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Increased cortical expression of FK506 binding protein-51 in HIV-associated neurocognitive disorders

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Abstract FK506 binding protein (FKBP)-51 and FKBP52 act as molecular chaperones to control glucocorticoid receptor (GR) sensitivity. Dysregulation of proteins involved in GR-mediated signaling can lead to maladaptive stress response and aging-related cognitive decline. As HIV infection is related to chronic stress, we hypothesized that altered cortical expression of these proteins was associated with HIV-associated neurocognitive disorders (HAND). We used quantitative immunohistochemistry to assess expression levels of these proteins in the mid-frontal gyrus of 55 HIV-infected subjects free of cerebral opportunistic diseases compared to 20 age-matched non-HIV controls. The immunoreactivity normalized to the neuroanatomic area measured (IRn) for FKBP51 was increased in HIV subjects both in the cortex and subcortical white matter ($p<0.0001$, U test), while no significant alterations were observed for GR or FKBP52. Notably, the cortical FKBP51 IRn was higher in HAND subjects than in cognitively normal HIV subjects ($p=0.02$, U test). There was also a trend for increasing cortical FKBP51 IRn with the increasing severity of HAND ($p=0.08$, Kruskal–Wallis test). No significant changes in FKBP51 IRn were found with respect to hepatitis C virus infection, lifetime methamphetamine use, or antiretroviral treatment in HIV subjects. In conclusion, the increased cortical expression of FKBP51 (an inhibitor for GR activity) might represent negative feedback in an attempt to reduce GR sensitivity in the setting of chronic stress-induced elevation of GR-mediated signaling inherent in HIV infection. The further increased FKBP51 expression might lead to maladaptive stress response and HAND.

Keywords FKBP4 · FKBP5 · HIV dementia · Immunophilin · NR3C1

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Introduction

In the current era of highly active antiretroviral therapy (HAART), HIV-associated neurocognitive disorders (HAND), predominantly in milder forms, continue to affect the clinical outcome of HIV infection, even in the setting of systemic viral suppression (Heaton et al. 2011; Nath et al. 2008). The dramatically improved survival due to HAART has led to a growing number of older HIV-infected individuals potentially carrying a greater risk of HAND (Becker et al. 2004). The differential susceptibility to the development and severity of HAND may be explained by individual differences in HIV variants, host genetic polymorphisms,

and comorbid factors such as chronic adverse effects of antiretroviral treatment, substance use, and aging-related systemic and brain disorders, which may interact with each other in contributing to neural injury, specifically synaptodendritic degeneration (Jayadev and Garden 2009). Nonetheless, it remains unclear which molecular pathways are primarily or specifically involved in the pathophysiology of HAND.

Chronic stress is inherent to the clinical course of HIV infection (Hand et al. 2006; Reif et al. 2011). One factor that may account for the susceptibility to HAND is the individual profile in the stress–response system (specifically glucocorticoid signaling), which in turn is dependent on host genetic variants and epigenetic modifications associated with early-life psychosocial experiences, as it has been proposed in aging-related cognitive decline (Goosens and Sapolsky 2007; Oitzl et al. 2010). In the brain, the glucocorticoid receptor (GR) is ubiquitous and particularly enriched in the prefrontal cortex, hippocampus, hypothalamus, and amygdala in correspondence with its role in modulating the hypothalamic–pituitary–adrenal (HPA) axis activity (Lupien et al. 2005). The GR-mediated effects of glucocorticoids are aimed at ending ongoing stress reaction, facilitating recovery, and promoting memory storage (de Kloet et al. 2005). These are accomplished by the activation or repression of a plethora of target genes in different functional classes such as cellular metabolism and energy production, signal transduction, and protein synthesis, trafficking, and turnover (Datson et al. 2001). In mammalian cells, the sensitivity of GR to its hormone ligands is dependent on the relative levels of FK506 binding protein (FKBP)-51 and FKBP52 immunophilins, where the former inhibits and the latter facilitates GR trafficking along microtubule tracks to the nucleus (Grad and Picard 2007; Wochnik et al. 2005). Both FKBP51 and FKBP52 are also involved in the regulation of microtubule-associated protein tau phosphorylation, microtubule assembly, and neurite outgrowth (Chambraud et al. 2010; Cioffi et al. 2011; Jinwal et al. 2010). FKBP51 negatively regulates the Akt/mTOR (mammalian target of rapamycin) complex-1 signaling that mediates the initiation of protein translation (Wang 2011). Accordingly, dysregulation of GR and its molecular chaperones can lead to maladaptive stress response and aging-related cognitive decline (Bizon et al. 2001; Soontornniyomkij et al. 2010; Touma et al. 2011; Wei et al. 2007).

To study the relationship between intracellular GR-mediated signaling and HAND, we applied quantitative immunohistochemistry to assess cellular expression in the isocortex of three functionally related proteins in the GR-mediated signaling (i.e., GR, FKBP51, and FKBP52). We hypothesized that altered cortical expression of these proteins was associated with HAND.

Methods

Study cohort

We assembled 55 autopsy HIV brains, free of cerebral opportunistic infections and neoplasms, obtained during 1999–2008 from the California NeuroAIDS Tissue Network, which were processed according to the protocols adopted by the National NeuroAIDS Tissue Consortium, and included clinical, laboratory, and neuropsychological data. The University of California, San Diego Human Research Protections Program approved the current project and all study participants provided written informed consent to participate. Written consent to autopsy was also obtained.

Neurocognitive functioning was reflected by a composite measure derived from a comprehensive neuropsychological battery assessing speed of information processing, attention/working memory, learning, recall memory, verbal fluency, abstract/executive functioning, and motor/psychomotor speed, with statistical correction for demographic variables (i.e., age, sex, ethnicity, and education), as described previously (Levine et al. 2008). Both the number and severity of deficits across the neuropsychological battery were considered. A clinical diagnosis of HAND was made according to standard criteria and classified with increasing severity into (1) asymptomatic neurocognitive impairment (ANI) requiring at least mild impairment (1 standard deviation below the mean of demographically corrected normative scores) in at least two cognitive domains, (2) mild neurocognitive disorder (MND) requiring, in addition to criteria for ANI, impairment in everyday functioning, and (3) HIV-1-associated dementia (HAD) requiring at least moderate impairment (2 standard deviation below the mean of demographically corrected normative scores) in at least two cognitive domains with marked difficulty in everyday functioning (Antinori et al. 2007). Forty-six of the HIV subjects (84%) underwent neuropsychological testing within a median of 17.2 weeks before death (interquartile range [IQR] of 24.1 weeks). There were 24 subjects who were diagnosed with HAND, including ANI (n=7), MND (n=14), and HAD (n=3). In addition, 11 subjects were affected by neuropsychological impairment due to other or undetermined causes, and 11 subjects were classified as cognitively normal.

Histories of antiretroviral treatment recorded in 46 HIV subjects (84%) within a median of 16.0 weeks (IQR 18.6 weeks) prior to death were grouped into *no treatment* (n=17), *non-HAART regimens* (n=4), and *HAART regimens* (n=25). In the no-treatment group, four subjects had never received antiretroviral drugs. The antiretroviral regimens and their durations varied markedly among HIV subjects on treatment (data not shown).

We used the Psychiatric Research Interview for Substance and Mental Disorders (Hasin et al. 1996) or

Composite International Diagnostic Interview (Robins et al. 1988) to ascertain lifetime substance use disorders. Both were structured diagnostic interviews that yielded DSM-IV diagnoses. Participants were classified with none, past, current, or past and current substance abuse and/or dependence across a number of common drugs of abuse: methamphetamine, cocaine, opiates, cannabis, and alcohol. Of 42 HIV subjects evaluated for methamphetamine use (76%), 16 met criteria for lifetime methamphetamine use (combining *abuse vs. dependence* and *current vs. past* categories) at their final premortem visit.

A diagnosis of major depressive disorder (MDD) was made according to DSM-IV standard criteria and recorded as *lifetime* MDD and (at the patients' final premortem visit) *current* MDD. Of 42 HIV subjects evaluated for MDD (76%), 24 met criteria for lifetime MDD; however, only 6 (of these 24 subjects) were documented to have current MDD.

The general autopsy findings were primarily consistent with acquired immune deficiency syndrome, including opportunistic infections (e.g., with cytomegalovirus, pneumocystis, cryptococcus, and aspergillus) and neoplasms (e.g., non-Hodgkin's lymphomas and Kaposi's sarcoma); other findings included hepatitis C virus (HCV) infection (in 12 of 49 HIV subjects who underwent serological testing), hepatic cirrhosis, and bronchopneumonia. Of the 55 HIV brains studied, 24 showed no significant histopathologic changes, 9 with Alzheimer type II gliosis, 9 with parenchymal vascular lesions (e.g., lacunar infarcts, microinfarcts, and microhemorrhages), 2 with focal white matter rarefaction, 5 with microglial nodules, 1 with a colloid cyst, and 1 with subependymal gray-matter heterotopia. Seven had evidence of HIV encephalitis (i.e., presence of multinucleated giant cells and HIV-1 p24-immunoreactive microglia).

Non-HIV controls with no history of neurological diseases were obtained during 1999–2007 from the California NeuroAIDS Tissue Network (n=11) and the University of California, Los Angeles Division of Neuropathology (n=9). The neuropathologic examination of the control brains revealed in most instances no significant histopathologic changes, except for Alzheimer type II gliosis, lacunar infarcts, microinfarcts, cavernous and venous angiomas, and rare microglial nodules. Seven of the controls had organ transplantation (i.e., heart [n=3], lung [n=2], liver [n=1], and allogeneic unrelated stem cell [n=1]); detailed information on specific immunosuppressive medications (including dosage in the final period of life) used in these patients was not available.

Immunohistochemistry

The primary antibodies were rabbit polyclonals against GR (H-300, Santa Cruz Biotechnology, Santa Cruz, CA, USA, #sc-8992, 1:75 dilution in Dako antibody diluent, DakoCytomation, Carpinteria, CA, USA), FKBP51 (Abcam, Cambridge, MA, USA, #ab2901, 1:200), and FKBP52 (ProteinTech Group, Chicago, IL, USA, #10655-1-AP, 1:100). Five-micrometer-thick formalin-fixed paraffin-embedded tissue sections were deparaffinized with xylene and rehydrated through graded ethanol series and water. Antigen retrieval was carried out in an autoclave at 121°C for 20 min with 10 mM Tris/1 mM EDTA-2Na/0.05% Tween 20 buffer (pH 9). The tissue sections were treated for 30 min with 0.3% hydrogen peroxide/PBS to quench endogenous peroxidase activity and then incubated for 30 min with 5% normal goat serum/PBS (Vector Laboratories, Burlingame, CA, USA). Following 24-h incubation at 4°C with the primary antibody, the immunoreactive signals were detected with the peroxidase-labeled polymer-conjugated goat anti-rabbit IgG secondary antibody (Envision+™, DakoCytomation, 40 min at room temperature) and diaminobenzidine (ImmPACT™ DAB Peroxidase Substrate, Vector Laboratories, 6 min). All the sections were dehydrated through graded ethanol series, rinsed in xylene, and mounted with Cytoseal 60 (Richard-Allan Scientific, Waltham, MA, USA). For the negative reagent control, the primary antibody was omitted.

Quantification of immunoreactivity

Tissue sections immunostained with DAB were digitally scanned using a microscope slide scanner (Aperio ScanScope® GL, Vista, CA, USA) equipped with a 20x objective lens (yielding the resolution of 0.5 μm per pixel). Using the ImageScope™ software (Aperio), a square of 3,000×3,000 μm^2 was extracted from the cortex and subcortical white matter separately. The immunoreactivity was quantified by means of two-dimensional image analysis using the Image-Pro® Analyzer software (version 6.3, Media Cybernetics, Bethesda, MD, USA). Images of same size and resolution were used for outlining the neuroanatomic area of interest (AOI), i.e., the isocortical layers II-VI for GR, FKBP51, and FKBP52, and the subcortical white matter for FKBP51 and FKBP52, as previously described (Soontornniyomkij et al. 2010). For measuring DAB intensity within the AOI, the same setting of histogram-based RGB color segmentation was applied to all sections for each immunostaining. The values of immunoreactivity normalized to AOI (IRn) were calculated from three statistical measurement values, as previously described (Soontornniyomkij et al. 2010).

Statistical analysis

Although the age in each of 2 subject groups was not inconsistent with a Gaussian distribution (p>0.1,

Kolmogorov–Smirnov [KS] test), there was significant difference between the two variances ($p<0.0001$, F test). The postmortem intervals in the HIV group were not normally distributed ($p<0.0001$, KS test), nor were the IRn values for GR, FKBP51, and FKBP52 in each subject group ($p<0.02$, KS test). Therefore, we chose to use non-parametric statistical methods. To determine associations among categorical proportions, the Chi-square (χ^2) and Fisher's exact tests were used. The Mann–Whitney U test was employed to compare continuous variables between two independent groups. For three independent groups, we used the Kruskal–Wallis (KW) test followed by the Dunn's post hoc test. The Spearman's rank correlation (ρ) test was employed to evaluate the linear relationship between two continuous variables in a given group. The GraphPad InStat 3 for Macintosh software (GraphPad Software, La Jolla, CA, USA) was used to perform all statistical analysis. All p values calculated were two-tailed and considered statistically significant at a threshold of $p<0.05$.

Results

Cohort characteristics

The proportion of female subjects was lower in the HIV group ($n=7$ of 55) than in the non-HIV control group ($n=9$ of 19, $p=0.003$, Fisher's exact test). Between HIV and control groups, there was no significant difference in age at death (median 45.0 and 47.5 years, IQR 13.5 and 25.5 years, $n=55$ and 20, respectively; $p=0.88$, U test) or in postmortem intervals (median 12.0 and 15.0 h, IQR 10.0 and 12.5 h, $n=54$ and 19, respectively, $p=0.38$, U test). Across the three age-at-death groups (i.e., ≤39, 40–49, and ≥50 years), no significant difference in age distribution was found between HIV ($n=19$, 19, and 17, respectively) and control ($n=8$, 4, and 8, respectively) groups ($p=0.47$, χ^2 test for independence).

The age at diagnosis was not different between HAND subjects and HIV subjects with normal cognition (median 39.9 and 46.4 years, IQR 12.0 and 14.4 years, $n=24$ and 11, respectively, $p=0.59$, U test). Across the three age-at-diagnosis groups (i.e., ≤39, 40–49, and ≥50 years), no significant difference in age distribution was observed between the HAND subgroup ($n=12$, 6, and 6, respectively) and the normal-cognition subgroup ($n=3$, 3, and 5, respectively, $p=0.38$, χ^2 test for independence).

The clinical diagnosis of HAND was not significantly associated with histologic evidence of HIV encephalitis at autopsy ($n=2$ of 24) compared to the diagnosis of normal cognition in HIV subjects ($n=3$ of 11, $p=0.30$, Fisher's exact test).

Immunohistologic patterns

In the cortex, immunoreactivity signals for GR, FKBP52, and FKBP51 were observed in neurons in the cortical layers II–VI. Both GR (Fig. 1a) and FKBP52 (Fig. 1b) stained the nucleus, perikaryal cytoplasm, and apical dendrite. In contrast, FKBP51 (Fig. 1c–e) stained the perikaryal cytoplasm and apical dendrite, but not the nucleus. In the subcortical white matter, GR stained oligodendroglia, while both FKBP52 and FKBP51 stained axonal fibers. There was a linear correlation between the cortical FKBP51 IRn and that in the white matter both in the control and HIV groups ($\rho=0.83$ and 0.54, respectively, $p<0.0001$ both), as was there for the FKBP52 IRn ($\rho=0.62$ and 0.53, $p=0.003$ and <0.0001, respectively).

In the HIV group, the age at death was not significantly correlated with the IRn for GR (cortex), FKBP51 (cortex and white matter), or FKBP52 (cortex and white matter) [$\rho=-0.04$, −0.19, −0.23, 0.06, and 0.006; $p=0.79$, 0.16, 0.09, 0.69, and 0.97, respectively], nor was the age at death in the control group ($\rho=0.13$, 0.28, −0.06, 0.06, and 0.02; $p=0.57$, 0.23, 0.81, 0.79, and 0.94, respectively). Among the three age-at-death groups (i.e., ≤39, 40–49, and ≥50 years) in all of HIV and control subjects, there was no significant difference in each of the IRn for GR (cortex), FKBP51 (cortex and white matter), or FKBP52 (cortex and white matter) [$p=0.14$, 0.30, 0.39, 0.10, and 0.77, respectively, KW test, Table 1].

The postmortem interval in the HIV group did not show significant correlation with the IRn for GR (cortex), FKBP51 (cortex and white matter), or FKBP52 (cortex and white matter) [$\rho=-0.02$, −0.22, −0.04, −0.06, and −0.17; $p=0.88$, 0.10, 0.76, 0.68, and 0.23, respectively], nor did the postmortem interval in the control group ($\rho=0.31$, −0.34, −0.44, −0.01, and 0.43; $p=0.20$, 0.15, 0.06, 0.95, and 0.06, respectively).

In the HIV group, there was no significant difference between female and male subjects in each of the IRn for GR (cortex), FKBP51 (cortex and white matter), or FKBP52 (cortex and white matter) [$p=0.33$, 0.85, 0.61, 0.67, and 0.28, respectively, U test], nor was there in the control group ($p=0.09$, 0.84, 0.90, 0.16, and 0.78, respectively, U test, Table 1).

Since chronic immunosuppressive treatment (e.g., with FK506 or rapamycin) in subjects with organ transplantation might affect the brain expression of proteins under study, we sought to test this possibility. Among non-HIV controls, the IRn for GR (cortex), FKBP51 (cortex and white matter), and FKBP52 (cortex and white matter) was not significantly different with respect to the presence or absence of organ transplantation ($p=0.48$, 0.59, 0.49, 0.21, and 1, respectively, U test, Table 1).

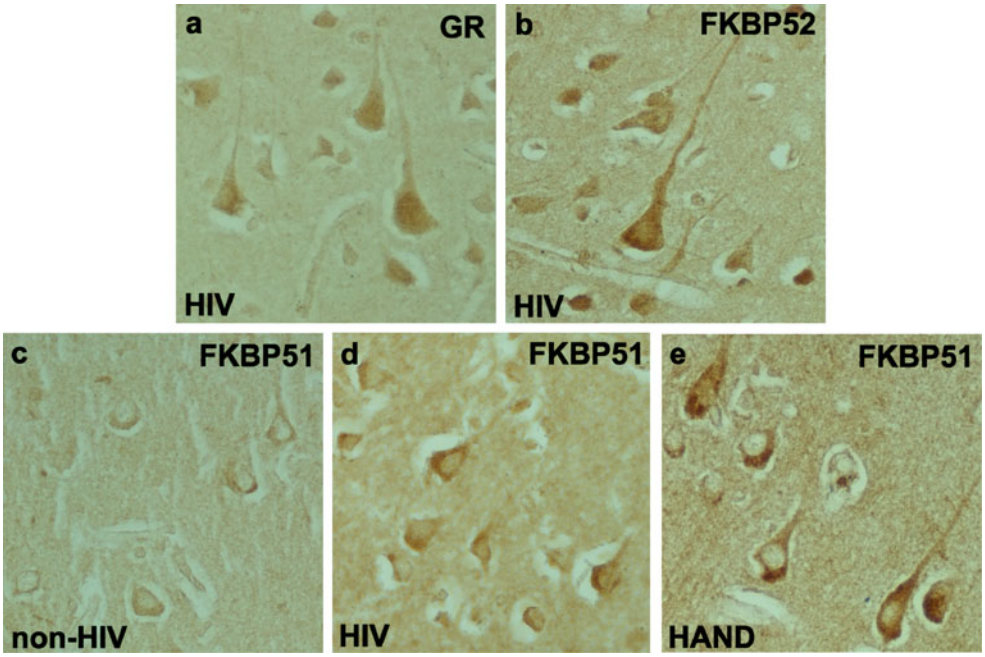

Fig. 1 Immunoreactivity patterns for the glucocorticoid receptor (*GR*, **a**), FK506 binding protein (*FKBP*)-52 (**b**), and FKBP51 (**c**–**e**) in isocortex sections are depicted. The neuronal FKBP51 immunoreactivity appears more intense from the non-HIV group (**c**) through the normal-cognition HIV subgroup (**d**) to the HIV-associated neurocognitive disorders (*HAND*) subgroup (**e**). Images in panels **a**, **b**, and **d** are adjacent sections from the same tissue block. Original magnification, ×400

Increased FKBP51 immunoreactivity in HIV subjects

The FKBP51 IRn was markedly increased in the cortex of HIV subjects compared to non-HIV controls ($p<0.0001$, *U* test), as was that in the white matter ($p<0.0001$, *U* test, Fig. 2a, Table 1).

The FKBP52 IRn showed a trend for decreasing in the cortex but not in the white matter of HIV subjects compared to controls ($p=0.07$ and 0.99, respectively, *U* test). There was no significant difference in the cortical GR IRn between these two groups ($p=0.24$, *U* test).

Higher FKBP51 immunoreactivity in HAND subjects

Among HIV subjects who underwent neuropsychological testing, those affected by HAND showed higher FKBP51 IRn in the cortex compared to HIV subjects with normal cognition ($p=0.02$, *U* test, Fig. 2b, Table 1). In the white matter, no significant difference in the FKBP51 IRn was found between these two subgroups ($p=0.85$, *U* test, Fig. 2b).

On comparison analysis of the cortical FKBP51 IRn across four subcategories of cognitive status with increasing severity (i.e., normal, ANI, MND, and HAD) in the HIV group, the apparent difference did not reach statistical significance ($p=0.10$, KW test). Nonetheless, there was a trend for increasing cortical FKBP51 IRn with increasing severity of HAND when MND ($n=14$) and HAD ($n=3$) subcategories were combined ($p=0.08$, KW test, Fig. 2c). The similar analysis of FKBP51 IRn in the white matter showed no significant differences ($p=0.54$ and 0.58, respectively, KW test).

The FKBP52 IRn distribution in the cortex and white matter was not significantly different between HAND subjects and cognitively normal HIV subjects ($p=0.47$ and 0.54, respectively, *U* test), nor was that of the cortical GR IRn ($p=0.45$, *U* test). Either in the cortex or white matter, there was no significant difference in FKBP52 IRn across the four subcategories of cognitive status ($p=0.88$ and 0.92, respectively, KW test), nor was there when MND and HAD subcategories were combined ($p=0.72$ and 0.80, respectively, KW test). No significant difference in cortical GR IRn was found across the four subcategories of cognitive status ($p=0.89$, KW test) or when MND and HAD subcategories were combined ($p=0.73$, KW test).

No changes in FKBP51 immunoreactivity with other HIV-related factors

The FKBP51 IRn in the cortex and white matter (Table 1) was not significantly different with regard to the diagnosis of lifetime MDD ($p=0.38$ and 0.33, respectively, *U* test), HIV encephalitis ($p=0.61$ and 0.16, respectively, *U* test), HCV infection ($p=0.44$ and 0.27, respectively, *U* test), and lifetime methamphetamine use ($p=0.60$ and 0.15, respectively, *U* test), nor was that with respect to antiretroviral treatment ($p=0.35$ and 0.09, respectively, KW test). Nonetheless, there was a trend for increasing FKBP51 IRn in the white matter of HIV subjects with current MDD ($p=0.07$, *U* test), while no significant difference was seen in the cortex ($p=0.65$, *U* test).

Either in the cortex or white matter, the FKBP52 IRn was not significantly different with regard to the diagnosis of lifetime MDD ($p=0.45$ and 0.91, respectively, *U* test),

Table 1 Median (interquartile range) values of the immunoreactivity normalized to the neuroanatomic area measured (*IRn*)

	n	GR IRn	FKBP51 IRn		FKBP52 IRn	
		Cortex	Cortex	WM	Cortex	WM
HIV infection						
(−)	20	0.42 (1.46)	0.48 (1.24)	13.25 (22.65)	1.42 (4.45)	4.25 (12.52)
(+)	55	0.89 (2.24)	7.68 (22.28)	35.89 (13.22)	0.71 (1.84)	2.09 (13.06)
Cognitive status[a]						
Normal	11	0.96 (1.58)	4.96 (7.77)	33.81 (19.91)	0.82 (1.75)	8.22 (14.57)
HAND	24	0.72 (1.20)	15.86 (24.19)	36.37 (8.57)	0.69 (1.71)	2.05 (8.95)
ANI	7	0.82 (1.54)	7.65 (17.47)	40.37 (5.72)	0.51 (0.78)	2.01 (10.60)
MND	14	0.72 (0.96)	22.30 (18.42)	35.96 (7.51)	0.84 (1.92)	1.78 (8.23)
HAD	3	0.41 (1.48)	5.47 (9.62)	29.00 (10.54)	0.82 (1.22)	2.88 (5.56)
MND or HAD	17	0.64 (1.04)	20.46 (23.84)	35.89 (8.76)	0.82 (1.95)	2.09 (8.61)
Lifetime MDD[a]						
(−)	18	0.85 (1.92)	12.62 (24.32)	36.56 (12.45)	0.69 (1.64)	6.76 (9.60)
(+)	24	0.45 (0.88)	6.93 (9.41)	33.98 (16.99)	0.88 (2.71)	2.31 (14.91)
Current MDD[a]						
(−)	35	0.80 (1.34)	6.94 (18.59)	34.42 (16.61)	0.93 (2.30)	6.88 (13.21)
(+)	6	0.59 (1.42)	8.89 (4.77)	40.27 (5.23)	0.57 (1.11)	1.47 (17.80)
HIV encephalitis[a]						
(−)	48	0.87 (2.46)	7.66 (17.66)	35.87 (13.70)	0.80 (2.33)	1.87 (12.63)
(+)	7	1.91 (1.97)	29.79 (36.55)	44.09 (15.38)	0.50 (0.37)	9.00 (9.25)
HCV infection[a]						
(−)	37	0.80 (1.71)	9.86 (22.82)	37.07 (14.26)	0.78 (2.51)	7.66 (15.50)
(+)	12	1.10 (2.33)	5.62 (22.22)	34.92 (13.41)	0.61 (0.96)	1.30 (3.02)
Lifetime METH use[a]						
(−)	26	0.44 (1.65)	8.77 (18.52)	36.89 (12.53)	0.80 (1.74)	6.54 (13.18)
(+)	16	0.91 (0.80)	6.06 (9.48)	33.98 (15.11)	0.94 (2.42)	4.85 (13.49)
Antiretroviral treatment[a]						
No	17	1.82 (1.90)	8.66 (24.32)	37.52 (7.64)	1.20 (2.80)	9.00 (13.79)
ART	4	1.11 (0.94)	5.37 (5.47)	25.97 (6.17)	1.92 (1.10)	18.97 (27.08)
HAART	25	0.43 (0.78)	6.94 (15.92)	33.67 (19.06)	0.61 (1.19)	1.68 (8.20)
Age-at-death group (years)						
≤39	27	0.43 (1.43)	6.28 (21.91)	34.52 (24.40)	0.54 (1.02)	1.91 (11.29)
40–49	23	1.84 (2.07)	7.68 (21.66)	31.64 (17.42)	1.44 (2.70)	2.88 (13.00)
≥50	25	0.86 (1.21)	2.48 (7.66)	22.72 (25.29)	0.82 (2.52)	6.65 (14.40)
Sex (HIV group)						
Female	7	1.33 (1.95)	8.08 (18.61)	33.67 (7.63)	0.78 (1.82)	12.34 (22.52)
Male	48	0.83 (2.17)	7.66 (21.85)	36.37 (14.43)	0.68 (1.84)	2.05 (10.79)
Sex (non-HIV group)						
Female	9	0.29 (0.36)	0.52 (0.91)	3.28 (22.23)	0.68 (1.03)	1.00 (14.27)
Male	10	1.34 (1.90)	0.30 (1.65)	14.31 (21.96)	1.75 (9.69)	6.98 (9.55)
Organ transplantation[b]						
(−)	13	0.43 (2.12)	0.43 (1.88)	12.48 (14.89)	0.68 (3.93)	2.12 (12.15)
(+)	7	0.26 (1.26)	0.52 (0.91)	16.51 (23.00)	1.51 (3.84)	6.39 (14.17)

GR glucocorticoid receptor, *FKBP* FK506 binding protein, *WM* subcortical white matter, *HAND* HIV-associated neurocognitive disorders, *ANI* asymptomatic neurocognitive impairment, *MND* minor neurocognitive disorder, *HAD* HIV-1-associated dementia, *MDD* major depressive disorder, *HCV* hepatitis C virus, *METH* methamphetamine, *HAART* highly active antiretroviral therapy regimens, *ART* antiretroviral therapy: non-HAART regimens, (−) absence, (+) presence

[a]HIV group only

[b]Non-HIV group only

current MDD (p=0.46 and 0.96, respectively, U test), HIV encephalitis (p=0.24 and 0.56, respectively, U test), and lifetime methamphetamine use (p=0.89 and 0.87, respectively, U test), nor was that with respect to antiretroviral treatment (p=0.43 and 0.28, respectively, KW test). The FKBP52 IRn in the white matter was markedly lower in HCV-HIV co-infected

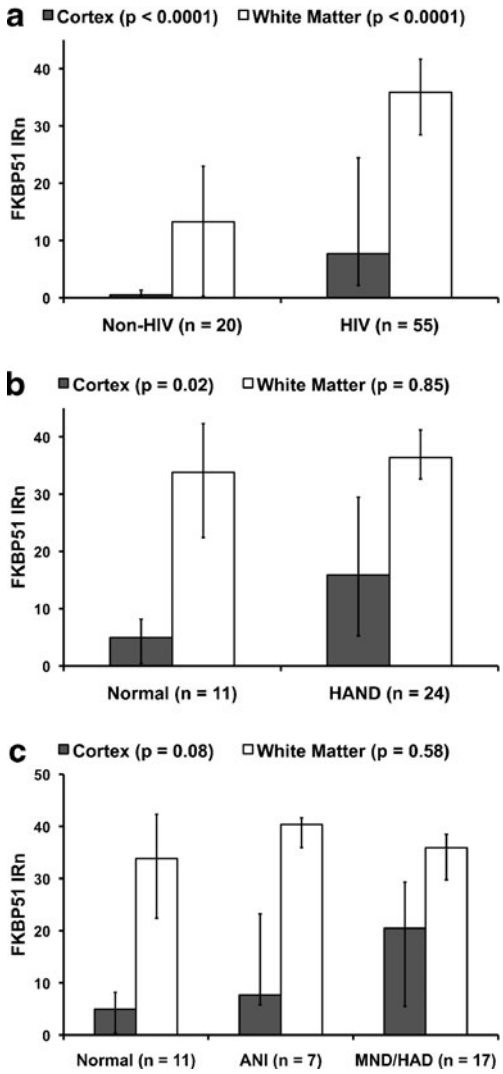

Fig. 2 **a**, **b**, **c** Median with respective interquartile range (*error bar*) values of the immunoreactivity normalized to the neuroanatomic area measured (*IRn*) for FK506 binding protein-51 (*FKBP51*) are shown. The *p* values are two-tailed (Mann–Whitney *U* test [**a**, **b**] and Kruskal–Wallis test [**c**]). *HAND* HIV-associated neurocognitive disorders, *ANI* asymptomatic neurocognitive impairment, *MND* minor neurocognitive disorder, *HAD* HIV-1-associated dementia

subjects (p=0.01, *U* test), while no significant difference was found in the cortex (p=0.21, *U* test).

The cortical GR IRn was not significantly different with regard to the diagnosis of lifetime MDD (p=0.19, *U* test), current MDD (p=0.68, *U* test), HIV encephalitis (p=0.87, *U* test), HCV infection (p=0.57, *U* test), and lifetime methamphetamine use (p=0.32, *U* test). For antiretroviral treatment, the distribution of GR IRn was significantly different across the three subgroups (i.e., *no treatment*, *non-HAART regimens*, and *HAART regimens*; p=0.02, KW test). On Dunn's post hoc analysis, the GR IRn was significantly lower in the HAART subgroup than in the no-treatment subgroup (p<0.05).

Discussion

Dysregulation of proteins involved in GR-mediated signaling (i.e., GR, FKBP51, and FKBP52) might contribute to maladaptive stress response and aging-related cognitive decline (Bizon et al. 2001; Soontornniyomkij et al. 2010; Touma et al. 2011; Wei et al. 2007). We studied whether altered cortical expression of these proteins could also be associated with HAND. The patterns of neuronal immunoreactivity for GR and FKBP52 (both in the nucleus and cytoplasm), and for FKBP51 (only in the cytoplasm) shown in the present study were consistent with the proposed mechanism of GR heterocomplex trafficking from the cytoplasm to the nucleus, i.e., upon GR binding to glucocorticoids, FKBP51 in the GR heterocomplex is competitively replaced by FKBP52 to facilitate translocation to the nucleus (Grad and Picard 2007; Wochnik et al. 2005). By using quantitative immunohistochemistry, we found that FKBP51 expression was increased in HIV subjects compared to non-HIV controls both in the cortex and subcortical white matter. More importantly in our study, the cortical FKBP51 expression was higher in subjects with HAND than in HIV subjects with normal cognition. In addition, there was a trend for increasing FKBP51 expression in the cortex with the increasing severity of HAND. We did not observe significant alterations in FKBP51 expression with respect to HCV infection, lifetime methamphetamine use, or antiretroviral treatment in HIV subjects, suggesting that increased cortical expression of FKBP51 is relatively specific to HAND. In addition, the FKBP51 immunoreactivity did not appear to be affected either by the age at death or postmortem interval.

Our interpretation is that chronic stress inherent in the clinical course of HIV infection (Hand et al. 2006; Reif et al. 2011) induces hypercortisolemia (Christeff et al. 1997) and thereby elevation of GR-mediated signaling, which in turn enhances neuronal expression of FKBP51 [an inhibitor for GR activity (Grad and Picard 2007)] as the intracellular ultra-short negative feedback in an attempt to attenuate GR sensitivity (Jääskeläinen et al. 2011). The finding of higher cortical FKBP51 expression in HAND subjects than in cognitively normal HIV subjects may reflect a response to chronic stress of greater extent, potentially leading to maladaptive stress reactions of the HPA axis and cognitive decline. Unfortunately, premortem data on the circulating cortisol levels (e.g., free cortisol in plasma or saliva) were not available in our present study to support the existence of chronic stress in HIV and specifically in HAND subjects (Hellhammer et al. 2009). The mechanistic scenario we are proposing is supported by other previous studies in animal models showing that mRNA expression of the *Fkbp5* gene (encoding FKBP51) was induced by dexamethasone treatment or different stress paradigms (e.g., 30-min restrained stress and 24-h food deprivation) in specific brain regions of

young adult mice (Scharf et al. 2011). Also, chronic corticosterone exposure decreased DNA methylation and increased mRNA expression of the *Fkbp5* gene in the mouse hippocampus, as well as decreased mRNA expression of the *Nr3c1* gene [encoding GR] (Lee et al. 2010). However, we did not observe any significant alteration in GR protein expression in the mid-frontal cortex of HAND subjects.

In response to elevated GR-mediated signaling, in addition to the intracellular ultra-short negative feedback mechanisms described above, the HPA axis activity is regulated by direct negative feedback action of glucocorticoids and indirect trans-synaptic inhibitory projections of the forebrain and brainstem onto parvocellular neurons in the hypothalamic paraventricular nucleus that produce corticotropin-releasing hormone (Jankord and Herman 2008). Enhanced FKBP51 expression in the forebrain as seen in HAND subjects in our study might lead to increased GR resistance and decreased efficiency of the forebrain negative feedback of the HPA axis activity (Binder 2009). Responses of the HPA axis to psychological or physiological stress are also modulated by sex steroids and may be different with respect to sex (Kudielka and Kirschbaum 2005). Although the proportion of female subjects was lower in the HIV group than in the control group, we did not find significant differences in FKBP51 immunoreactivity between female and male subjects either in the HIV or control group.

As a scaffolding protein, FKBP51 affects the Akt/mTOR kinase signaling pathway by bringing PHLLP phosphatase closer to the Akt-Ser473 site to facilitate the dephosphorylation of Ser473, which in turn down-regulates Akt/mTOR signaling (Wang 2011). Increased cortical FKBP51 expression as seen in HAND subjects in our study might negatively regulate the initiation of protein translation mediated by mTOR complex-1 signaling and thereby contribute to HAND, as the long-lasting forms of synaptic plasticity and memory are dependent biochemically on the expression of new proteins both somatically and dendritically (Hoeffer and Klann 2010).

Our finding of FKBP51 and FKBP52 expression in the white-matter axonal fibers is consistent with previous animal studies showing FKBP51 expression localized in axonal tracts along with tau protein (Jinwal et al. 2010). Both of these immunophilins have been shown to regulate tau phosphorylation and microtublule assembly (important for protein trafficking and neurite outgrowth) in a somewhat opposing manner (Cioffi et al. 2011). By forming complexes with heat shock protein-90, FKBP51 enhances tau dephosphorylation and thereby tubulin polymerization, while FKBP52 binds preferentially to phosphorylated tau and promotes microtubule destabilization (Chambraud et al. 2010; Cioffi et al. 2011; Jinwal et al. 2010).

Both genetic variants and epigenetic modifications in non-coding regions of the *FKBP5* gene may influence basal and glucocorticoid-induced expression of this gene, leading to differences in GR sensitivity (Jääskeläinen et al. 2011). Single nucleotide polymorphisms (SNPs) associated with enhanced *FKBP5* expression (e.g., T allele in rs1360780) following GR-mediated signaling might lead to increased GR resistance and decreased efficiency of the feedback inhibition of the HPA axis activity (Binder 2009). On the other hand, healthy human subjects carrying SNPs associated with lower *FKBP5* expression (e.g., C allele in rs1360780) were shown to react with stronger suppression of plasma cortisol following low-dose dexamethasone treatment (Touma et al. 2011). The high-induction *FKBP5* alleles have been over-represented in individuals affected by MDD, bipolar disorder, and post-traumatic stress disorder (Binder 2009). SNPs in the *FKBP5* gene may interact with HIV-related factors to influence the susceptibility to HAND. It is of interest to explore in future studies whether particular SNPs in the *FKBP5* gene are associated with enhanced FKBP51 expression observed in HAND subjects, similar to those reported in mood disorders in the general population (Binder et al. 2004; Jääskeläinen et al. 2011) and in individuals exhibiting delayed recovery from psychosocial stress (Ising et al. 2008). In the present study, we did not observe any significant alteration in FKBP51 expression by immunohistochemistry with regard to a *lifetime* diagnosis of MDD in HIV subjects. When considering a *current* MDD diagnosis (made at the patients' final premortem visit), which was more temporally approximate to the postmortem brain examination, we found a trend for higher FKBP51 expression in the white matter but not in the cortex of HIV subjects with *current* MDD.

A trend for decreasing cortical FKBP52 expression in the HIV group compared with the control was seen in our present study. This FKBP52 immunoreactivity finding on tissue sections appeared inconsistent with previous data from the mRNA and protein analysis of tissue homogenates (Tatro et al. 2009), which might be explained by differences in subject cohorts and quantitative methods used. In the present study using computer-assisted image analysis, the RGB color segmentation was set to measure immunoreactivity signals confined primarily to the soma and apical dendrites of cortical neurons, with exclusion of the neuropil. In contrast, the measurement in tissue homogenates would include all components of the cortex.

It remains to be determined whether chronic immunosuppressive therapy (e.g., with FK506 or rapamycin) alters the brain expression of FKBP51, FKBP52, or other immunophilins such as FKBP12. In the present study, detailed data on specific immunosuppressive medications (including dosage in the final period of life) used in non-HIV subjects with organ transplantation were not available. Nonetheless, the expression levels of FKBP51 and FKBP52 did not appear to change with the history of organ transplantation.

In conclusion, the increased cortical expression of FKBP51 in HIV subjects compared to that in controls might represent intracellular ultra-short negative feedback in an attempt to reduce GR sensitivity in the setting of chronic stress-induced elevation of GR-mediated signaling inherent in HIV infection. The further increased FKBP51 expression might lead to maladaptive stress response and HAND. Notably, the increased cortical FKBP51 expression might serve as a marker of disease progression as we found a trend for increasing FKBP51 expression with the increasing severity of HAND. This neuronal FKBP51 up-regulation may provide an avenue of therapeutic intervention with immunophilin ligands such as FK506, rapamycin, and non-immunosuppressive analogs for neuroprotective and neuroregenerative effects as shown in cell systems of HIV-1 protein-induced neurotoxicity (Caporello et al. 2006; Steiner et al. 2007), as well as in animal models of ischemic stroke (Chauhan et al. 2011) and methamphetamine-induced dopaminergic neurotoxicity (Koike et al. 2005).

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