J. Neurovirol. (2016) 22:327–335
DOI 10.1007/s13365-015-0401-8

Expression of *CHRFAM7A* and *CHRNA7* in neuronal cells and postmortem brain of HIV-infected patients: considerations for HIV-associated neurocognitive disorder

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Received: 3 July 2015 / Revised: 13 October 2015 / Accepted: 27 October 2015 / Published online: 13 November 2015

Abstract Despite the recent advances in antiretroviral therapy, human immunodeficiency virus type 1 (HIV-1) remains a global health threat. HIV-1 affects the central nervous system by releasing viral proteins that trigger neuronal death and neuroinflammation, and promotes alterations known as HIV-associated neurocognitive disorders (HAND). This disorder is not fully understood, and no specific treatments are available. Recently, we demonstrated that the HIV-1 envelope protein $gp120_{IIIB}$ induces a functional upregulation of the α7-nicotinic acetylcholine receptor (α7) in neuronal cells. Furthermore, this upregulation promotes cell death that can be abrogated with receptor antagonists, suggesting that α7 may play an important role in the development of HAND. The partial duplication of the gene coding for the α7, known as *CHRFAM7A*, negatively regulates α7 expression but its role in HIV infection has not been studied. Hence, we studied both *CHRNA7* and *CHRFAM7A* regulation patterns in various $gp120_{IIIB}$ in vitro conditions. In addition, we measured *CHRNA7* and *CHRFAM7A* expression levels in postmortem brain samples from patients suffering from different stages of HAND. Our results demonstrate the induction of *CHRNA7* expression accompanied by a significant downregulation of *CHRFAM7A* in neuronal cells when exposed to pathophysiological concentrations of $gp120_{IIIB}$. Our results suggest a dysregulation of *CHRFAM7A* and *CHRNA7* expressions in the basal ganglia from postmortem brain samples of HIV+ subjects and expand the current knowledge about the consequences of HIV infection in the brain.

Keywords gp120 · HIV · HAND · Nicotinic acetylcholine receptor · CHRNA7 · CHRFAM7A

Introduction

The human immunodeficiency virus type 1 (HIV-1) is considered one of the principal pandemics of the twenty-first century with approximately 34 million of subjects infected globally (Joint United Nations Programme on HIV/AIDS (UNAIDS) 2013). In addition to developing acquired immunodeficiency syndrome (AIDS), infected individuals may also develop neurological complications known as HIV-associated neurocognitive disorders (HAND). HAND include asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HAD) (Antinori et al. 2007). HAD results in disabling cognitive impairment accompanied by motor dysfunction, speech problems, and overt behavioral changes (González-Scarano and Martín-García 2005; Clifford and Ances 2013). Although the incidence of HAD has decreased (Bhaskaran et al. 2008), the prevalence of HAND, mostly of the milder forms of neurocognitive impairment (ANI and MND), could be as high as 50 % of patients (Sacktor et al. 2002; Cysique et al. 2004; Heaton et al. 2011). Moreover, the high prevalence of HAND occurs despite administration of combined antiretroviral therapy

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(cART) (Mothobi and Brew 2012). For instance, under cART, HAND persist despite systemic or brain viral load reduction or control (Cysique and Brew 2011; Koneru et al. 2014).

HIV is unable to infect neurons due to their lack of primary CD4 receptors; however, neuronal expression of both CCR5 and CXCR4 secondary receptors could allow viral interactions (Hesselgesser et al. 1997). Several hypotheses have emerged to explain the cause of HAND including the neurotoxic properties of viral proteins and the severe uncontrolled chronic neuroinflammation (Kong et al. 1996; Heaton et al. 2011). Particularly, the HIV-1 viral envelope protein gp120 has been reported to have various neurotoxic properties in vitro and in vivo including the inhibition of adult neural progenitor cells proliferation, neuronal damage and induction of apoptosis, and cell death of human neuronal cells (Toggas et al. 1994; Meucci and Miller 1996; Hesselgesser et al. 1998; Jana and Pahan 2004; Bardi et al. 2006; Okamoto et al. 2007; Ballester et al. 2012). Moreover, the severity of brain damage correlates with gp120 levels (Desai et al. 2013).

The alpha7 nicotinic acetylcholine receptor (α7) is one of the most common receptors expressed in the mammalian brain (Dani and Lester 2001). The α7 subunit is encoded by the *CHRNA7* gene in chromosome 15 and is composed of ten exons (Gault et al. 1998). Interestingly, the *CHRNA7* has a counterpart gene named *CHRFAM7A* (Gault et al. 1998). The *CHRFAM7A* gene product, dupα7, exerts a regulatory/inhibitory role on the α7 ion channel activity and expression (de Lucas-Cerrillo et al. 2011; Araud et al. 2011), although a recent work has challenged these results showing that dupα7 and α7 can form functional heteropentamers with altered responses to choline and varenicline (Wang et al. 2014). This may be due to differences in the expression system used that could influence ion channel functionality and assembly—the first study used oocytes while the most recent used Neuro2a cells, and the use of α7's chaperone RIC-3 in Neuro2a cells but not in oocytes. For a comprehensive review about dupα7 refer to (Costantini et al. 2014). Notwithstanding, although the α7 has been amply studied in CNS, very little is known about its role in the neuropathology of HIV infection. We recently demonstrated that $gp120_{IIIB}$ induces a functional α7 upregulation in neuronal cells and that the expression of $gp120_{IIIB}$ in the brain of a transgenic mouse model also induces the overexpression of α7 in the brain, particularly in the striatum, basal ganglia's primary input (Ballester et al. 2012). Moreover, we found that the activation of upregulated α7 in these neuronal cells induces cell death in a calcium-dependent manner (Ballester et al. 2012). In light of the possible role of α7 in the HIV neuropathogenesis, we evaluated the mRNA expression patterns of *CHRNA7* and *CHRFAM7A* and the expression ratio *CHRNA7*:*CHRFAM7A* upon $gp120_{IIIB}$ application in a human neuronal cell line and in postmortem brain samples from HIV-infected patients expressing different severity stages of neurocognitive impairment.

Materials and methods

Reagents

All reagents were purchased from Sigma - Aldrich unless otherwise specified.

Cell culture and treatments

SH-SY5Y neuronal cell line was obtained from ATCC (Manassas, VA). Cells were incubated at 37 °C with 5 % CO_2 in DMEM/F-12 media supplemented with 10 % fetal bovine serum, 1 % penicillin/streptomycin, and 1.2 g of $NaHCO_3$. Cultures were performed in 12-well plates followed by treatments with $gp120_{IIIB}$ (Fitzgerald Industries International, Concord, MA) at 0.0015, 0.015, 0.15, 1.5, or 15 nM for the indicated time. For time-dependent experiments, the concentration of $gp120_{IIIB}$ was 0.15 nM. The CXCR4 antagonist, AMD3100 (EMD Chemicals, Inc., Gibbstown, NJ), was used at 1 μM and added 10 min prior to $gp120_{IIIB}$ application. The range of gp120 concentrations tested here was based on gp120 quantification studies using plasma, serum, and tissues from HIV-infected subjects (Gilbert et al. 1991; Oh et al. 1992; Santosuosso et al. 2009; Rychert et al. 2010). To our knowledge, there are no quantification studies to determine gp120 in the brain or cerebrospinal fluid (CSF). However, there is a robust body of evidence demonstrating that indeed gp120 is present in the central nervous system and CSF, even though no evidence of quantification is available in the literature (Buzy et al. 1989; Rolfs and Schumacher 1990; Ruță et al. 1998; Cashion et al. 1999; Jones et al. 2000; Ohagen et al. 2003; Pillai et al. 2006). Moreover, the existence of anti-gp120 antibodies in the CNS unequivocally attests to its presence (Lucey et al. 1993; Di Stefano et al. 1996; Trujillo et al. 1996).

RNA extraction and quantitative RT-PCR assay

In SH-SY5Y neuronal cells, total RNA samples were extracted using TRIzol Reagent (Invitrogen Corporation, Carlsbad, CA). To eliminate possible genomic contamination, extracted RNA was treated with DNase using the Ambion DNA-free kit (Ambion, Austin, TX). Quantification of total RNA was performed using a Nanodrop system (Thermo Scientific, Waltham, MA). The cDNA synthesis was carried out using 0.75 μg of total RNA with the iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, Inc., Hercules, CA) following the manufacturer's instructions. After optimization of the PCR conditions, real-time PCR experiments were performed using the iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA) in a Mastercycler® Ep Realplex Thermal Cycler (Eppendorf, NY). *CHRNA7* and GAPDH primers where used at a final concentration of 400 nM, *CHRFAM7A*

primers at 100 nM together with 100 ng of cDNA. Primers were designed using IDT Designer Software (Integrated DNA Technologies, Inc.). The primers employed to amplify the genes of interest from cells and tissue samples were the following: *CHRNA7* forward, 5′-GCTCCGGGACTCAACATG-3′; reverse, 5′-GGGATTGTAGTTCTTGACCAGC-3′; *CHRFAM7A* forward, 5′-CCGAAGTTACTGGCCTCTATC-3′ reverse, 5′-CTGAGTCGTGTAGATAAGCTCTC-3′, and for GAPDH: forward, 5′-GCTCTCTGCTCCTCCTGTTC-3′, reverse, 5′-GACTCCGACCTTCACCTTCC-3′. All primers were used with an annealing temperature of 55 °C.

Tissue processing and RNA extraction

Postmortem brain tissues from HIV-infected patients were obtained from the Texas NeuroAIDS Research Center (IRB#: 98–402), California NeuroAIDS Tissue Network (IRBs#: 00000353, 00000354, 00000355, and 000002758), and UCLA National Neurological AIDS Bank (IRB#: 10000525). Tissue samples were pulverized in liquid nitrogen under RNase-free conditions. RNA extraction was performed using TRIzol Reagent (Invitrogen, Eugene, OR) following manufacturer's instructions. The RNA integrity was assayed in 1 % electrophoresis agarose gel. Samples were processed for qRT-PCR as described above.

Statistical analyses

To evaluate the statistical significance of changes in expression levels of *CHRNA7* and *CHRFAM7A* in neuronal cells, we used one-way ANOVA followed by Holm-Sidak's multiple comparison test which allowed corrections for multiple comparisons with a fixed alpha value (0.05). Spearman correlation was used to identify correlations between *CHRFAM7A* and *CHRNA7* expression levels in neuronal cells. The detected outliers were excluded from analysis. Statistical analysis was conducted using the GraphPad Prism 6 software (GraphPad Software, San Diego, CA, www.graphpad.com).

Results

$gp120_{IIIB}$ promotes the downregulation of *CHRFAM7A* in neuronal cells

Neuronal cells were exposed to various concentrations of $gp120_{IIIB}$ including those within the pathophysiological range quantified in HIV-infected patients (0.0015, 0.015, and 0.15 nM) (Gilbert et al. 1991; Oh et al. 1992; Santosuosso et al. 2009; Rychert et al. 2010). Measurements of *CHRNA7* and *CHRFAM7A* levels after addition of pathophysiological relevant $gp120_{IIIB}$ concentrations show that the *CHRFAM7A* was downregulated in a dose-dependent manner, and that the expression of *CHRNA7* was induced (Fig. 1a). Noteworthy is that this effect in *CHRFAM7A* expression levels is sustained even when supraphysiological concentrations of $gp120_{IIIB}$ were used (15 nM). Further evaluation shows that *CHRNA7*:*CHRFAM7A* expression ratios increase with the $gp120_{IIIB}$ treatment (Fig. 1b).

A pathophysiological dose of $gp120_{IIIB}$ time-dependently dysregulates *CHRNA7* and *CHRFAM7A* expression in neuronal cells

CXCR4 is a coreceptor employed by HIV to infect immune cells and is expressed by neurons (Hesselgesser et al. 1997). Neuronal cells exposed to $gp120_{IIIB}$ (0.15 nM) at different time points showed that the α7 gene, *CHRNA7*, was upregulated after 12 h post $gp120_{IIIB}$ exposure whereas *CHRFAM7A*

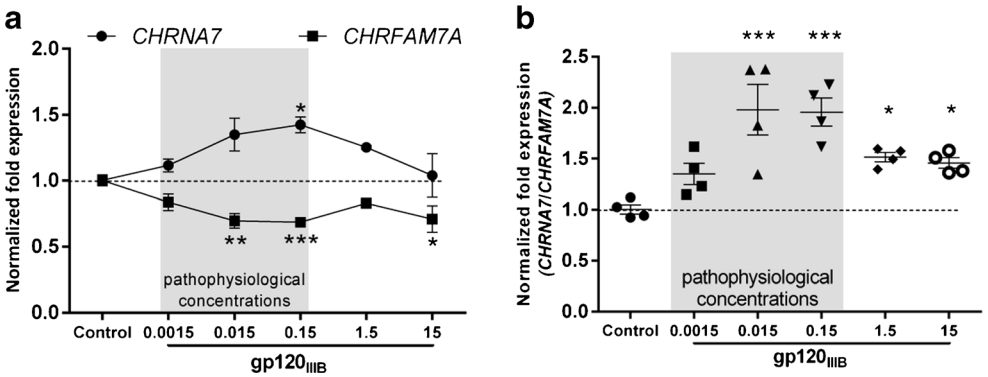

Fig. 1 $gp120_{IIIB}$ induces the downregulation of *CHRFAM7A* in neuronal cells. **a** Neuronal cells were incubated with $gp120_{IIIB}$ 0.0015, 0.015, 0.15, 1.5, and 15.0 nM for 12 h. A downregulation of *CHRFAM7A* was observed under pathophysiological (0.015 and 0.15 nM) and supraphysiological doses (15 nM). **b** *CHRNA7*:*CHRFAM7A* expression ratio shows a significant increase under pathophysiological and supraphysiological conditions as compared to control. In panels **a** and **b**, results were normalized to the control. *$P \leq 0.05$, **$P \leq 0.01$, *** $P \leq 0.001$. Statistical analysis one-way ANOVA followed by Holm-Sidak's multiple comparison tests, *error bars* represents SEM. For all panels, $n=4$ independent experiments

downregulation initiated as early as 15 min post $gp120_{IIIB}$ application, and lasted for 24 h (Fig. 2a). Moreover, a ratio analysis demonstrates an early increase in the *CHRNA7*:*CHRFAM7A* expression (Fig. 2b).

A CXCR4 antagonist abrogates the $gp120_{IIIB}$-induced dysregulation of *CHRNA7*

To determine whether the *CHRNA7* and *CHRFAM7A* dysregulation depends on CXCR4 stimulation, an antagonist (AMD3100) was applied prior to $gp120_{IIIB}$ addition. Our results show that CXCR4 blockade abrogates $gp120_{IIIB}$-induced upregulation of *CHRNA7* (Fig. 3). Unexpectedly, *CHRFAM7A* was downregulated by AMD3100 in the absence of $gp120_{IIIB}$ (Fig. 3a).

The *CHRNA7* and *CHRFAM7A* expression levels in the basal ganglia of HIV-infected subjects

It is known that the basal ganglia is an area of the brain that is severely affected in HIV-infected patients (Woods et al. 2009) and contains some of the brain's highest viral load (Kure et al. 1990). We recently found that the *CHRNA7* gene product, α7, is upregulated in the striatum (a component of the basal ganglia) of mice expressing $gp120_{IIIB}$ in the brain (Ballester et al. 2012). Here, we examined *CHRNA7*, *CHRFAM7A*, and *CHRNA7*:*CHRFAM7A* levels in the basal ganglia of HIV-infected postmortem basal ganglia samples representing different stages of neurological impairment. Table 1 summarizes the subject characteristics. Evaluation of *CHRFAM7A* and *CHRNA7* genes in HIV+ patients shows that *CHRNA7* is significantly expressed at higher levels than *CHRFAM7A* (Fig. 4a), which is consistent with what we observed in the neuronal cells (Fig. 1a). The *CHRNA7*:*CHRFAM7A* ratio of the HIV+ group was increased in these patients (Fig. 4b). Examination of *CHRNA7* levels in basal ganglia from HIV-infected subjects suffering from different stages of cognitive impairment showed no significant differences (Fig. 4c). In terms of *CHRFAM7A*, at first glance, patients with normal cognition are not different from HIV− (Fig. 4d) but detailed examination of the distribution of *CHRFAM7A* levels in normal cognition patients demonstrates two distinguishable groups identified as subgroups A and B (Fig. 4d). Evaluation of these groups revealed that subgroup A is upregulated while subgroup B is downregulated for *CHRFAM7A* expression (Fig. 4d). Furthermore, examination of *CHRFAM7A* levels in the minor cognitive-motor disorder (MCMD) group suggests that only HIV+ patients with low *CHRFAM7A* levels develop MCMD (Fig. 4e). Ratio analysis demonstrates no significant differences in the *CHRNA7*:*CHRFAM7A* expression ratio in the basal ganglia of these patients, and a linear trend analysis showed a non-significant (P=0.08) increment in *CHRNA7*:*CHRFAM7A* with increasing cognitive impairment severity (Fig. 4f).

Discussion

HIV-infected patients suffer from cognitive disorders associated with the infection. In a previous report, we demonstrated that $gp120_{IIIB}$ is capable of inducing a functional upregulation of the α7 in neuronal cells and that this upregulation promotes cell death in a calcium-dependent manner (Ballester et al. 2012). In the current study, we expand these observations demonstrating that $gp120_{IIIB}$ induces the upregulation of the α7 gene *CHRNA7* and the downregulation of its partial duplication, *CHRFAM7A*, in neuronal cells. The significant reduction in *CHRFAM7A* expression could imply that dupα7's dominant negative effect on α7's functionality may be concomitantly reduced, thus providing a modulatory/regulatory explanation for our previous observations (Ballester et al. 2012). Because of dupα7's dominant negative regulatory

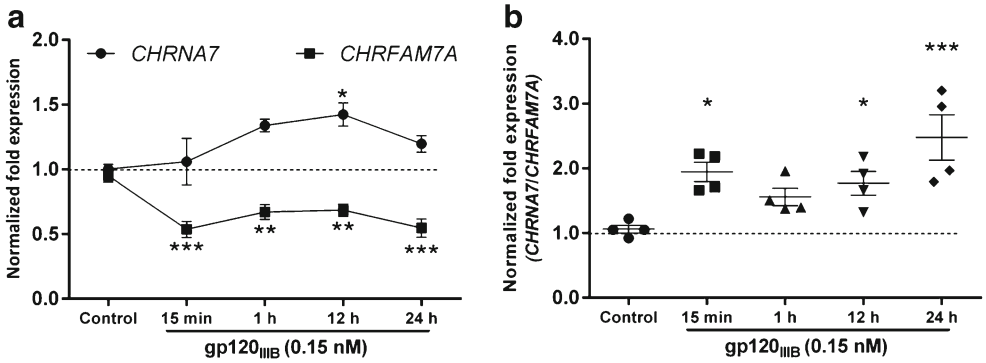

Fig. 2 Time-dependent responses of *CHRNA7* and *CHRFAM7A* in neuronal cells exposed to HIV-1 $gp120_{IIIB}$. **a** Neuronal cells were incubated with $gp120_{IIIB}$ (0.15 nM) at various time points. As compared to untreated control cells, downregulation of *CHRFAM7A* was observed at all time points while *CHRNA7* was upregulated after 12 h of $gp120_{IIIB}$ application. **b** *CHRNA7*:*CHRFAM7A* expression ratio showed a significant increase after 15 min, 12 h, and 24 h post $gp120_{IIIB}$ application. Results were normalized and compared to the control cells. *P≤0.05, **P≤0.01, ***P≤0.001. n=4 independent experiments. Statistical analysis: one-way ANOVA followed by Holm-Sidak's multiple comparison test

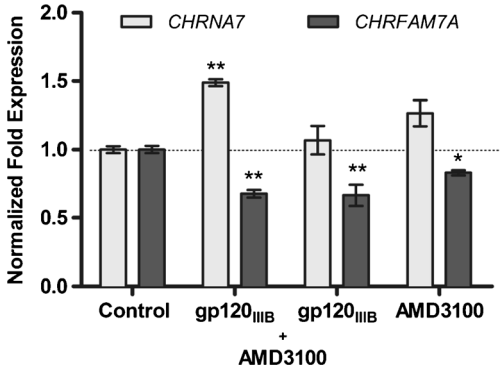

Fig. 3 Dysregulation of the *CHRFAM7A*:*CHRNA7* ratio by $gp120_{IIIB}$ is dependent on CXCR4-gp120 interaction. Neuronal cells were incubated with 0.15 nM $gp120_{IIIB}$ for 12 h with and without a pre-incubation of AMD3100. The *CHRNA7* upregulation induced by $gp120_{IIIB}$ was obliterated by AMD3100 treatment. AMD3100 treatment alone downregulates the *CHRFAM7A*. Results were normalized and compared to the control cells. $*P \leq 0.05$, $**P \leq 0.01$. $n=3$ independent experiments. Statistical analysis: one-way ANOVA followed by a Holm-Sidak's multiple comparison tests

effect on α7, we evaluated the *CHRNA7*:*CHRFAM7A* ratio as indicative of the α7 functionality and found that $gp120_{IIIB}$ indeed does modify the ratio. In our study, we also used different concentrations of $gp120_{IIIB}$ to better understand its effects on *CHRNA7* and *CHRFAM7A* expressions. Our results show that the greatest observed reduction in *CHRFAM7A* expression together with a *CHRNA7* induction occurs within the pathophysiological range of $gp120_{IIIB}$ documented for HIV-infected patients.

We also studied the kinetics of the $gp120_{IIIB}$-induced *CHRNA7* and *CHRFAM7A* dysregulation. Our results demonstrate that the $gp120_{IIIB}$ first induces a reduction in *CHRFAM7A* expression (15 min) followed by *CHRNA7* induction (12 h), shedding light on the regulatory/modulatory mechanism behind the α7 upregulation which points to an early regulatory mechanism (before 15 min) by the *CHRFAM7A* gene. These results, together with our previous published observations demonstrating that the functional upregulation of α7 in neuronal cells promote cell death and that the α7 upregulation appears to be restricted to the basal ganglia (Ballester et al. 2012), are consistent with: (i) the neuronal apoptosis and cell death in the presence of $gp120_{IIIB}$ (X4), gp120 R5, and supernatants containing HIV-1 (Hesselgesser et al. 1998; Catani et al. 2000; Xu et al. 2004), (ii) the neuronal apoptosis identified in postmortem brain from adults and pediatric HIV-infected patients (Adle-Biassette et al. 1995; Gelbard et al. 1995), (iii) the basal ganglia neuronal density reduction in HIV-infected patients (Everall et al. 1995), (iv) autopsy studies of patients with HAD showing that the greatest burden of neuropathology is found in the basal ganglia (Brew et al. 1995), (v) the large accumulation of gp120 in humans' basal ganglia (Jones et al. 2000), and (vi) the neuronal dysfunction and cellular destruction identified in a transgenic mice expressing gp120 in the brain (Corboy et al. 1992; Toggas et al. 1994; Berrada et al. 1995).

Although neurons do not express CD4, they express functional CXCR4 and CCR5 coreceptors enabling gp120 to interact with them and activate signaling pathways leading to neuronal cell death (Kaul et al. 2005; Kaul et al. 2007). The role of CXCR4 in the gp120-mediated neurotoxicity can be direct, through the activation of neuronal receptors by gp120, or indirect through the stimulation of glial cells leading to release of neurotoxic factors (Ghafouri et al. 2006). The activation of CXCR4 by SDF-1α (CXCR4 endogenous agonist)

Table 1 Subject characteristics

	HIV−		HIV+	
	(*n*=5)	Normal cognition (*n*=16)	MCMD (*n*=9)	HAD (*n*=13)
General characteristics				
[a]Age (years)	51.0 (48.0, 51.0)	53.0 (46.0, 59.0)	44.0 (40.5, 58.5)	51.0 (44.5, 54.5)
Gender	5 males	15 males/1 female	9 males	13 males
Treatment	N/A	RTV (1), 3TC (5), CBV (1), DRV (1), NVP (1), ABC (1), NA (6)	RTV (1), APV (1), CBV (2), 3TC (1), NA (4)	ZVD (1), FTC (1), RTV (1), APV (1), CBV (1), EFV (1), ABC (1), 3TC (1), NA (5)
Viral-immune profile				
[a]CD4 cell count (cells/mm^3)	NA	171.0 (18.0, 352.0)	26.5 (7.8, 159.8)	247.0 (25.0, 365.0)
[a]Plasma HIV RNA (log 10)	N/A	4.6 (2.6, 5.2)	5.1 (4.3, 5.8)	4.3 (2.6, 5.1)

[a] Values presented as median (25th, 75th percentiles)

N/A not applicable, *NA* information not available, *RTV* ritonavir (Norvir), *3TC* lamivudine (Epivir), *CBV* zidovudine+lamivudine (Combivir), *DRV* TMC-114, darunavir (Prezista), *NVP* nevirapine (Viramune), *ABC* abacavir (Ziagen), *APV* amprenavir (Agenerase), *ZDV* AZT, zidovudine (Retrovir), *FTC* emtricitabine (Coviracil; Emtriva), *EFV* efavirenz (Sustiva)

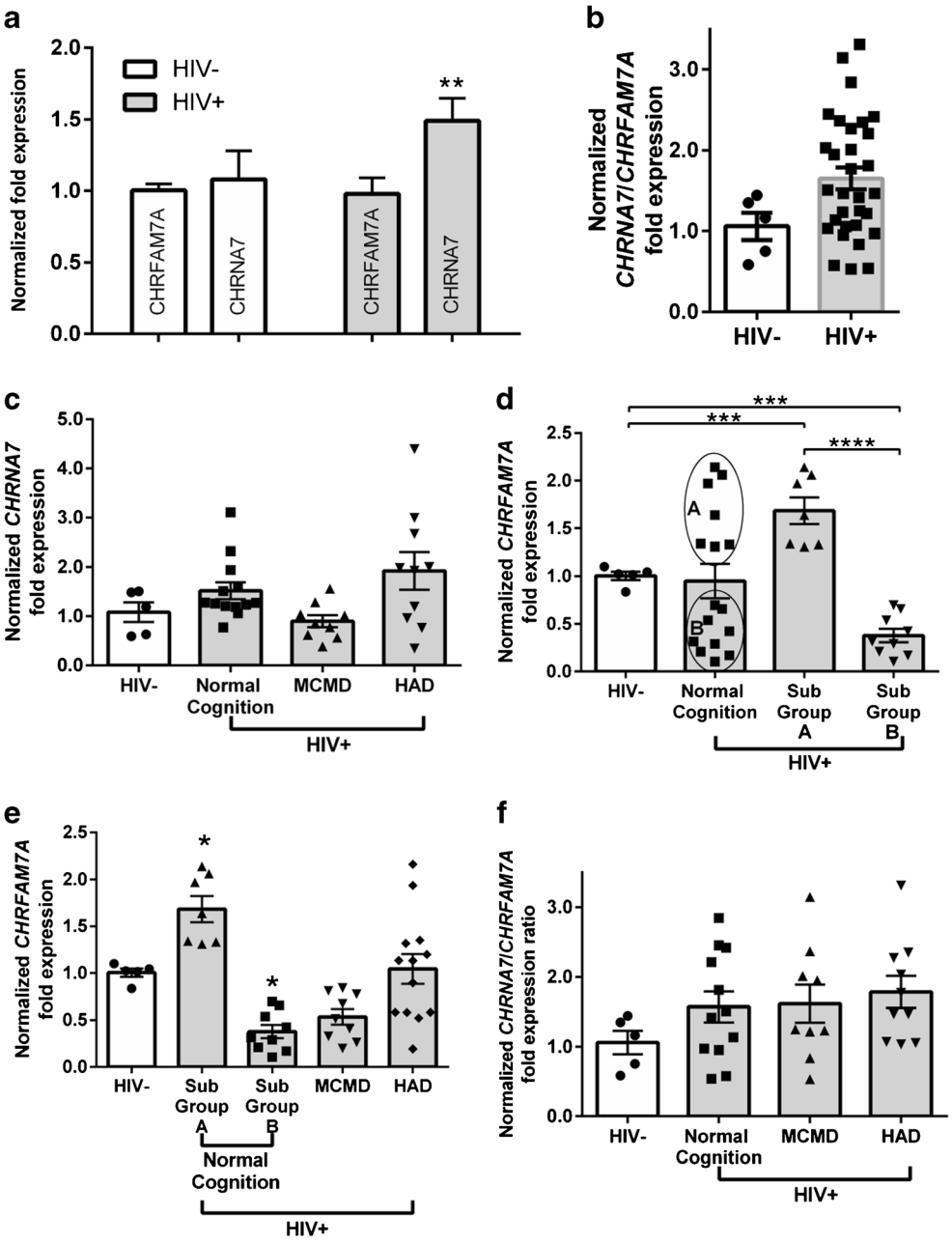

Fig. 4 Dysregulation of the *CHRFAM7A* transcript in the basal ganglia of HIV+ individuals. **a** HIV-infected subjects exhibited significant higher levels of *CHRNA7*, HIV− (n=5) and HIV+ (n=31). **b** *CHRNA7*:*CHRFAM7A* expression ratio appears higher in HIV-infected subjects, HIV+ (n=5) and HIV− (n=31). **c** Analysis of *CHRNA7* mRNA levels in HIV-infected individuals. For control, n=5, normal cognition n=13, MCMD n=9, and HAD n=11. **d** Two subgroups of HIV+ individuals with normal cognition exhibited different responses in *CHRFAM7A* regulation and are significantly different when compared to HIV− individuals. For control, n=5, normal cognition n=16, subgroup A n=7, and subgroup B n=9. **e** No significant changes were detected in *CHRFAM7A* levels in MCMD- or HAD-suffering patients when compared to HIV− whereas normal cognition subgroups are significant different from HIV− subjects. For control, n=5, subgroup A n=7, subgroup B n=9, MCMD n=9, and HAD n=13. **f** Analysis of variance followed by a linear trend test comparing all groups (P=0.08). For control, n=5, normal cognition n=10, MCMD n=9, and HAD n=12. For all panels, results were normalized to HIV− individuals. *P≤0.05, **P≤0.01, *** P≤0.001

or gp120 has been implicated in the mechanism for neuronal dysfunction during HAD (Hesselgesser et al. 1998; Zheng et al. 1999). Herein, we report alterations in the gene expression of a cholinergic receptor and its partial duplication which are both amply distributed through the brain. Dysregulation of these genes under neuropathological settings is not new. For instance, the ratio of *CHRNA7*:*CHRFAM7A* mRNA levels is different in bipolar subjects when compared to unaffected controls (De Luca et al. 2006). Moreover, in vitro studies have demonstrated that two pro-inflammatory mediators, characteristic of HIV-1 infection, LPS and IL-1β, decrease *CHRFAM7A* expression leading to the suggestion that chronic pro-inflammatory responses might change the *CHRFAM7A*:*CHRNA7* expression ratio (Benfante et al. 2011; van der Zanden et al. 2012). $gp120_{IIIB}$ is unable to promote *CHRNA7*:*CHRFAM7A* alterations in the presence of AMD3100, an antagonist of CXCR4, suggesting that the $gp120_{IIIB}$-induced *CHRNA7*:*CHRFAM7A* dysregulation is CXCR4-dependent.

The cognitive impairments observed in HIV-infected subjects are the consequence of neurological alterations in the brain that compromise neural tracts resulting in significant damage and alterations of specific areas. The basal ganglia, which is one of the most severely affected areas in these

patients (Berger and Nath 1997; Berger and Arendt 2000; Berger et al. 2000; von Giesen et al. 2001; Woods et al. 2009), contains cholinergic neurons and interneurons that express α7 (Azam et al. 2003; Bonsi et al. 2011). To better understand the clinical implications of our findings, analysis of *CHRNA7* and *CHRFAM7A* genes was performed on post-mortem basal ganglia samples from HIV-infected individuals with different levels of neurological impairment severity. Our results demonstrate that regardless of the neurological impairment severity, the *CHRNA7* was not significantly altered as compared to HIV− subjects. However, comparing the expression of the *CHRNA7* and *CHRFAM7A* genes within HIV-infected patients reveals that the *CHRNA7* expression is significantly increased in these patients (Fig. 4a). Interestingly, a closer look at the *CHRFAM7A* gene expression levels revealed two distinct populations within the normal cognition group: subgroups A and B. Of note, a significant increase was detected in the expression of the *CHRFAM7A* gene in subgroup A when compared to the HIV− group and subgroup B, and a significant reduction in the expression of *CHRFAM7A* in subgroup B was detected when compared to the HIV− group and subgroup A. In addition, comparing the *CHRFAM7A* expression in both subgroups reveals a statistically significant difference. A provocative hypothesis on the existence of these two discernible subgroups within the normal cognition group is that the patients exhibiting elevated levels of *CHRFAM7A* are less likely of suffering from HIV-associated cognition problems, and those with low levels of *CHRFAM7A*, within subgroup B, are more susceptible to develop neurological impairment as lower *CHRFAM7A* expression levels could imply a potentiation of the α7 receptor expression, increased calcium influx, and ultimate neuronal cell death (de Lucas-Cerrillo et al. 2011; Araud et al. 2011; Ballester et al. 2012). Because the tissues employed in this study were collected before patients presenting ANI were distinguished from patients displaying normal cognition, subgroups A and B could comprise patients with either normal cognition or ANI. It is tempting to hypothesize that subgroup A comprise patients with normal cognition, and subgroup B comprise patients that presented ANI as patients presenting ANI are known to progress to more severe stages (Grant et al. 2014). Taking this into account, our results may imply that alterations in the expression of *CHRNA7* and *CHRFAM7A*, or the *CHRNA7:CHRFAM7A* ratio might be detrimental to the cognitive performance of these patients.

In this study, we tested the hypothesis that higher levels of neurological impairment could be associated with alterations in *CHRNA7* or *CHRFAM7A* expression levels. Whether this dysregulation is responsible for the destruction of cholinergic neurons within the basal ganglia of HIV-infected patients remains to be determined. However, the available evidence points in that direction. For instance, (i) the basal ganglia of HIV-infected patients is compromised (Berger and Nath 1997; Berger et al. 2000; von Giesen et al. 2001; Woods et al. 2009) and (ii) the α7 upregulation in the basal ganglia of transgenic mice expressing gp120 in the brain predispose this area to cell death events similar to what was detected in α7-upregulated neuronal cells (Ballester et al. 2012). Together, this evidence leads us to suggest that the alterations in the *CHRNA7*:-*CHRFAM7A* expression might be implicated in the basal ganglia alterations observed in HIV-infected subjects with neurological impairments. This interpretation is supported by several lines of evidence showing that the motor dysfunction suffered by subjects, under pathological circumstances, involves compromised basal ganglia interneurons (Bonsi et al. 2011) reminiscent of MCMD-suffering patients.

In conclusion, we showed that $gp120_{IIIB}$ is capable of dysregulating the *CHRNA7*/*CHRFAM7A* expression in neuronal cells. Moreover, this dysregulation was detected in post-mortem brain samples recovered from HIV-infected patients with different stages of HAND. The present study is limited in that the results from HIV+ patients basal ganglia may be hindered by the lack of statistical power to detect small changes in expression levels as statistically significant given the dispersion in the data, and that the normal cognition group may actually include HIV+ patients that presented asymptomatic neurocognitive impairment (ANI) because the tissues were collected before ANI was established as a classification category of HIV-induced neurocognitive disorders. Nevertheless, our results raise fundamental questions about the role of α7 and dupα7 in HIV-induced neurological disorders and warrant further statistically powered investigations using an increased number of brain samples from HIV-infected subjects under different stages of HAND. In addition, further studies aimed at exploring the CCR5 tropic gp120 ($gp120_{JRFL}$) effects on α7 expression in neuronal cells are warranted. It would be interesting to determine whether CCR5 stimulation influences α7 expression as occurs with the CXCR4 tropic-specific $gp120_{IIIB}$. In fact, it is known that activation of these G-protein-coupled receptors produces similar signaling pathways (Davis et al. 1997; Lee et al. 2003) that, in the presence of gp120, could lead to death of neuronal cells (Catani et al. 2000); therefore, it would not be surprising that both gp120s produce similar responses.

Acknowledgments We are very grateful for Dr. Valerie Wojna's valuable suggestions and in depth review of this manuscript. We thank the National NeuroAIDS Tissue Consortium (NNTC) and the Data Coordinating Center (DCC) which is a project funded by the National Institute of Mental Health and the National Institute of Neurological Disorders and Stroke under the following grants: U24MH100928 (California NeuroAIDS Tissue Network), U24MH100930 (Texas NeuroAIDS Research Center), and U24MH100929 (UCLA National Neurological AIDS Bank). This research was supported by the National Institutes of Health-NINDS and National Institute of General Medical Sciences (NIGMS) grants (1P20GM103642 and 2U54NS43011 to José A. Lasalde-Dominicci), the RISE Program from NIGMS (2R25GM061151 to Manuel

Delgado-Vélez and Orestes Quesada), and MARC Program from NIGMS (5T34GM007821 to Orestes Quesada and Felix M. Ramos). Research reported in this publication was supported by the National Institute on Minority Health and Health Disparities of the National Institutes of Health under Award Number U54MD007587 and the National Institute of Mental Health (NIMH) grant Number P30MH075673-07 to Carlos A. Báez-Pagán. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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