visual-spatial analysis) ($P = 0.005$), Learning ($P = 0.02$), and TOVA visual attention ($P = 0.04$). When adjusted for viral load, Sequential and Simultaneous Processing remained significantly different. Results were similar comparing by *total* HIV-subtype.

Conclusion—HIV subtype A children demonstrated poorer neurocognitive performance compared to D. Subtype-specific neurocognitive deficits may reflect age-related differences in the neuropathogenesis of HIV. This may have important implications for when to initiate ART and the selection of drugs with greater CNS penetration.

Keywords

HIV clades; cognitive ability; neuropsychology; CD activation; viral load; attention; memory; motor; children; encephalopathy; home environment

INTRODUCTION

HIV-subtype B is the predominant HIV-subtype in the U.S, Latin America, Europe, and Australia. In East Africa subtypes A, C, and D are the predominant subtypes while subtype C is predominant in India and much of Asia [1]. Subtype specific differences in co-receptor tropism and viral fitness may underlie the differential impact on HIV disease progression [2]. Studies of HIV-infected adults in East Africa have suggested that subtype D is associated with more rapid CD4 decline, greater rise in viral load, and more rapid progression to AIDS or death [3,4]. Differences in neurovirulence among HIV-subtypes have also been postulated because of markedly different prevalence rates of HIV-associated neurocognitive disorders (HAND) in various parts of the world [5]. However, disease prevalence between geographically distinct regions may also be explained by differences in host genetics and available healthcare resources.

Sacktor and colleagues (2009) examined the relationship between HIV-subtype and the severity of HIV associated cognitive impairment among HIV-infected adults initiating antiretroviral therapy in a single clinic in Uganda [6]. Using subtype assignments that were generated by sequence analysis of the gag and gp41 regions, 8 of 9 (89%) HIV-infected adults with subtype D had dementia, compared to 7 of 33 (24%) HIV-infected adults with subtype A ($P = 0.004$). They concluded that HIV dementia may be more common among adults with subtype D than those with subtype A, providing the first evidence that HIV subtypes may differ in terms of neurocognitive pathogenesis. However, there has been no study investigating potential differences in the impact of HIV by subtype in children and in the context of ongoing neurocognitive development. In Uganda, where HIV-subtypes A and D are in co-circulation, there is a unique opportunity to investigate the neuropsychological impact of these different subtypes in children.

The purpose of the present study is to compare the neurocognitive impact between HIV-subtypes A and D in a cohort of HIV-infected ART-naïve Ugandan children. Because *env* is the region of the virus important to co-receptor usage and cellular/tissue tropism [7], we hypothesized that children with HIV-subtype D in the envelope region will exhibit greater impairment of neurocognitive function.

METHOD

Participants

One hundred and two ART-naïve children 6–12 years old (64 boys, 38 girls; mean 8.9 years) were enrolled from an ongoing prospective cohort of HIV-infected Ugandan children based in Kampala, Uganda [8]. Neuropsychological assessments were conducted in a private room in a wing of the patient ward that afforded quiet and privacy. Assessments were conducted in the local language of the children (Luganda) by native speakers. Assessors were unaware of the HIV-subtype or clinical status of the children.

Clinical and socioeconomic data were obtained from the cohort study database. Socioeconomic status and quality of home environment was assessed upon enrollment. All children received comprehensive care, including CD4 measures, HIV RNA levels, and complete blood counts every 12 weeks. Children had access to medical care 7 days a week and all symptoms are evaluated using standardized protocols and diagnostic codes. ART was initiated per Uganda country guidelines. Children selected for this analysis were ART-naïve at the time of neurocognitive testing,

Informed written consent was obtained from the child's parent and assent from older children (age > 7 yrs). IRB approval for this study was obtained from Makerere University, University of California, San Francisco and Michigan State University.

Kaufman Assessment Battery for Children – (2nd edition) (KABC-2)

The K-ABC has been used by Boivin with Ugandan pediatric cerebral malaria survivors [9], HIV-positive Congolese (Zairian) children [10], and Ugandan school-age HIV survivors [11]. The KABC-2 maintains its factor structure with Ugandan cerebral malaria survivors [12]. The K-ABC has been used as well with HIV-infected American children [13,14]. The primary outcome variables were the global scores of Sequential Processing (memory), Simultaneous Processing (visual-spatial processing and problem solving), Learning (immediate and delayed memory), and Planning (executive reasoning). We did not administer the Knowledge (crystallized intelligence) portion of this test because of problems in cultural suitability.

Test of Variables of Attention (TOVA)

Both the auditory and visual versions of the Test of Variables of Attention (TOVA) were administered. The TOVA is a visual continuous performance test used in the diagnosis and monitoring of children and adults with attention deficit disorders. In our prior use of this computerized assessment, the TOVA provided the most sensitive measures of the persisting neurocognitive effects of CM in Ugandan children [15]. Following instructions and practice trials, the Auditory and Visual TOVA each take about 22 minutes to administer. We began our neurocognitive assessment sessions with the TOVA so that vision and hearing deficits for any of our study children would immediately become evident, allowing us to exclude the child from further study participation. In the present analysis, we used TOVA D prime signal detection measure (signal detection sensitivity) for overall attention performance.

Bruininks/Oseretsky Test (BOT) for Motor Proficiency (2nd Ed)

This is regarded as one of the most comprehensive and sensitive instruments for motor assessment. Testing involves game-like tasks that hold the child's interest and are not verbally complex. Composite scores include fine manual control, manual coordination, body coordination, strength and agility and total composite score. Its use in this study is particularly relevant because of the pronounced motor impairment that seems to accompany HIV-related neurocognitive delay compared with impairment from severe malaria [10,16].

Home Observation for the Measurement of the Environment (HOME)

The middle childhood version of the Caldwell Home Observation for the Measurement of the Environment (HOME) [17] was used to assess the stimulation and learning opportunities offered by the child's home environment. It is important to control for this measure when evaluating the impact of HIV-subtype on neurodevelopmental and psychosocial outcomes. This is especially true when assessing such outcomes in African children developmentally at risk for a number of factors related to poverty. Along with the HOME, we also used a socioeconomic evaluation scale of physical quality of the home environment (SES score) previously used with Ugandan children.

Subtype assignment

HIV-subtype was determined by application of the multi-region hybridization assay from plasma HIV RNA (MHA) [18]. The MHA is particularly useful for identifying recombinant forms since five different targets dispersed across the HIV genome are simultaneously assessed [19]. This protocol generates five short amplicons in *gag*, *pol*, *vpu*, *env* (*gp120*) and *gp41* by polymerase chain reaction (PCR) using pan-subtype primers. Each amplicon is then distributed to separate second-round Taqman (Applied Biosystems, Foster City, California) kinetic PCR

reactions, employing subtype-specific Taqman probes. The assay was originally developed for use with DNA from peripheral blood mononuclear cells, but it has also been used with rt-PCR amplifying RNA in plasma [18]. The second generation of this assay has 90% sensitivity and 98% specificity when compared to the “gold standard” of full genome sequencing. [19]. Because of variability in performance, this assay does not generate a subtype in every region for every strain. Therefore, subtype was defined for this analysis in two ways: 1) *env* defined by hybridization in the envelope region and 2) *total* using the subtype assignment determined in two or more gene regions (may or may not include *env*); if subtypes differed by region within a strain it was considered recombinant.

CD4⁺ T-cell counts, immune activation, and viral load testing

CD4 measures were available within a median (mean; range) of 28 (32, 0–212) days from the time of neuropsychological assessment. HIV viral load data were available within a median (mean; range) 30 (54, 0–245) days). CD4 T-cell and CD8 T-cell activation level testing was performed on EDTA anti-coagulated whole blood using a fresh lyse-no wash flow cytometry procedure. Blood was incubated with monoclonal antibodies including CD3, CD8, CD4, CD38, and HLA DR, then processed and acquired on a multi-laser bench top flow cytometer. For each run of patient samples, a separate sample of stabilized blood product (CD-Chex, Streck Laboratories) was processed. CD4 T cell and CD8 T cell activation levels were defined as % CD38 and HLA-DR co-expression [20].

Statistical Analyses

Children were grouped by *env* and *total* subtype (A, C, D, A/D recombinant) and compared on all demographic and clinical measures using the Student *t* test for two independent samples from SPSS Base program v17.0 (Table 1). Because the testing modules can vary as a result of cultural specific factors, Z-scores were generated using age adjusted norms from a cohort of 122 non HIV children from a separate study in the greater Kampala area. Subtype A and D children were then compared on all global performance measures for the KABC-2, TOVA, and BOT-2 (Table 2), again first by *env* subtype and then by *total* subtype. Age, gender, weight-for-age z scores (CDC2000, Epi Info), HOME total score, and viral load were used as covariates for ANCOVA comparisons between A and D on our principal neurocognitive outcomes.

RESULTS

HIV-subtype determination in the *env* region was possible for 54 (52%), with *total* subtype defined for 70 (68%) children (Table 1). Taken together, children with any subtype determination did not differ from those children in whom subtype was indeterminate, demographically or by weight adjusted for age z scores (WAZ) (Based on Epi Info CDC 2000 norms). No children had signs of symptoms of CNS infection on the day of testing or within 3 months before or after the testing date. No child had been diagnosed with pulmonary tuberculosis with 120 days of testing and only one child had been diagnosed with malaria within 60 days of testing. Subtype A and D infected children had comparable WHO staging [21] (Table 2). While nutrition/malnutrition were not directly measured in the SES or HOME surveys, the SES provided a measure of the most locally important influence on nutritional status, financial resources and our clinical data on weight /height ratios provides one measure of the physical consequences of malnutrition. For either of these variables, there was no significant difference between groups infected with subtype A HIV and those with subtype D (by either definition).

There were no differences in CD4 count or %, comparing by *env* A, to D/indeterminate or A, to D /recombinant/indeterminate when using *total* subtype. However, children with subtype A had higher viral load levels than those with indeterminate or D when defining subtype by

env (Table 1). When using *total* subtype, those with A had significantly higher viral loads than those with indeterminate subtype, and higher (but not reaching statistical significance) compared to subtype D.

Mean and standard deviation raw score performance totals were compared for children infected with each of the subtypes with subtype being a composite of two or more gene regions (Table 3). Subtype A infected children ($n = 37$) performed more poorly than those with subtype D infection ($n = 24$) on KABC-2 Simultaneous Processing (visual-spatial analysis and problem solving) and Learning using raw score totals adjusted for age, gender, weight-for-age z score, and HOME quality in an ANCOVA analysis. On KABC-2 performance, the recombinant (A/D) children ($n = 9$) resembled the subtype A children in level of performance. When log viral load was included as a covariate in the ANCOVA comparison between subtypes A and D, KABC-2 differences did not remain significant.

Of the viral genes assessed for subtype determination, *env* harbors the most direct determinants of co-receptor usage (CXCR4 versus CCR5) and cellular/tissue tropism [7]. Because this could influence the efficiency of viral replication in the CNS, we used subtype determination from this particular gene region as the basis for comparing neuropsychological performance of children with infection by subtypes A ($n = 37$) and D ($n = 16$). For the unadjusted t score comparison between subtypes A and D in Table 4, we used performance measures standardized by age using a non-HIV control group at Mulago Hospital ($n = 104$). For the adjusted t score, ANCOVA means and standard errors were computed from global raw scores using age, gender, weight-for-age z score, HOME total score, and viral load as covariates.

On the KABC-2 measures standardized by age, subtype A infected children performed significantly more poorly than those with subtype D. There were significant differences on KABC-2 Sequential Processing ($P = 0.01$), Simultaneous Processing ($P = 0.005$), and Learning ($P = 0.02$) (Table 4). The subtype A group also performed more poorly on the TOVA D prime signal detection measure of attention ($P = 0.04$). After adjusting for viral load along with the other covariates, K-ABC-2 Sequential ($P = 0.05$) and Simultaneous Processing ($P = 0.007$) remained significant. Simultaneous Processing was the only ANCOVA cognitive performance that remained statistically significant after adjusting for the number of comparisons for the KABC-2 performance measures.

The only evaluative basis for diagnosing behavioral or learning disability for our study children is to use our present neuropsychological and cognitive assessments as the basis for that determination. Since normative data does not exist for Ugandan children for our tests, we can define impairment for a study child as any score that is 2 or more SDs below the mean for the non HIV-infected control children for that year of age. Using this criterion, 7 of the subtype A children (19%) based on *tot* were impaired on at least one of the global neurocognitive domains listed in Table 3, compared to 4 subtype D children (17%) and 1 subtype C child (100%). The proportion of children with at least one domain of impairment did not differ significantly between subtypes A and D.

DISCUSSION

The impact of the viral genetic complexity that characterizes the HIV epidemic in sub-Saharan Africa on the disease course of individual patients is not well understood. In the current work, we found evidence that HIV-subtype influences neurocognitive disability in HIV infected Ugandan children. Whether we used a combination of gene regions (*tot*), or just the *env* region to determine subtype, children with subtype A did more poorly on cognitive ability and visual attention than those infected with subtype D. The differences were especially pronounced on KABC-2 Simultaneous Processing (visual-spatial analysis and problem solving). It should also

be noted that Simultaneous Processing was the K-ABC performance domain where ART-naïve HIV-positive children have shown the most significant deficits compared to HIV-negative children, both in DR Congo [10] and Uganda [11]. Simultaneous Processing remained significantly lower for the subtype A children after adjusting for the higher viral load apparent for subtype A children as determined by the *env* region.

In contrast to our findings, Sacktor et al. (2009) concluded that in Ugandan adults, those with subtype D infection had a greater level of neurocognitive deficits compared to those with subtype A. This stark difference in subtype association may partly be due to the fact that the adults in that study were at a more advanced disease stage and qualified for initiation of ART. Subtype D is known to have a greater immuno-virulence in East African adults, and the immuno-compromise associated with this aspect of subtype D disease may have contributed to the higher prevalence of HAND among Ugandan adults (although CD4 counts were not different between groups). Our children were ART-naïve because they were at an earlier stage of disease with lesser degrees of laboratory and clinically defined immunodeficiency.

In preliminary study, we have not observed a higher mortality in children infected with subtype D children in our cohort. It should also be noted that in Sacktor et al., infection with subtype D was associated with an increased risk of HIV dementia when subtype was defined by the *gp41* envelope gene. In Sacktor et al., the same statistical association of subtype D and HIV dementia was not evident in the *gag* gene. These results suggest that determinants within the *env* region may be more critical for conferring an increased risk of HIV dementia compared to the *gag* gene in HIV-infected individuals with subtype D. In fact, use of the *env* gene for our subtype determination resulted in more robust neuropsychological differences favoring subtype D children in our study. Sacktor and colleagues also suggest that neuropsychological differences among subtype D and A may depend on differences in co-receptor usage and syncytium inducibility mediating differences in neurovirulence.

Immunologically asymptomatic HIV disease is thought to be associated with more encephalopathy in children compared to adults [22]. Coupled with the impact of the virus on developing neural networks in children during sensitive periods of development [23], the neurocognitive effects of HIV-subtype may fundamentally differ in children compared to adults. Subtype A HIV may be more directly encephalopathogenic than HIV-subtype D in children, while in adults, infection with subtype D HIV could result in a greater degree of neuropsychological impairment related to heightened immunopathology associated with HIV-subtype D infection. .

There are several well defined virologic differences between HIV-Subtypes A and D. Subtype D strains have an increased number of positively charged amino acid substitutions in the *envelope* region [24]. Studies suggest an increased prevalence of CXCR4 tropism in subtype D isolates. In one such study of Ugandan adults who had not yet developed AIDS; a surprisingly high 44% (12/27) of those with subtype D harbored CXCR4-tropic virus, compared to only 17% (4/23) of those with subtype A [25]. There is a need to determine CXCR4 tropism of isolates from children in the cohort and determine if subtype D is associated with an increased prevalence of CXCR4 tropism.

Higher degrees of CCR5 tropism associated with subtype A isolates of treatment-naïve individuals could confer an advantage in infecting macrophages, the principal cells of transport of the virus through the blood-brain barrier into the central nervous system [7] and microglia, the other major target cell for HIV in the CNS. This could result in CCR5-tropic strains being more "encephalopathogenic", particularly during sensitive periods of brain development in early childhood. In contrast, CXCR4-tropic strains may be more immunopathogenic over the

long term because they infect a wider range of target cells in lymphoid tissue but at the same time, they may replicate less efficiently in the CNS.

However, the relationship between HIV subtype and neuropathogenesis is likely to be more complex than this simple association and involve other factors. Although subtype D HIV may be predisposed to CXCR4 tropism, subtype A strains can also acquire this phenotype in advanced disease. What is more, many potential target cell types express both CCR5 and CXCR4 co-receptors [26]. In HIV-subtype C infection, the viral regulatory gene product Tat has been implicated as another variable affecting the development of HAND [27,28].

In a murine model, HIV-1 Tat mediates differences in monocyte recruitment between subtype C and subtype B infected macrophages resulting in attenuation of astrogliosis and relative preservation of neuronal network integrity during infection with subtype C [29]. In this same study, HIV-subtype B was associated with greater memory impairment. In vitro, subtype C Tat induces apoptosis in primary human neurons to a lesser degree than subtype B Tat. The determinants of this difference in subtype C neurotoxicity was mapped to the dicysteine motif in the 5' 1st exon of Tat [30]. Such comparisons between subtype A and D *tat* should be done.

Additional study of the neuropsychological associations of HIV-subtypes in younger infants and children in Uganda are warranted. There is also a need to further validate subtype associations with independent markers of neuro-inflammation (e.g. monocyte chemotactic protein-1 or MCP-1) and the neurocognitive deficits associated with subtype A infection in Ugandan children. In addition, given recent reports of a high prevalence of neurocognitive impairment in western adult populations [31], and pediatric populations despite HAART [32], there is a need to determine how HAART differentially affects neurocognitive responses in subtype A and D infected children.

Conclusions

In contrast to adults, children with HIV-subtype A demonstrated poorer neurocognitive performance compared to D in this cross sectional study. Some of this effect may be the result of higher viral loads, but likely also reflects subtype- and age-specific differences in the neuropathogenesis of HIV. These findings support the need to monitor the long-term developmental effects of HIV infection of different subtypes in children through longitudinal follow-up to define a neurodevelopmental trajectory.

While the current data are not sufficient to alter clinical practice, they strongly point to a need for such additional study. If future studies confirm that HIV subtype A is more neuropathogenic for children in the long-term, such findings could have more direct clinical implications. For example, they might suggest that HIV subtype should be considered in deciding the timing of ART initiation and that the encephalopathic risk of a given subtype should be considered in selecting among antiretroviral drugs with varying CNS penetration characteristics [33]. At a time when universal treatment remains highly controversial [34,35], it is important to develop more precise tools to stratify the risk of HIV infected children for both immunological and non-immunological harm from deferred treatment.

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Table 1

Demographic and immunological characteristics (mean (standard deviation)) of HIV-infected children, stratified by HIV-subtype in the *env* region and by *Total* subtype.

Characteristic	HIV-subtype				
	A	C	D	A/D	Unknown
Patients by <i>env</i> HIV-subtype					
No. of patients	37	1	16		48
Age, years	8.7 (1.8)	9.7	8.5 (1.7)		9.2 (1.9)
Education, years	3.2 (2.0)	3.0	3.6 (1.7)		3.9 (2.1)
Percentage of male subjects	62.2	100	62.5		62.5
Caldwell HOME score total	23.5 (6.9)	24.0	23.6 (5.9)		23.2 (7.1)
Socioeconomic status total	9.3 (3.8)	9.00	9.3 (3.2)		8.7 (2.9)
Weight for age z score	-1.4 (1.3)	-0.2	-1.0 (1.1)		-1.2 (1.0)
CD4 lymphocyte count, cells/mL	675 (369)	1347	709 (331)		670 (309)
CD4 %	25.3 (7.9)	43.0	28.0 (8.6)		27.2 (9.2)
Log plasma HIV RNA level, copies/mL	5.01 (0.62)	3.29	4.57 (0.59)*		4.60 (0.67)#
CD4 Activation	12.8 (5.8)	3.7	12.8 (9.2)		11.7 (5.7)
CD8 Activation	42.8 (13.5)	31.8	42.6 (12.6)		42.2 (13.6)
Patients by <i>Total</i> HIV-subtype, with >= 2 regions hybridized					
No. of patients	37		24	9	32
CD4 lymphocyte count, cells/mL	638 (359)		707 (391)	879 (273)	667 (271)
CD4 %	24.7 (7.9)		28.3 (9.5)	30.3 (6.2)	27.1 (9.5)
Log plasma HIV RNA level, copies/mL	4.98 (0.62)		4.68 (0.54)	4.71 (0.68)	4.45 (0.75)##
CD 4 Activation	13.6 (5.5)		12.6 (9.0)	10.7 (3.9)	10.8 (5.7)
CD 8 Activation	45.7 (12.6)		45.0 (13.4)	38.1 (8.8)	37.9 (14.0)

* $P = .02$ for subtype A vs subtype D using Student t test for two independent samples.

** $P < .01$ for subtype A vs subtype D.

*** $P = .001$ for subtype A vs subtype D.
$P = 0.003$ for subtype A vs unknown group.
$P = 0.002$ for subtype A vs unknown group.

Table 2

The number and percent of children at each WHO (2005) stage of HIV disease is presented by *env* subtype (A, D). All children were ART-naïve at the time of neuropsychological assessment.

<i>env</i> Subtype	WHO HIV Stage*			
	I	II	III	IV
A	5 (13.5%)	20 (54.1%)	11 (29.7%)	1 (2.7%)
C	1 (100%)	0 (0%)	0 (0%)	0 (0%)
D	3 (18.8%)	10 (62.5%)	3 (18.8%)	0 (0%)

Fisher's Exact test, $P = 0.73$ (excluding subtype C)

* **Stage I:** HIV disease is asymptomatic and not categorized as AIDS.

Stage II: include minor mucocutaneous manifestations and recurrent upper respiratory tract infections.

Stage III: includes unexplained chronic diarrhea for longer than a month, severe bacterial infections and pulmonary tuberculosis.

Stage IV: includes pneumocystis pneumonia, toxoplasmosis of the brain, extrapulmonary tuberculosis, candidiasis of the esophagus, trachea, bronchi or lungs, Kaposi's sarcoma, and CMV infection; these diseases are used as indicators of AIDS.

Table 3

Adjusted global raw score means (*M*) plus and minus the standard error (*SE*) are presented for the subtype groups (A, D, A/D Recombinant) as determined by *tot*, which is a composite of two or more gene regions (*env*, *gag*, *pol*, *vif*, and *gp41*). Covariates for ANCOVA are age, gender, weight-for-age *z* score (CDC2000 Epi Info), and Caldwell HOME total score. Significant differences between subtypes A and D for ANCOVA analyses are denoted in subtype D column (*, **). Statistically significant ANOVA *P* values are in bold.

Neuropsychological Test	Adjusted mean score $\pm$ standard error by HIV composite subtype				
	A (n = 37)	D (n = 24)	AD (n = 9)	ANCOVA <i>P</i>	Observed Power
KABC-2 ^a Sequential Processing	29.1 $\pm$ 0.9	31.3 $\pm$ 1.1	26.1 $\pm$ 1.8	0.037	0.71
KABC-2 Simultaneous Processing	37.2 $\pm$ 1.7	43.7 $\pm$ 2.0 *	36.9 $\pm$ 3.4	0.040	0.62
KABC-2 Learning	92.8 $\pm$ 5.9	116.3 $\pm$ 7.1 **	89.4 $\pm$ 11.8	0.028	0.67
KABC-2 Planning/Reasoning	11.0 $\pm$ 1.2	12.2 $\pm$ 1.4	9.1 $\pm$ 2.4	0.46	0.18
TOVA Visual D Prime	2.5 $\pm$ 0.1	2.8 $\pm$ 0.2	2.5 $\pm$ 0.3	0.44	0.19
Signal Detection					
TOVA Auditory D Prime	1.1 $\pm$ 0.3	1.0 $\pm$ 0.4	0.7 $\pm$ 0.6	0.87	0.07
Signal Detection					
BOT-2 ^c Motor Control	58.9 $\pm$ 1.8	56.9 $\pm$ 2.2	54.6 $\pm$ 3.6	0.53	0.15
BOT-2 Manual Coordination	44.7 $\pm$ 1.7	46.0 $\pm$ 2.1	48.6 $\pm$ 3.6	0.61	0.13
BOT-2 Body Coordination	50.7 $\pm$ 1.1	52.1 $\pm$ 1.3	55.5 $\pm$ 2.3	0.18	0.36
BOT-2 Strength/Agility	29.9 $\pm$ 1.2	32.1 $\pm$ 1.4	36.2 $\pm$ 2.5	0.076	0.52

^aKaufman Assessment Battery for Children, 2nd Edition;

^bTests of Variables of Attention;

^cBruininks-Oseretsky Test of Motor Proficiency, 2nd Edition.

* Bonferroni pairwise comparison between subtypes A and D: *P* = 0.05, effect size = 6.8 (*SE* = 3.9)

** Bonferroni pairwise comparison between subtypes A and D: *P* = 0.04, effect size = 27.0 (*SE* = 13.7)

The ANCOVA adjusted raw score means plus and minus the standard error are presented for subtypes A and D determined by the *env* gene. Covariates are age, gender, weight-for-age z score (CDC2000 Epi Info), Caldwell HOME total score, and viral load. The Student *t* test probability and the ANCOVA *t* values are presented with statistically significant values remaining significant after Bonferroni adjustment for number of simultaneous comparisons in bold.

Table 4

Neuropsychological Test	A (n = 37)	D (n = 16)	<i>p</i> [/] z scores	<i>P</i> adjusted	Observed Power	Effect Size ± SE
KABC-2 ^a Sequential Processing	28.8 ± 0.8	31.9 ± 1.2	0.01	0.05	0.50	3.0 ± 1.5
KABC-2 Simultaneous Processing	27.2 ± 1.7	46.3 ± 2.6	0.005	0.007	0.79	9.0 ± 3.2
KABC-2 Learning	96.5 ± 6.2	114.3 ± 9.6	0.02	0.14	0.31	17.8 ± 11.8
KABC-2 Planning/Reasoning	11.7 ± 1.3	12.4 ± 2.1	0.40	0.76	0.06	0.8 ± 2.6
TOVA ^b Visual D Prime	2.5 ± 0.1	2.9 ± 0.2	0.04	0.11	0.35	0.4 ± 0.2
TOVA Auditory D Prime	1.2 ± 0.3	0.8 ± 0.5	0.63	0.42	0.12	0.5 ± 0.6
BOT-2 ^c Total Score	186.3 ± 4.6	194.2 ± 7.2	0.22	0.37	0.14	8.0 ± 8.8

^aKaufman Assessment Battery for Children, 2nd Edition;

^bTests of Variables of Attention D prime (signal detection measure of overall attention performance)

^cBruininks-Oseretsky Test of Motor Proficiency, 2nd Edition total score

[/]*P* value for Student *t* test for z scores using *M* and *SD* for year of age for Ugandan non-HIV control children at Mulago Hospital (n = 104) without HIV. No covariates were used in this comparison.