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Envelope gene evolution and HIV-1 neuropathogenesis

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Abstract

In the era of combined antiretroviral therapy (cART), HIV-associated neurocognitive disorders (HAND) account for 40 to 56% of all HIV^+ cases. During the acute stage of HIV-1 infection (<6 months), the virus invades and replicates within the central nervous system (CNS). Compared to peripheral tissues, the local CNS cell population expresses distinct levels of chemokine receptors, which levels exert selective pressure on the invading virus. HIV-1 *envelope* (*env*) sequences recovered from the brains and cerebrospinal fluid (CSF) of neurocognitively impaired HIV^+ subjects often display higher nucleotide variability as compared to non-impaired HIV^+ subjects. Specifically, *env* evolution provides HIV-1 with the strategies to evade host immune response, to reduce chemokine receptor dependence, to increase co-receptor binding efficiency, and to potentiate neurotoxicity. The evolution of *env* within the CNS leads to changes that may result in the emergence of novel isolates with neurotoxic and neurovirulent features. However, whether specific factors of HIV-1 evolution lead to the emergence of neurovirulent and neurotropic isolates remains ill-defined. HIV-1 *env* evolution is an ongoing phenomenon that occurs independently of neurological and neurocognitive disease severity; thus HIV *env* evolution may play a pivotal and reciprocal role in the etiology of HAND. Despite the use of cART, the reactivation of latent viral reservoirs represents a clinical challenge because of the replenishment of the viral pool that may subsequently lead to persistent infection. Therefore, gaining a more complete understanding of how HIV-1 *env* evolves over the course of the disease should be considered for the development of future therapies aimed at controlling CNS burden, diminishing persistent viremia, and eradicating viral reservoirs. Here we review the current literature on the role of HIV-1 *env* evolution in the setting of HAND disease progression and on the impact of cART on the dynamics of viral evolution.

Keywords

HIV; envelope; viral evolution; HIV-associated neurocognitive disorders

Introduction

In the United States (US) there are an estimated 1.2 million people infected with the human immunodeficiency virus, type 1 (HIV-1) (CDC Report, 2014). Approximately 40 to 56% of

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HIV$^+$ individuals in the US also suffer from neurological and neurocognitive morbidities, collectively known as HIV-associated neurocognitive disorders (HAND) (1, 2). Fortunately, new antiretroviral regimes reduced AIDS-related mortality (3) and mitigate the most severe neurological complications (4). However, the milder forms of HAND remain prevalent among subjects, despite the use of combination antiretroviral therapy (cART) (2). In the context of HAND, HIV-1 envelope *(env)* gene diversity is of particular interest because of the ability of this kind of diversity to provide the infecting virus with a greater capacity to persistently burden the central nervous system (CNS). In this review we discuss relevant literature which describes the HIV-1 *env* gene evolution and how that evolution affects the course of HIV-related neuropathology.

HIV-1 associated neurocognitive disorders (HAND) and disease severity

HAND comprises an array of sub-syndromic neurocognitive abnormalities which are further classified based on the extent of disease severity (5). HIV-associated dementia (HAD) is the most severe form and is characterized by overt symptoms of dementia, behavioral dysfunction, memory loss, and reduced overall neurocognitive performance (5). For the mild neurocognitive disorders (MND), the symptomatic features are impaired behavioral and cognitive functions, slow movements, motor incoordination, personality changes, and mild abnormal memory (5). MND has a prevalence of 12%, whereas the prevalence of HAD is 2%, being the less common diagnosis (2). The diagnosis criteria from the American Academy of Neurology (AAN) were modified to include the category of asymptomatic neurocognitive impairment (ANI) (5). Even though ANI is the mildest manifestation of HAND, it is currently the most prevalent and accounts for 33% of the individuals diagnosed with HAND (2). The clinical relevance for ANI patients is that subjects are 3 times more prone towards symptomatic decline than are those without any level of neurocognitive impairment (6).

The neuropathological etiologies of HAND development include marked neuronal loss, altered metabolic and neurotransmitter balance, and failure of immune responses (reviewed by (7-10)). The molecular mechanisms and crucial events of the host responses that can trigger the development of the neuropathological features of HAND have been reviewed elsewhere (7, 8). HIV-1 primarily infects CD4$^+$ cells (11, 12). HIV-1 migrates into and invades the CNS via HIV-infected circulating peripheral immune cells (including monocytes and T-cells) (13). The pattern of viral trafficking suggests that HIV-1 crosses the blood–brain barrier, spreads from macrophages, and expands within meningeal tissues towards deep brain parenchyma (13, 14). The characterization of viral isolates from cerebrospinal fluid (CSF) revealed that efficient viral replication takes place within long-lived CNS cells (15). Subsequent viral shedding released from invading and perivascular macrophages permits infection of resident glial cells such as microglia and astrocytes (16).

HIV-1 CSF variants recovered during acute stages of infection (<6 months) suggest early CNS involvement (17). In the early stages of CNS neuropathology, detectable changes in inflammatory markers occur prior the appearance of neurologically-related symptoms (18). Changes in neurocognitive status and the loss of neuronal integrity markers can be detected during the acute stage of infection (9, 19). Immunochemical staining of brain sections of

HIV$^+$ patients who have died from non–HIV-related causes revealed that the clinical hallmarks of HAND were astrocytosis and microglial nodules, at the time of death (20). Interestingly, neurological involvement associated with HIV infection may be of prognostic value in terms of determining mortality risk during the course of infection. Findings from a large cohort study (n = 1,651) indicate that HIV-infected subjects with at least 1 comorbid neurological involvement have a higher mortality risk than do those without neurological comorbidity (21). Thus, HIV CNS burden, with or without apparent development of HAND, represents an important clinical challenge because of the relatively high risk of mortality throughout the course of the disease.

The role of the HIV-1 envelope in CNS infection

During the course of the disease, viral factors can influence the neurocognitive impairment outcome (11). The HIV-1 viral genome encodes for proteins that are required for viral infectivity and pathogenesis. The well-studied HIV-1 envelope *(env)* encodes for the heavily glycosylated spike protein HIV-1 gp120 and the transmembrane protein HIV-1 gp41. The primary targets of HIV-1 are the circulating cells of the monocyte lineage and lymphoid lineage. Infection is accomplished by engaging, first, the CD4 receptor and then subsequently one or both of the chemokine co-receptors: CCR5 (R5) or CXCR4 (X4) (22, 23). In the case of neurons, some express CXCR4 (24) but are not productively infected by HIV-1 (25). Despite the lack of infection, neurons are not spared from the neurotoxic damage caused by viral proteins such as gp120. For example, the presence of gp120 induces neuronal apoptosis in murine models of HIV neuropathology and in primary human brain cultures (26, 27). These effects depend on the genetic make-up of gp120 and are useful in discriminating between neurotropic and neurovirulent isolates.

In general, HIV-1 *env*s are categorized (based on the preferred co-receptor utilized for cellular entry) as being X4-, R5-, or dual-tropic (D-tropic) isolates (22, 23). Within the same host, HIV-1 *env* displays high nucleotide sequence variation at the time of viral sampling (28-31). There are 5 variable regions, termed V1 through V5, that are embedded between 5 constant regions (C1 – C5) and that influence viral co-receptor preference and cellular tropism (23, 32). The hypervariable region 3 (V3) has been the subject of extensive study because of its usefulness in inferring and predicting viral phenotype (33-36). Mainly, genetic variability within the V3, and to a lesser extent genetic diversity in regions outside V3 (i.e., hypervariable regions V1/V2 and V4/V5), confers viral isolates with the phenotypic capacity to engage the CD4 receptor and either the CCR5 (37) or the CXCR4 co-receptor to mediate cellular entry (23).

Co-receptor tropism is influenced by the collective net charges of the amino acid side chains in V3. The occurrences of positively (H+K+R) and negatively (D+E) charged amino acids are summed to obtain the overall net charge (38). R5 variants are favored when the net positive charge in the V3 loops is lower than 5, whereas X4-using and D-tropic strains are favored by a net charge of 5 or greater in V3 (34, 39). R5-tropic *env*s are the predominant variant forms found before the onset of the neurological manifestation of and during the acute and early chronic stages of the natural course of HIV-1 infection (40). In contrast, CXCR4-tropic (X4-tropic) envelopes emerge at the latest stages of disease (40) and are

associated with the marked depletion of $CD4^+$ lymphocytes in individuals whose infection consist primarily of these envelopes compared to those individuals harboring mainly R5 viral population (41). Despite variable tropism, HIV-1 *env*s from the brain display a preference for CCR5 during the early stages of infection (20). However, a subsequent switch in co-receptor usage from R5 to X4 is common as the HIV-infection progresses along its natural course (41).

Sequence studies comparing brain-derived *env*s from impaired versus non-impaired HIV^+ patients have led to a better understanding of the neurotropic and neurovirulent mechanisms of HIV-1 (20, 26, 42, 43). The above-mentioned studies have also highlighted an important link between genetic variability within *env* and the occurrence of neurotropic genotypes that can influence neurologic progression. Assessments of the biological functions of the motifs located within *env*s from neurocognitively impaired patients have provided a clearer understanding of the impact of HIV-1 genetic variance at play during HAND pathogenesis (26). Cross-sectional and longitudinal studies of subjects with neurocognitive impairment have shown that *env*s obtained from plasma and CSF exhibited changes in their nucleotide and amino acid sequences (15, 17, 44). A classic clinical study found that HIV-1 sequences from CSF were more genetically diverse within the C2V3 region of HIV^+ subjects with severe neurocognitive impairment than they were in those same regions of HIV^+ subjects without cognitive impairment (45). However, other studies show that the autologous genetic diversity within V3 in CSF *env*s is usually lower than plasma *env* genetic diversity (46, 47).

Interestingly, the characterization of sequences from the CNS viral pool provides evidence that neurotropic HIV-1 isolates, to some extent, may govern the neuropathogenic development of HAND. Recovered HIV-1 strains from CSF are often genetically unique and exhibit variable cellular tropism and co-receptor preference (15, 17, 20, 46, 48). A comparison of full-length *env* sequences obtained by single genome amplification in CSF unraveled the neuroadaptive features of the CNS viral population in subjects with or without neurocognitive impairment (49). A sequence comparison of autologous plasma *env*s revealed that genetic determinants for CSF *env*s contain shorter V1/V2 loops and a lower mean number of glycosylation sites (49). Evering *et al* 2014 concluded that neuroadaptation ensued as a consequence of immune selection in the CNS. Loss of CNS immune control appears to be important in the occurrence of neurotropic isolates (46). Phylogenetic analyses of HIV-1 *env* sequences from neurocognitively impaired brains identified macrophages as the culprit sources of viral brain infection (11). Additional phylogenetic evidence indicates that the rate of HIV-1 *env* evolution in meningeal tissues and parenchymal brain regions is non-specific and is associated with the expansion of macrophage infection (14). Hence, HIV-1 macrophage infection (11, 50, 51) and altered immune responses (45, 46) are associated with the neuropathological progression of neurocognitive impairment. In sum, these observations provide indirect support that HIV-1 gene evolution influences HIV-related neuropathology and that cell-type–specific *env* strains can affect neuropathogenesis.

HIV-1 CNS evolution and HIV-1 *env* diversity over the course of infection

Lentiviruses demonstrate extensive genetic evolution throughout the course of infection (52). Not surprisingly, HIV-1 evolves within the tissues of the invaded host as a viral strategy to

mediate persistent infection. HIV-1 genetic evolution ensues, generating diverse phenotypes with distinct pathological features (53). Among the contributing factors needed for HIV-1 to evolve are deficiencies in the error proofing activity of the HIV-1 reverse transcriptase (54), the genetic recombination of the compartmentalized autologous virus (28, 30), and the emergence of escape variants from antibody-mediated immune neutralization (45, 46).

The precise time that *env* evolution occurs in CNS remains unknown, but a few studies that have examined *env* sequences suggest that the emergence of a diverse viral population ensues before the manifestation of neuropathological disease (18, 20, 55). HIV-1 *env* subpopulations isolated from peripheral blood samples are produced by cells with similar life spans (56). In recently infected subjects, independent viral populations with low genetic variability are detected within less than 1 year (57). Tissue-specific evolutionary patterns of viral isolates differ in HIV-infected subjects with or without HAND (58, 59). The rate of genetic evolution is seemingly higher in the lymphoid tissues of patients with a HAND diagnosis (59). A phylogenetic model addressing the dynamics of viral gene flow in the CNS indicates that HIV-1 evolution in the *env* gene develops in a non-specific manner (14). The consequence of HIV *env* evolution is the occurrence of compartmentalized isolates with distinct tissue-specific genetic features which can induce functional phenotypic changes in each isolate. Therefore, the recovery of compartmentalized variants unique to the CNS (17) is a subject of clinical interest, as is their individual contribution to influencing HAND pathogenesis.

It is widely accepted that the CNS milieu promotes adaptive pressures on the invading virus. The CNS is characterized by the variable anatomical and cellular distribution of the chemokine receptors CXCR4 and CCR5 utilized by HIV (60, 61). Astrocytes and microglia exhibit low levels of CXCR4 and CCR5 expression compared to other chemokine receptors (61). Neurons express CXCR4 but not CCR5, and the expression pattern of the former is distinct according to the brain regions sampled (60). For example, both HIV^+ and HIV^- brains are immunoreactive to CXCR4 within the cytoplasm of some hippocampal and brainstem neurons (24). Nonetheless, the predominant expression of CXCR4 levels is detected in regions associated with the limbic system and basal ganglia (60). Therefore it is probable that chemokine receptor availability expressed by glial and neuronal cells can contribute to CNS viral evolution, resulting in a “bottleneck effect” that selectively favors viral isolates with lower coreceptor dependence and higher coreceptor affinity.

In addition to the selective pressures from the widespread chemokine receptor expression variability and the distinct cell populations in CNS, HIV-1 evolution results from compromised immune responses during HAND. Patients with HAD demonstrated higher viral diversity in CNS than did matched HIV^+ controls without such neuropathology (45). CNS viral diversity resulted from the inability of serum from HAD patients to neutralize recombinant virus containing C2V3 regions that was isolated from the brain of HIV^+ neurocognitively impaired patients (45). The antibody-mediated neutralization response against R5 and X4 isolates was tested in virus-containing media by using dilutions of each patient's sera against the HIV-1 strains NL4-3 (X4) and YU-2 (R5) (45). Sera from HAD patients mainly failed to neutralize recombinant HIV-1 NL4-3 that contained C2V3 from the brains of patients suffering from HAD (45). These observations were further supported by S.

K. Pillai, et al. (46), who showed that the CSF of 18 of the subjects studied had reduced neutralization activity against the R5-tropic JR-CSF and the X4-tropic NL4-3 strains compared to autologous plasma.

Accumulated evidence indicates that heterogeneity both within and without the V3 region influences the extent of CNS infection by providing isolates with cell type-specific tropism. For example, astrocytes were previously considered to be secondary players in HIV-1 neuropathology but now the extent of astrocytic involvement is better understood during neuropathology (16, 62). Astrocytic infection is more prominent in subjects with HIV-1 encephalitis and such a finding correlates best with increase neuropathologic markers (16, 62). Microdissection techniques in combination with single-cell PCR methods have made it possible to identify astrocyte-specific sequences within V3 in some subjects with HAND (62). In 1 patient, the astrocyte-specific HIV-1 *env*s sequences contained a key proline residue at position 13 within V3 (62).

Interestingly, mechanistic studies show that macrophage tropic (M-tropic) strains recovered from CNS tissues have altered mechanisms that facilitate efficient entry into macrophages. M-tropic strains are characterized by their low CD4-dependence and by their increased capacity to mediate efficient cellular fusion (43, 48, 63). *In vitro* studies focused on unraveling the driving mechanisms of M-tropism in HIV-1 *env*s have identified some sequence characteristics that cause isolates to efficiently enter macrophages (26, 42, 50, 51, 64). One key mechanism consists of the combined changes in potential N-linked glycosylation sites (PNLGs). PNLGs are amino acid motifs in which a glycan moiety is added to an asparagine found within the triplet amino acid sequence N-X-(65). Glycosylation occurs in the 1st arginine (N) residue of the sequence motif, if it is followed by any amino acid (X) in the 2nd position (except proline), and is followed by either a threonine (T) or serine (S) in the 3rd position (66). A shift in the number and location of PNLGs is critical to confer variable phenotypes on HIV envelopes. CSF sequences have a reduced number of PNLGs compared to sequences from plasma sources (46). The modulation of PNLGs can dramatically affect HIV-1 phenotype to provide M-tropism and the capacity to evade immune neutralization (50, 64, 67). Specifically, one instance in which *env* acquires increased neurotropism occurs because of the loss of a PNLG at position 386 of the V4 region (64). Conversely, results from a study comparing autologous evolution between CSF and plasma in 4 subjects with HAND versus 5 without HAND found significant genetic differences, though only in the C4 region (59). Thus it appears that amino acid composition as well as PNLGs spanning from the constant region 4 (C4) to V5 play key roles in both antibody evasion (67) and enhanced viral infectivity (50, 51, 59). These findings highlight the relevance of the occurrences of position-specific amino acid residues that are critical for specific cell-type tropism.

HIV-1 gp120 tropism and the severity of neurovirulence and neurotoxicity

R5-utilizing M-tropic *env*s not only show reduced CD4 dependence but also show high affinity for CCR5 (43). In addition to these features, R5-M-tropic isolates, such as gp120 BaL, can induce neuronal apoptosis. For instance, the injection of recombinant HIV-1 gp120 BaL into rat striatum resulted in prominent toxicity to the rat's neurons and a distinctive

neurotoxic profile that is linked to HIV-1 tropism (27, 68). The neuronal apoptosis induced by HIV-1 gp120 BaL was restricted to the site of the injection (i.e., the brain) (27). X4-utilizing M-tropic strains demonstrate alterations in the conformational mechanism whereby HIV-1 gp120 sequentially interacts with CD4 and CXCR4 in a more efficient manner (38). In addition, The HIV-1 gp120 IIIB is the representative X4-tropic strain and triggers a wider neuronal apoptotic effect that is distributed locally and distally from the site of the injection (27). Furthermore, *in vitro* infectivity assays using lymphotropic SF2 and macrophage-tropic SF128A HIV-1 strains showed that infection of microglia but not of astrocytes was highly dependent on the strain used (69). Neurovirulent strains of HIV-1 gp120, namely, MACS-1Br, MACS-1spln, and UK1-br, induced neuronal apoptosis in primary neuronal cultures to a greater extent than did autologous *env*s obtained from lymph nodes (26).

In clade C isolates, neurotoxicity is mainly driven by cysteine-rich motifs in HIV Tat. However, besides HIV Tat-induced neurotoxicity, variability in neurotoxicity caused by genetic differences in gp120 of clade C HIV has been reported to occur (70). Using the human neuronal cell line SH-SY5Y as a model of neuronal toxicity, the authors demonstrated that HIV clade C strains from India were less neurotoxic than clade C strain from southern Africa. This study provided new insight into why there are some geographical differences in neurological involvement between cases with clade C HIV infection (70). In addition to the variability in neurotoxicity among region-specific isolates from the same HIV subtypes, neurotoxicity appears to exhibit clade-specific variations. For example, conditioned-media from HIV-infected human macrophages is more potently neurotoxic to both rat and human neurons in groups from clade B HIV-1 than from clade C HIV-1 (71). A recent study addressing the neurotoxic mechanisms between clade B and clade C HIV gp120 demonstrated that the dopamine system is preferentially affected by HIV gp120 B but not by gp120 C (72). Specifically, clade B gp120 appears to promote neurotoxicity by impairing the expression of dopamine receptors (DRD-2), dopamine transporters (DAT), and the signaling proteins calcium/calmodulin kinases (CaMK) type 2 and 4 in astrocytes when compared to gp120 from clade C (72). Of particular interest, the downregulation of dopamine system proteins was synergistically induced by concomitant treatment with methamphetamine. However, in this mechanistic study, neurotoxicity was assessed using primary human astrocytes (72), and whether similar neurotoxic mechanisms are the case with neurons remains elusive. Not surprisingly, such differences in mediating neurotoxicity were located on the V3 and C3 regions. Computational analysis indicated that V3 and C3 variability led to a distinct spatial rearrangement of gp120 motifs between both clades (72). Genetic heterogeneity in HIV-1 *env* is not only critical for neurotropism and cell entry but also crucial for exerting potent neurotoxicity and for causing neuropathology. Thus, assessing HIV *env* genetic differences may be of prognostic value as doing so might provide viral markers that will allow health care practitioners to determine the risk of neuropathological development of HAND that is independent of a particular host's individual risk factors.

HIV-1 CNS viral reservoirs and combined antiretroviral therapy (cART)

New and potent antiretroviral regimens have improved the outlook of many AIDS patients, reducing their risk of mortality by half (3). Subjects on cART demonstrate improvement in their $CD4^+$ count, have reduced plasma viral load and have attenuated inflammatory

responses within the CNS (73). Hence, the initiation of cART regimens can significantly allay the early neuropathogenic development of HAND. The CHARTER study proved that the neuropathological features of HIV, such as microglial nodules and astrogliosis, were found with less frequency in asymptomatic brains than in brains of individuals in advanced stages of CNS involvement (4). Unfortunately, another population-based study showed there is no significant benefit for CNS-targeted cART regimen (74).

Phylogenetic studies of HIV-1 *env* evolution have provided evidence of the clinical challenges that still need to be addressed, even now, in the current antiretroviral era. (75). Viral reservoirs represent a continuous burden because of their ability to replenish the viral pool as a means of inducing persistent infection. For example, in some patients, C2V3 plasma variants have been recovered despite those patients having undergone successful therapy (76). Reactivation of HIV-1 strains occurs because of therapy failure or after antiretroviral treatment interruption (77). The interruption of antiretroviral regimens often triggers rebound viremia that is genetically similar to isolated latent reservoirs (78).

Changes in the genetic composition within the V3 region of *env* can show there to be either an increase or decrease in genetic diversity (77). Moreover, the genetic complexity of the gp120 V3 region of the viruses in 17 out of 27 patients who interrupted antiretroviral therapy changed over a period of 12 weeks (77). The genetic diversity of the HIV-1 viral pool often remains low during suppressive antiretroviral therapy (75, 79). In early seroconverters, diversity within V3 from plasma isolates is influenced by cART. After 60 weeks of therapy, cART restricts V3 diversity to different extents. Interestingly, HIV-1 viral divergence within V3 decreases, indicating that the broad genetic makeup within this region may shift back to be similar to that of the founder virus (80). Viral evolution studies further noted that the reactivation of “ancestral” virus may originate in long-lived cells (79). Amplified variants from 6 patients undergoing suppressive antiretroviral therapy after 2 years showed genetic similarities to the ancestral virus, suggesting that ongoing replication may go undetected even in the setting of cART (75). However, the exact mechanism as to why this genetic diversity shifts to resemble that to the founder virus remains unknown.

The cellular source of viral reservoirs was identified using a macaque model of SIV infection and of rapid CNS disease progression (81). This report showed that the latently harbored virus originated from a minor subset of resting memory $CD4^+$ T-cells in the blood. Macaques receiving antiretroviral therapy had a significant decline of latently infected $CD4^+$ T-cells by 175 days post-inoculation compared to therapy-naïve SIV-infected controls (81). In a longitudinal study, the impact of cART on the phylodynamic changes of viral reservoir population was investigated in 8 patients. Phylogenetic analysis of single genome HIV-1 sequences before the initiation of cART revealed that few genetic changes occurred after a period of 4 to 12 years in therapy (82). The effect of cART on the phylodynamic changes did not differ between individuals treated during early infection and those treated at later stages of infection. The study concluded that, after suppressive long-term cART, stable genetic HIV variants are found in resting memory $CD45RO^+/CD27\ (^{+/-})$ T-cells (82).

The study of HIV-1 evolution remains an important means of understanding both the contribution of viral heterogeneity to HAND disease and the association between clinical

neurocognitive outcomes and viral neuropathogenesis. A more complete understanding HIV-1 evolution may represent a key aspect in the eventual successful eradication of HIV-1 infection. In fact, a large sequence database from clinically well-defined HIV cases has been established in an effort to identify and better understand the viral genetic characteristics (83).

Conclusions

We have reviewed the relevant literature on HIV-1 viral evolution in the setting of the neuropathogenic comorbidity of HAND. HIV-1 viral evolution appears to be an ongoing phenomenon independent of neurological and neurocognitive disease severity (18, 20, 55, 84). An important consequence of viral evolution could be the subsequent emergence and appearance of neurovirulent strains. Based on clinical findings, the extent of viral evolution appears to vary according to the severity of HAND (84, 85). Among the culprits of disease progression, evidence points to HIV-1 *env* variability as a key factor that triggers and sustains neuropathology. Thus, HIV-1 *env* evolution plays a key role in and significantly influences HAND progression. To date, neurological comorbidities due to HIV-1 infection continue to be a significant clinical challenge, despite ongoing long-term cART, as the reactivation of latent virus may continue to burden HIV^+ subjects (77). Although cART does not eradicate HIV-infection, studies should aim to understand whether *env* evolution in viral reservoirs causes the neurotoxic and neurovirulent attributes in neuronal and glial cultures and animal models to be retained.

Taken in sum, our review highlights the importance of studying genetic variability within HIV-1 *env* as an additional risk factor for the development of NI. In addition, we recommend that future research emphasize those *env* genetic signatures with the potential to be neurotropic during early and asymptomatic stages so that tools for predicting neuropathological outcomes can be developed. In conclusion, there is a better understanding of HIV-1 virus evolution within the *env* gene during the onset of disease, and this knowledge should be exploited in the development of future therapies aimed at controlling CNS burden, diminishing persistent viremia, and eradicating viral reservoirs.

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