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Differential Contribution of HIV-1 Subtypes B and C to Neurological Disorders: Mechanisms and Possible Treatments

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Abstract

With the introduction of combinatory antiretroviral therapy, patients infected with human immunodeficiency virus type 1 (HIV-1) can live much longer than before. However, the identification of HIV-associated neurocognitive disorder (HAND), especially HIV-associated dementia in 15-20% of patients infected with HIV-1, indicates additional complexity. These disorders turn out to be subtype dependent. Recently, many studies are ongoing trying to understand how the virus induces neuronal injury which could lead to neurological dysfunction. Most of these studies are focusing on the HIV-1 release of proteins such as Tat. However, the exact role of these proteins and their involvement in neuronal degeneration remains unidentified; this is especially true since viral proteins from different HIV-1 subtypes differ in their ability to cause neuronal damage. This review describes the role of different HIV-1 subtypes, identifies probable pathways involved in neuronal damage, the contribution of different HIV-1 subtypes to the progression of HAND, and potential treatments for HAND. **(AIDS Rev. 2019;21:76-83)**

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Key words

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Introduction

Patients infected with human immunodeficiency virus type 1 (HIV-1) (35.1 million adults and 1.8 million children [https://www.WHO.int/hiv/data/en/]) including those using the effective but not curative combinatory antiretroviral therapy (cART) suffer from deregulation and impairment of organs such as heart, kidney, and brain[1]. Studies involving 37,000 HIV-infected patients using cART (from 2003 to 2013) showed that comorbidity increased with age in those patients compared to uninfected patients and having the same age[2].

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AIDS-related deaths have been reduced by 51% since 2004 (the peak of HIV-related deaths) and have further decreased from 1.4 million in 2010 (https://www.hiv.gov/hiv- basics/overview/data-and-trends/global-statistics).

HIV is divided into two types, HIV-1 and HIV-2, among which HIV-1 is the most common. HIV-1 is further separated into three groups: major Group M (major), non-major Group N (non-M, non-O), and Group O (outlier). By far, more than 90% of AIDS cases derive from the infection of H IV-1 Group M (https://www.avert.org/professionals/hiv-science/types-strains#footnote1_gl1pyu4 and References within). Group M is divided into subtypes (clades), among these are the most studied subtypes: A, B, C, and D. Subtype C is the most prevalent subtype in Asia, while subtypes A and D are found mainly in Africa. Subtype B is the predominant type in the developed world, including the United States and European countries. Some of the subtypes are described to be more virulent or more resistant to different drugs[3]. Epidemiological research found that disease progression is closely related to the different viral subtypes[4]. Furthermore, the progression and severity of HIV-associated neurocognitive disorder (HAND), which is common in patients even in the cART era, is found to be different in patients infected with different HIV-1 subtypes. This review will discuss the role of these subtypes and their contribution in the progression of HAND.

Impact of HIV-1 subtypes on the progression of neurological disorders

Approximately 30-50% of HIV-infected patients develop some neurological dysfunction, ranging from mild-to-severe symptoms. HAND is now classified into three categories: minor cognitive/motor disorder (impairment in more than one cognitive abilities); mild cognitive/motor complex (CMC) (cognitive abnormality with mild functional disorder), and HIV-associated dementia (HAD). HAD is the most severe HAND identified by severe functional impairment resulting in dramatic reductions in the ability to care for oneself, work efficiency, and quality of life.

HAND symptoms result from neuronal deregulation directly or indirectly brought on by HIV-1 infection in the central nervous systems (CNS). HIV does not infect neurons; however, HIV-1 does infect microglia and brain-resident macrophages. These infected cells produce and release toxic diffusible factors that can lead to neuronal death (Fig. 1). In addition to exerting a toxic effect on neurons through macrophages and microglia, HIV viral proteins are found to cause neuronal dysfunction and neuronal death in recent studies. Tat, one of the six regulatory viral proteins controlling the ability of HIV to infect cells, is detectable in the serum of HAND patients. TAT protein and mRNA levels correlate with the severity of neurological dysfunction[5,6]. In PSAPP mice, Tat expression in astrocytes induced neurodegeneration, tau phosphorylation, and amyloid deposition in the brain – confirming the neurotoxicity of Tat[7]. Tat enters neurons by receptor-mediated endocytosis and directly induces apoptosis in both rat and human neurons. This apoptosis is caused by the expression of AMPAR through the release of tumor necrosis factor-α independent of nuclear factor κB activation[8]. Further studies found that nitric oxide synthase and endolysosomes are also involved in Tat-induced neurotoxicity[9,10]. Synapse loss brought on by HIV Tat is dependent on calcium influx through N-methyl-D-aspartate receptor (NMDAR) and the activity of mir-128a, which inhibits the expression of the presynaptic protein SNAP25[11].

In addition to Tat, Vpr, a second regulatory viral protein presents in the serum and cerebrospinal fluid (CSF) of HIV patients, causes a depolarization of neurons. Vpr causes an inward current by forming cation-selective ion channels across the cytoplasmic membrane, resulting in hippocampal neuronal death[12]. The first 40 N-terminal amino acids of Vpr are sufficient to create the ion channel which results in neuron death[13]. Besides the ability of the N-terminal region to induce neuronal death, the C-terminal fragment (70-96) is also found to cause neuronal apoptosis associated with the activation of caspase-3[14]. Similarly, caspase-8 activation is identified in human neuronal cultures after exposure to Vpr[15]. In *in vivo* studies done in neonatal mice, Vpr is injected through the ventricle and induces the loss of neurons and dendritic processes in the cortex, hippocampus, cerebellum, and choroids plexus[16]. Transgenic mice expressing Vpr in basal ganglia monocytoid cells exhibit behavior deficits. Neuronal injury is also observed in the basal ganglia of these mice, including the activation of caspase-3 as well as loss of synaptophysin, GABAnergic, and cholinergic neurons[17].

Gp120, a glycoprotein that forms part of the HIV-1 envelope, interacts with several receptors found in the CNS such as CD4, CCR5, CXCR4, and nAChR[18-20]. Gp120 kills neurons by stimulating the neurotoxic pathways mediated by nitric oxide, calcium, glutamate, and superoxide anions[21,22]. Besides these classical

neurotoxic pathways, gp120 reduces intracellular furin levels reducing the cleavage of pro-brain-derived neurotrophic factor (BDNF) into mature BDNF. This reduction in cleavage results in the imbalance between antiapoptotic and proapoptotic neurotrophins, contributing to neuronal injury[23]. Cell death brought by gp120IIIB found in subtype B HIV-1, has a specificity for the CXCR4 receptor, and could also be mediated by the calcium – highly permeable acetylcholine receptor α7-nAChR. The expression of α7-nAChR increases in gp120-treated SH-SY5Y cells and gp120-transgenic mice striatum[24]. The gp120IIIB-induced neurotoxicity in neurons requires the presence of CXCR4, phosphatase, and tensin homolog on chromosome 10 (PTEN) and p38 MAPK[25-28].

Until now, it is a common belief that HIV-1 virus cannot infect neurons but does infect brain macrophages. However, the HIV-Nef gene sequence was detected in hippocampal neurons isolated from postmortem HIV patients[29]. These findings corroborate with other reports that used in situ PCR and demonstrated the presence of viral proteins in neurons[30], suggesting the possibility that HIV-1 can infect neurons. If indeed neurons could be infected by the HIV, there would be additional neuronal damage since Nef has been found to bind directly to calmodulin. Calmodulin is involved in a wide range of cellular calcium-dependent signaling pathways by regulating the activity of many enzymes[31]. Furthermore, Nef causes an increase in K+ current evoked after membrane depolarization, either by direct binding to the K+ channel or through interacting with receptors regulating the K+ channel[32]. The possibility that Nef contributes to the neuronal damage reported in HIV-patients is further supported by human neuronal cultures after exposure to Nef that results in nuclear fragmentation and a decrease in cell number[33]. Furthermore, transgenic mice expressing Nef in astrocytes exhibit impaired spatial and recognition memory, supporting the contribution of Nef to HAND[34].

There have been several studies conducted to identify the relationship between HIV-1 subtypes, and the severity of neurocognitive impairments with individuals infected by different subtypes of HIV-1. In one of these studies, researchers found that among those HIV-infected adults in Uganda, HAD is more common in patients with subtype D (which is more CXCR4-tropic) than those infected with subtype A (which is more CCR5-tropic). On the other hand, in HIV-infected children with subtype A, they demonstrated no statistical difference than those infected with subtype D[35]. Besides the relationship of subtypes A and D with the neurocognitive disorder, subtype C is found to have slower replication kinetics in monocyte-derived macrophages, leading to lower levels of macrophage-mediated neurotoxicity[36]. The diminished neuroinvasive capacity of subtype C is contributed to the different primary conformation of Tat. Tat from subtype C does not cause neuron injury unless morphine was present and acted through glial cells expressing μ-opioid receptors[37]. Clinically, patients infected with subtype C show decreased incidence of cognitive deficits[38]. Severe combined immune deficiency mice injected with macrophages infected by subtype B display more severe cognitive deficits than mice infected with subtype C[39]. *In vitro* studies have found that subtype B Tat is more potent than subtype C Tat when inducing neuronal death (Fig. 2)[40].

Studies in recent years have discovered subtype-specific differences in the neurotoxicity and the induction of Aβ production in neurons by HIV-1 Tat protein from subtypes B and C[41]. These differences are attributed to the di-cysteine C30-C31 motif found in Tat from subtype B[42]. The mechanism why subtype C Tat protein is less neurotoxic might be attributed to the natural mutation found at cysteine 31 that is critical in mediating persistent excitation of the NMDAR. This persistent excitation is achieved by disrupting a disulfide bond on the NR1 subunit of the NMDAR that eventually leads to cognitive dysfunctions[43].

In addition to the lower toxicity of Tat protein from subtype C, gp120 protein from the same subtype was also found to be less toxic compared to that isolated from subtype B. As indicated in the results from human astrocytes, subtype B gp120 induces higher levels of glutamate, as well as prostaglandin E2 and thromboxane A2 receptor, the neuropathogenic byproducts of cyclooxygenase-2-mediated arachidonic acid metabolism[44]. Further, the capability of inducing CMC is hypothesized to be determined by the mutations in the gp120 sequences, which means that there exist some variations of gp120 specific for causing CMC[45]. Speaking of particular mutations in viral proteins responsible for causing cognitive dysfunction, a mutation at amino acid 77 in Vpr distinguishes HIV patients with (77Q) or without (77R) HAND. However, 77Q is dominant in HIV patients with dementia; the Vpr protein with the 77Q mutation is found to induce less neurotoxicity than Vpr with 77R[46].

Similar conditions apply to Nef, the three-dimensional structure of Nef in the brain of HAD patients infected with HIV-1 subtype B is found to be more identical to Nef from subtype D and less similar to Nef collected

from patients that do not have HAD. In addition, Nef from the brain of HAD patients is structurally different from Nef from the same patient's peripheral organs indicating the possible existence of specific genetic alterations that change the structure of Nef in the brain, which contributes to cognitive deficit[47].

Potential treatments of HAND

At present, there are no specific treatments for HAND. The treatment of HAND usually employs a multidiscipline approach with neurologists, HIV specialists, psychiatrists, and psychologist participating. Early studies identified reversal of brain metabolic abnormalities[48] and improvement in motor functions in patients receiving a higher dose of zidovudine (azidothymidine [AZT]), a nucleoside reverse transcriptase inhibitor (NRTI). AZT treatment resulted in lower dementia prevalence[49-52], indicating the activity of this antiretroviral drug in preventing HAND. Since AZT generally does not entirely stop HIV replication, only slowing replication down it is usually combined with other antiretroviral drugs. Studies have found that cART was able to reduce the incidence of HAD[53] and improve motor speed performance in patients receiving a specific cART combination[54]. However, some antiretroviral agents such as efavirenz (EFV) and rilpivirine (RPV), which fall in the non-NRTI (NNRTI) class, have CNS side effects that exacerbate neurological dysfunction[55,56]. Therefore, it is essential for physicians to consider the neurological health of HIV patients when choosing the most appropriate cART drug combination[57].

Protease inhibitors (PIs) are a class of antiretroviral drugs that inhibit the activity of a protease that cleaves nascent proteins for assembly of new virions. Among the series of PIs developed for HIV patients, some were tested for the effect on β-amyloid deposition *in vitro* and *in vivo*, a hallmark of dementia. Atazanavir, ritonavir, and saquinavir (SQV) were found to modestly inhibit Aβ degradation while lopinavir, nelfinavir (NFV), and ritonavir enhanced secretion of undigested Aβ after phagocytosis. Lopinavir, NFV, ritonavir, and SQV were found to inhibit Aβ40 production from primary human cortical neurons[58]. Indinavir, another HIV PI, alleviated memory deficits induced by celecoxib or streptozotocin by inhibiting brain AChE activity, as well as thiobarbituric acid reactive species levels and restoring reduced glutathione levels[59]. Ritonavir, in addition to slightly inhibiting Aβ degradation, induces the expression of P-glycoprotein, a blood–brain barrier (BBB) drug transporter, resulting in restricted entry of HIV PIs into the brain, leading to neurological dysfunction[60]. On the contrary, inhibition of P-glycoprotein at the BBB is found to increase the brain distribution of an HIV PI, NFV, supporting P-glycoprotein as a potential target for HAND[61].

Several factors limit the distribution of the drugs to the brain. First, cART can bound the plasma proteins and therefore be less available for the CNS[62]. Second, the BBB has a complex structure which is a major obstacle for an effective drug delivery and creates a poor pharmacokinetic profile[63]. Different approaches are developed to deliver cART through the barrier, including biotechnologies, prodrugs based, nanogels, liposomes, or chemical modification for CNS delivery[64].

NRTI has a low molecular weight and low protein binding and therefore reaches a good CSF concentration. Raltegravir (RGV) exhibits a good passage into CSF. Nevirapine (NVP) had the highest CSF/plasma penetration compared to other drugs. In the opposite, the penetrability of EFV in CSF is limited. Moreover, the PIs have, in general, a limited penetrability in the CSF due to the presence of efflux mechanisms and high plasma protein binding. However, their penetrability can be boosted by low-dose ritonavir. Some PIs like SQV, NFV, tipranavir, and atazanavir do not even reach therapeutical concentrations in CSF and can be were below the detection limit[65].

The dolutegravir resistance is more common in subtype B versus non-B clades (18.7% vs. 22%), due to a G140S substitution. In all the non-B, two nucleotides are required, raising the genetic barrier to the emergence of G140S mutations[66]. In the RGV population, HIV-1 subtype B develops the mutations N155H, Q148H/R/K, and Y143R/C/H. The mutation Q148H/R/K can be found with 13% of prevalence in all B subtypes versus 0% in non-B. However, some studies showed the same susceptibility among the integrase inhibitors, RGV, elvitegravir, and MK-2408 with HIV-1 subtype B and C, resulting in similar outcomes[67].

Despite having a low genetic barrier, EFV and NVP have been widely used. A single mutation in the binding pocket of the NNRTIs is sufficient to initiate a clinical failure[68]. The NNRTI resistance is caused by two mechanisms: the mutations K103N and E138K affecting the entry of the NNRTI from the binding pocket[69,70] or is altering the pocket geometry through the mutations Y181C and Y188L[71,72]. In tissue culture, under EFV pressure but not NVP or delavirdine, the HIV-1 clade C develops the V160M mutation, conferring high-level resistance to all NNRTIs[73]. There is no significant

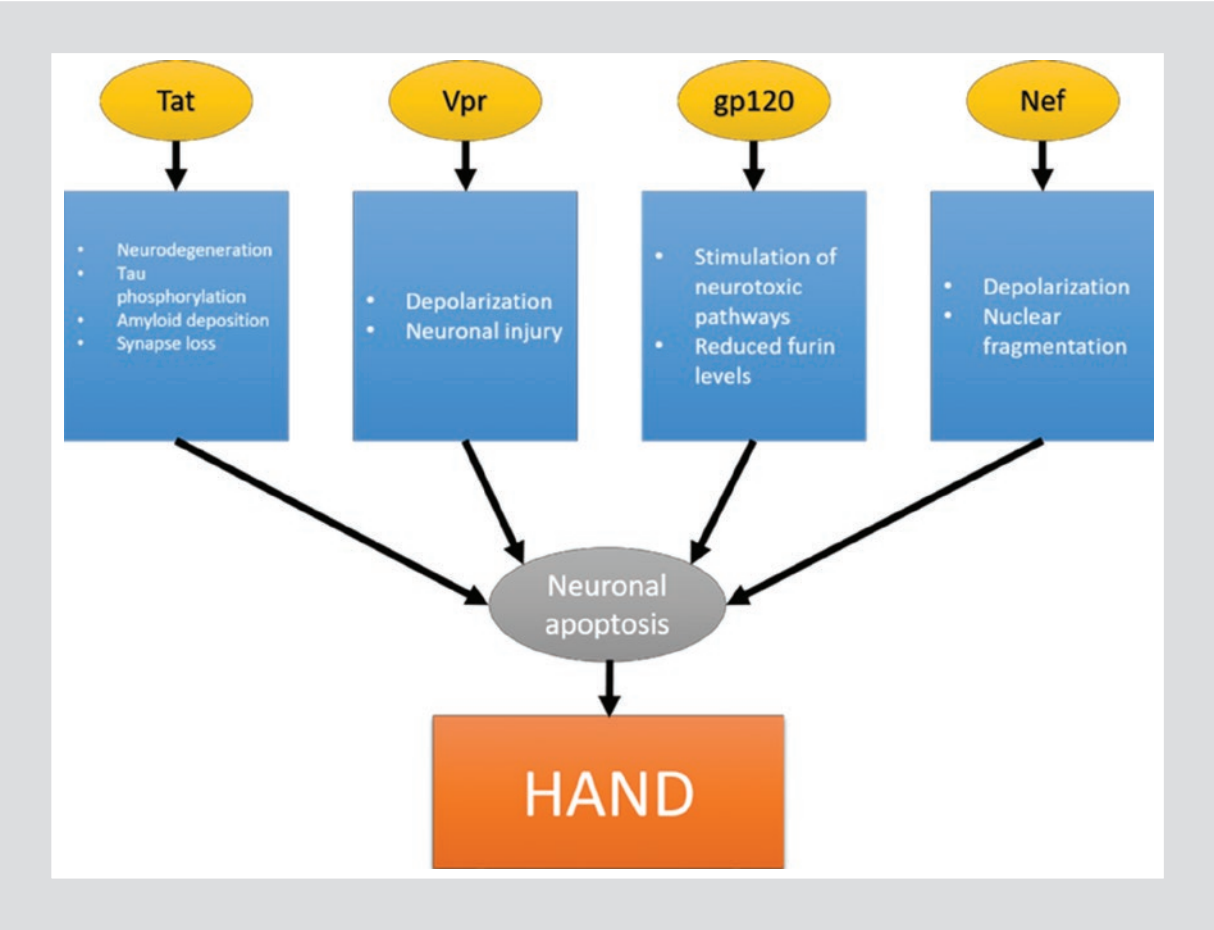

Figure 1. *Summary of HIV-1 proteins' contribution to the progression of HIV-associated neurocognitive disorder (HAND). The contribution of HIV-1 Tat, Vpr, gp120, and Nef to neuronal apoptosis and HAND.*

difference of V106M prevalence in patients receiving NVP between subtypes B and C[74]. V106M and Y181L are the major NNRTI drug-resistant mutations[75]. The prevalence of V106M is 14% in HIV-infected patients carrying subtype C, whereas a small percentage of subtype B-infected patients carries this mutation[76].

The prevalence of the other major mutation Y181C is 21% for the subtype B and only 12% for the subtype C, both B and C are likely to develop the Y181C mutation[77]. Some subtype C-infected patients carry mutation such as E138K/Q/R, M230I/L, and Y188L is resistant to etravirine or RPV, respectively, than those infected with subtype B. Moreover, RPV is associated with the E138A substitution occurring more frequently in subtypes C (5.9-7.5%) than B (0-2.3%)[78].

Besides antiretroviral drugs, other therapies are exhibiting promising effects toward reducing HAND. Memantine and ifenprodil, two GluN2B-preferring NMDAR antagonists, promoted the recovery from Tat-induced synaptic loss and cell survival through inhibiting Tat-induced activation of cell death pathways[79]. Furthermore, estrogen was found to delay Tat-induced neuronal apoptosis by inhibiting a mitochondrial apoptotic signaling pathway in an endoplasmic reticulum-sensitive manner[80] and decreases the neurotoxicity of factors released by Gp120-treated microglia[81-83]. Methylphenidate (Ritalin), the psychostimulant employed to alleviate the neuropsychiatric symptoms, was found to improve cognitive performance in patients diagnosed with HAND[84]. A suggested treatment for HAND is to promote the synaptic concentration of dopamine through inhibiting the neurotransmitter transporters involved in the uptake of dopamine in HIV patients with dopaminergic dysfunction[84-86].

Another obstacle in reducing HAND is the CNS hosting latent viruses as a reservoir in children and adults, contributing to the virus persistence in the brain. The CNS reservoir might reactivate after cART cessation and spread to other compartments. Some latency-reversing agents are available but present neurotoxic effects and BBB penetrability issues such as cART[87].

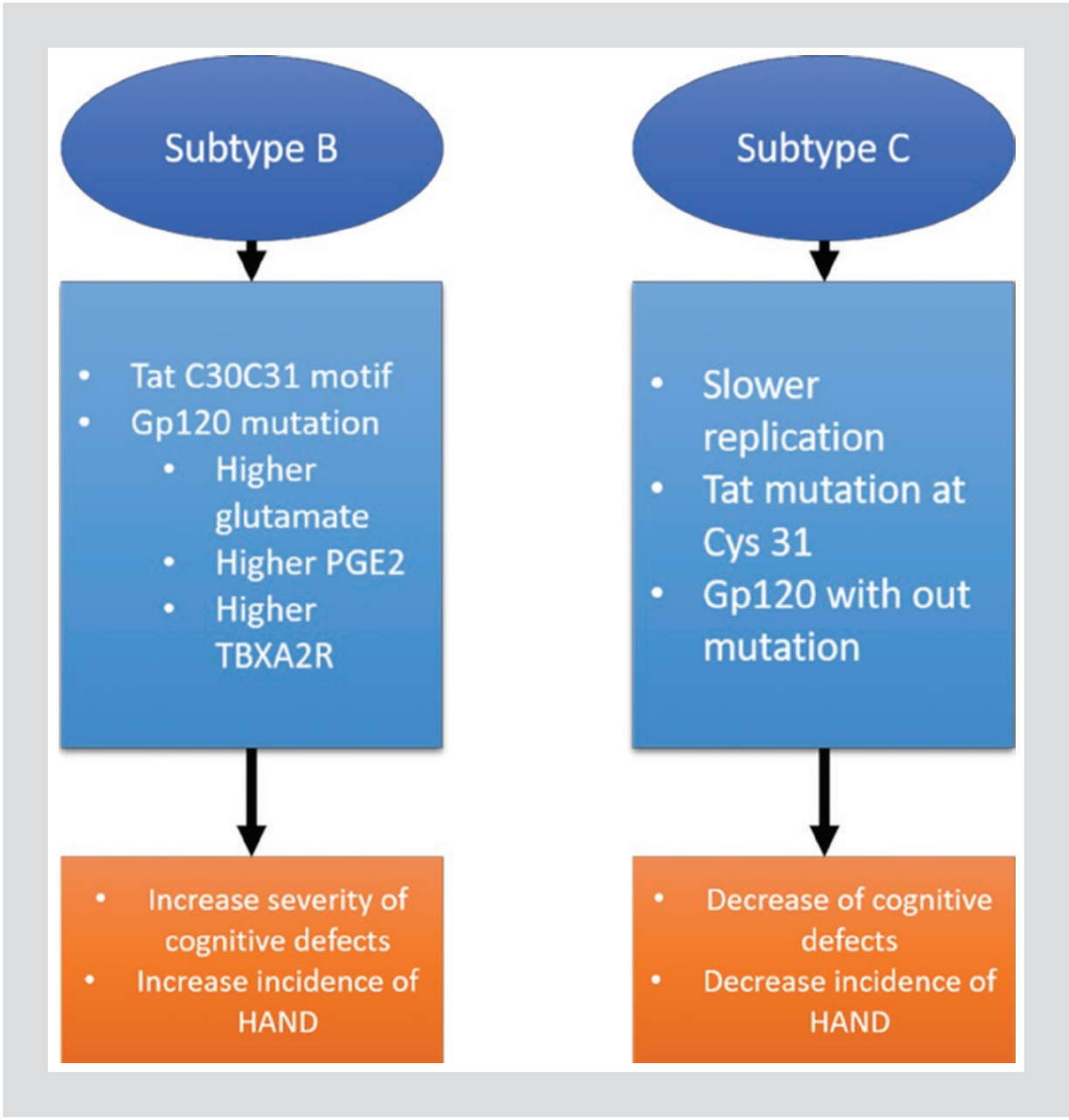

Figure 2. *Comparison of subtype B to subtype C. A comparison of HIV-1 subtype B and subtype C elements and their contribution to the severity and incidence of HIV-associated neurocognitive disorder.*

Conclusion

With the introduction of highly active antiretroviral therapy, the life span of HIV patients has been dramatically prolonged. However, the prevalence of HAND has also increased, especially the mild-to-moderate neurological dysfunction form. HAND is possibly caused by HIV-1 viral proteins resulting in CNS toxicity. The onset and progression of the neurological disorder vary with the age of patients and the different subtype of HIV-1 virus. Recent discoveries have demonstrated how different subtypes result in different through the viral proteins specific structures and sequences and results in different incidence and severity of HAND. More studies are required to understand better the mechanisms of how viral proteins cause HAND, as well as to explore the similarities and differences between different subtypes and degree of HAND. In addition, more research should be done to better identify the differences between HAND and neurodegenerative and neuropsychiatric diseases not caused by HIV infections. Equal importance should be

given to the development of appropriate treatments for HAND, either with improved regimens or neuroprotective and psychostimulant compounds or novel specific therapies for HAND.

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Conflicts of interest

None.

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