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

Félix M. Ramos, Manuel Delgado-Vélez, Ángel L. Ortiz, Carlos A. Báez-Pagán, Orestes Quesada, José A. Lasalde-Dominicci

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...
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 (Toghas 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 alpha 7 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 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 induces a functional α7 upregulation in neuronal cells and that the expression of gp120 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 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 % CO2 in DMEM/F-12 media supplemented with 10 % fetal bovine serum, 1 % penicillin/streptomycin, and 1.2 g of NaHCO3. Cultures were performed in 12-well plates followed by treatments with gp120 (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 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 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; Santosuoso 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; Rutá 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 were 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′-GCTCTGCTCCTTCACCTTCC-3′, reverse, 5′-GACTCGACCTTCACCTTCC-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, 0000354, 0000355, and 00002758), 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**

**gp120HIV promotes the downregulation of *CHRFAM7A* in neuronal cells**

Neuronal cells were exposed to various concentrations of gp120HIV 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 gp120HIV 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 gp120HIV were used (15 nM). Further evaluation shows that *CHRNA7*:*CHRFAM7A* expression ratios increase with the gp120HIV treatment (Fig. 1b).

**A pathophysiological dose of gp120HIV 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 gp120HIV (0.15 nM) at different time points showed that the α7 gene, *CHRNA7*, was upregulated after 12 h post gp120HIV exposure whereas *CHRFAM7A* supraphysiological conditions as compared to control. In panels a and b, results were normalized to the control. *P*≤0.05, **P**≤0.01, *** P**≤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.

![Fig. 1](https://example.com/figure1.png)
downregulation initiated as early as 15 min post gp120
 application, and lasted for 24 h (Fig. 2a). Moreover, a ratio
 analysis demonstrates an early increase in the
 \( \text{CHR}A7: \text{CHR}FAM7A \) expression (Fig. 2b).

A CXCR4 antagonist abrogates the gp120
-induced dysregulation of \( \text{CHR}A7 \)

To determine whether the \( \text{CHR}A7 \) and \( \text{CHR}FAM7A \) dysregulation depends on CXCR4 stimulation, an antagonist (AMD3100) was applied prior to gp120 addition. Our results show that CXCR4 blockade abrogates gp120-induced upregulation of \( \text{CHR}A7 \) (Fig. 3). Unexpectedly, \( \text{CHR}FAM7A \) was downregulated by AMD3100 in the absence of gp120 (Fig. 3a).

The \( \text{CHR}A7 \) and \( \text{CHR}FAM7A \) 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 \( \text{CHR}A7 \) gene product, \( \alpha_7 \), is upregulated in the striatum (a component of the basal ganglia) of mice expressing gp120 in the brain (Ballester et al. 2012). Here, we examined \( \text{CHR}A7 \), \( \text{CHR}FAM7A \), and \( \text{CHR}A7: \text{CHR}FAM7A \) 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 \( \text{CHR}FAM7A \) and \( \text{CHR}A7 \) genes in HIV+ patients shows that \( \text{CHR}A7 \) is significantly expressed at higher levels than \( \text{CHR}FAM7A \) (Fig. 4a), which is consistent with what we observed in the neuronal cells (Fig. 1a). The \( \text{CHR}A7: \text{CHR}FAM7A \) ratio of the HIV+ group was increased in these patients (Fig. 4b). Examination of \( \text{CHR}A7 \) levels in basal ganglia from HIV-infected subjects suffering from different stages of cognitive impairment showed no significant differences (Fig. 4c). In terms of \( \text{CHR}FAM7A \), at first glance, patients with normal cognition are not different from HIV− (Fig. 4d) but detailed examination of the distribution of \( \text{CHR}FAM7A \) 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 \( \text{CHR}FAM7A \) expression (Fig. 4d). Furthermore, examination of \( \text{CHR}FAM7A \) levels in the minor cognitive-motor disorder (MCMD) group suggests that only HIV+ patients with low \( \text{CHR}FAM7A \) levels develop MCMD (Fig. 4e). Ratio analysis demonstrates no significant differences in the \( \text{CHR}A7: \text{CHR}FAM7A \) expression ratio in the basal ganglia of these patients, and a linear trend analysis showed a non-significant \((P=0.08)\) increment in \( \text{CHR}A7: \text{CHR}FAM7A \) 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 is capable of inducing a functional upregulation of the \( \alpha_7 \) and cell death in a calcium-dependent manner (Ballester et al. 2012). In the current study, we expand these observations demonstrating that gp120 induces the upregulation of the \( \alpha_7 \) gene \( \text{CHR}A7 \) and the downregulation of its partial duplication, \( \text{CHR}FAM7A \), in neuronal cells. The significant reduction in \( \text{CHR}FAM7A \) expression could imply that dup\( \alpha_7 \)'s dominant negative effect on \( \alpha_7 \)'s functionality may be concomitantly reduced, thus providing a modulatory/regulatory explanation for our previous observations (Ballester et al. 2012). Because of dup\( \alpha_7 \)'s dominant negative regulatory.

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**Fig. 2** Time-dependent responses of \( \text{CHR}A7 \) and \( \text{CHR}FAM7A \) in neuronal cells exposed to HIV-1 gp120. **a** Neuronal cells were incubated with gp120 (0.15 nM) at various time points. As compared to untreated control cells, downregulation of \( \text{CHR}FAM7A \) was observed at all time points while \( \text{CHR}A7 \) was upregulated after 12 h of gp120 application. **b** \( \text{CHR}A7: \text{CHR}FAM7A \) expression ratio showed a significant increase after 15 min, 12 h, and 24 h post gp120 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.
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 up-regulation 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

<table>
<thead>
<tr>
<th>HIV−</th>
<th>Normal cognition</th>
<th>HIV+</th>
<th>MCMMD</th>
<th>HAD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=5)</td>
<td>(n=16)</td>
<td>(n=9)</td>
<td>(n=13)</td>
</tr>
<tr>
<td>General characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>51.0 (48.0, 51.0)</td>
<td>53.0 (46.0, 59.0)</td>
<td>44.0 (40.5, 58.5)</td>
<td>51.0 (44.5, 54.5)</td>
</tr>
<tr>
<td>Gender</td>
<td>5 males</td>
<td>15 males/1 female</td>
<td>RTV (1), 3TC (5), CBV (1), DRV (1), NVP (1), ABC (1), NA (6)</td>
<td>RTV (1), APV (1), CBV (2), 3TC (1), NA (4)</td>
</tr>
<tr>
<td>Treatment</td>
<td>N/A</td>
<td>RTV (1), 3TC (5), CBV (1), DRV (1), NVP (1), ABC (1), NA (6)</td>
<td>ZVD (1), FTC (1), RTV (1), APV (1), CBV (1), EFV (1), ABC (1), 3TC (1), NA (5)</td>
<td></td>
</tr>
<tr>
<td>Viral-immune profile</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>CD4 cell count</strong></td>
<td>NA</td>
<td>171.0 (180.0, 352.0)</td>
<td>26.5 (7.8, 159.8)</td>
<td>247.0 (25.0, 365.0)</td>
</tr>
<tr>
<td><strong>Plasma HIV RNA</strong></td>
<td>N/A</td>
<td>4.6 (2.6, 5.2)</td>
<td>5.1 (4.3, 5.8)</td>
<td>4.3 (2.6, 5.1)</td>
</tr>
</tbody>
</table>

*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)
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 $\text{CHRNA7}:\text{CHRFAM7A}$ 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-1$\beta$, decrease $\text{CHRFAM7A}$ expression leading to the suggestion that chronic pro-inflammatory responses might change the $\text{CHRFAM7A}:\text{CHRNA7}$ expression ratio (Benfante et al. 2011; van der Zande et al. 2012). gp120$_{\text{HIV}}$ is unable to promote $\text{CHRNA7}:\text{CHRFAM7A}$ alterations in the presence of AMD3100, an antagonist of CXCR4, suggesting that the gp120$_{\text{HIV}}$-induced $\text{CHRNA7}:\text{CHRFAM7A}$ 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 postmortem 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 gp120HIV is capable of dysregulating the CHRNA7/CHRFAM7A expression in neuronal cells. Moreover, this dysregulation was detected in postmortem 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 duxp7 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 (gp120JRFL) 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 gp120HIV. 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.

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Compliance with ethical standards

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

References


J. Neurovirol.


Kaul M, Ma Q, Medders KE et al (2007) HIV-1 coreceptors CCR5 and CXCR4 both mediate neuronal cell death but CCR5 paradoxically can also contribute to protection. Cell Death Differ 14:296–305. doi:10.1038/sj.cdd.4400266


