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LETTER TO THE EDITOR

# Amyloid-like fibrils derived from β-sheets of gp120 contribute to the neuronal pathology of HIV-associated neurocognitive disorders

**KEY WORDS**

HIV-associated neurocognitive disorders (HAND);
HIV-1 gp120;
Amyloid peptides;
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C−C chemokine receptors type 5 (CCR5)

**To the Editor:**

Despite advancements in combinational antiretroviral treatment for HIV-1, cognitive impairments are still experienced by over 50% of acquired immunodeficiency syndrome patients[1]. Unlike other classical forms of neurologic dementia such as Alzheimer's disease, HIV-associated neurocognitive disorder (HAND) predominantly manifests in younger individuals with HIV infection, which is categorized into asymptomatic neurocognitive impairment, mild neurocognitive disorder, and severe HIV-associated dementia, necessitating urgent development of effective diagnosis and treatment.

Amyloidoses are histological hallmarks of neurodegenerative diseases. These amyloidosis diseases commonly manifest as disease-specific amyloid misfolding, leading to spontaneous assembly into fibrillar aggregates. Amyloid fibril-forming proteins were found to be exclusively encoded by host cells. Previously, we have characterized the amyloid properties of HIV-1 glycoprotein gp120 and identified fibrogenic peptides EPs (enhancer peptides) and GAPs (amyloid peptides from HIV-1 gp120) as potential enhancers of HIV-1 infection[2,3]. In this study, we discovered the novel and neurotoxic GAPs in the central nervous system of transgenic gp120 (*Tg-gp120*) mice and patients with HAND. The central nervous system targeting potential GAPs, GP-3-6 and GP-18 were identified within conserved β-sheet structures of gp120. Mechanistic studies suggested GP-3-6 and GP-18 induced neurotoxicity by activating the neuronal C−C chemokine receptors type 5 (CCR5)/cAMP-response element binding protein (CREB) signaling. Furthermore, Maraviroc (MVC), an FDA-approved HIV-1 entry inhibitor targeting CCR5, ameliorated neuropathological injury in *Tg-gp120* mice and reduced neuronal cytotoxicity induced by GAPs.

## 1. Identification of amyloid fibrils and GAPs in brain tissue of *Tg-gp120* mice

Our overall line of research is shown in Fig. 1A. Neuropathologically, *Tg-gp120* mice, a transgenic mouse model of HIV-related brain injury induced by glial fibrillary acidic protein (GFAP)-primed overexpression of soluble gp120, showed significant loss of neuronal nuclei (NeuN), along with dendritic and presynaptic damage indicated by decreased expression of neuropil-positive microtubule-associated protein 2 (MAP-2) and synaptophysin (SYP) in the cortex and hippocampus (Supporting Information Fig. S1A−S1F). Meanwhile, gp120 overexpression led to progressive neuroinflammation with significant activation of astrocytic GFAP and microglial Iba1 compared with WT mice (Fig. S1G−S1L). After confirming gp120-induced nerve injury, we investigated the presence of amyloid fibrils using Thioflavin-S (ThS) staining, a fluorescent histochemical marker of dense core senile plaques. As *Tg-gp120* mice aged, amyloid aggregates were observed in the neocortical and the hippocampus's CA1, CA3, and dentate gyrus (DG) regions (Fig. 1B, Supporting Information Fig. S2A and S2B). Co-staining of brain sections with ThS and immunofluorescence (IF) revealed co-localized amyloid fibrils with GFAP (Fig. 1C), suggesting that gp120 may be responsible for promoting amyloid fibril formation through GFAP expression. Further transmission electron microscopy (TEM) results demonstrated the presence of characteristic amyloid-like fibrils in

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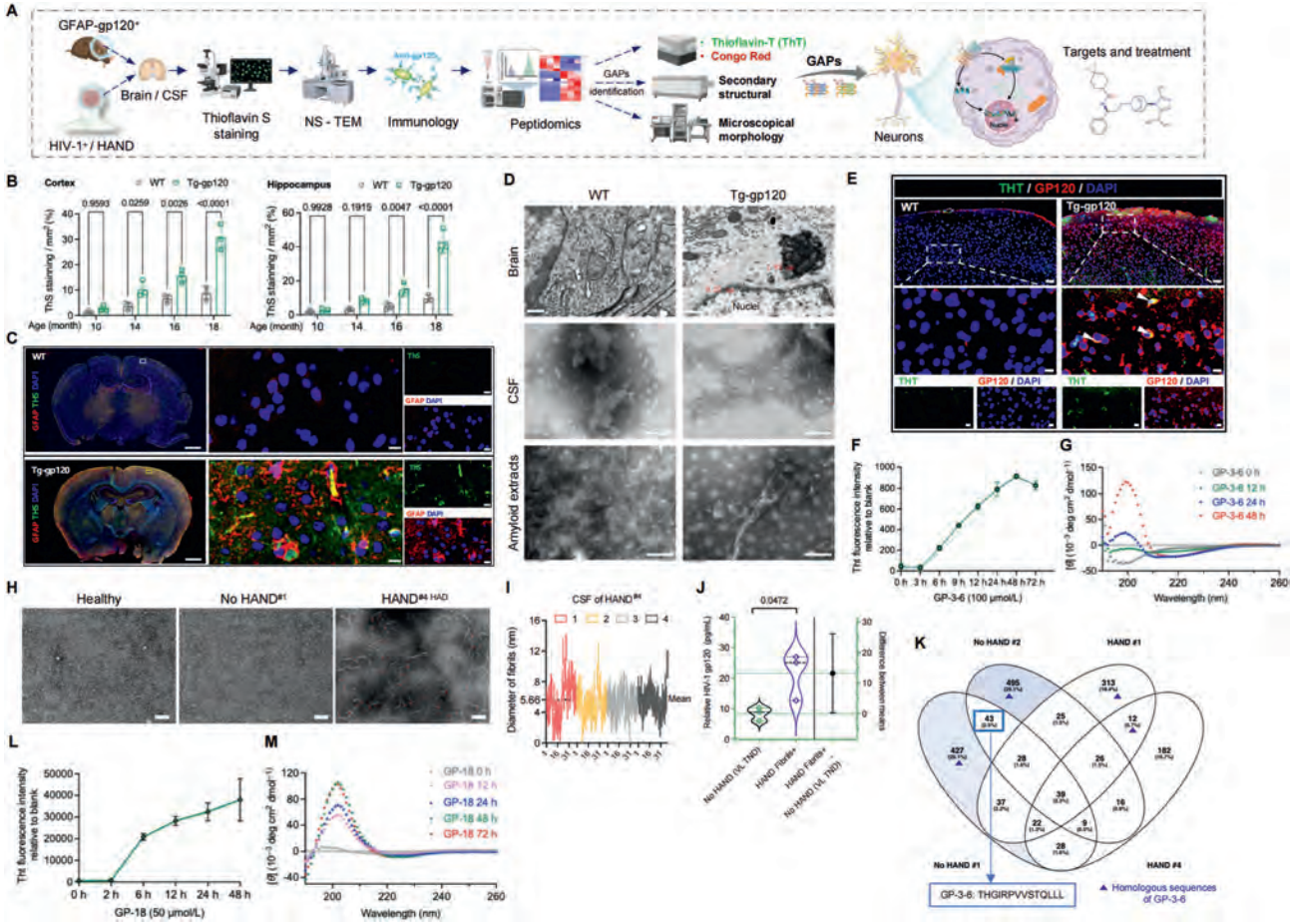


**Figure 1** Identification of amyloid-like fibrils and GAPs derived from gp120. (A) Technical roadmap and workflow for this study; (B) Amyloid fibrils quantification (positive ThS staining) ($n$ = 3); (C) Co-staining of ThS and anti-GFAP, scale bars from left to right are 500 μm, 10 μm, and 10 μm, 16-months-old mice; (D) TEM micrographs of amyloid fibrils. Scar bars: 500 nm (top), 100 nm (middle), and 100 nm (bottom); (E) Colocalization of gp120 (red) and ThT (green), scale bars from top to bottom indicate 50 μm, 10 μm, and 10 μm; (F) ThT binding; (G) CD spectra (GP-3-6, 200 μmol/L); (H) TEM of CSF samples. Scale bars, 50 nm; (I) Statistics of CSF amyloid filaments size distribution (#4 HAND); (J) Levels of gp120 in CSF samples ($n$ = 3); (K) Venn diagram of peptides examined by LC−MS/MS; (L, M) Fibrils formation of GP-18 was monitored by ThT staining (L) and CD spectra (M).

cortical astrocytes and neuronal spaces, in cerebrospinal fluid (CSF) samples, as well as in amyloid extracted[4] from aging *Tg-gp120* mice (Fig. 1D, Supporting Information Figs. S2C, S3A and S3B). These findings indicate the involvement of amyloidogenic gp120 in HAND pathogenesis.

The confirmation of fibril-forming protein was achieved through immunoblot and immunogold labeling analysis, which showed partial recognition of brain extracts from *Tg-gp120* mice by the gp120 antibody (Fig. S3C and S3D). These findings were consistent with the results obtained from ThT/IF/nuclei triple staining (Fig. 1E) and Congo red/IHC double staining (Fig. S3E). Additionally, we investigated the levels of endogenous $\beta$-amyloid (A$\beta$) and Tau protein, two pathological markers associated with HIV-induced neurodegeneration and Alzheimer's disease development. No difference was observed in A$\beta_{1-42}$ levels between *Tg-gp120* and WT mice in brain sections, CSF, or serum (Supporting Information Fig. S4A−S4D). However, phosphorylated Tau at Ser396 was specifically activated in 16-month-old *Tg-gp120* mice (Fig. S4E−S4H), prompting that gp120 may not be the sole source of amyloid material. Furthermore, we performed LC−MS/MS analysis to identify peptides derived by gp120 (Supporting Information Fig. S5). Initially, a total of 12 highly abundant peptides were discovered and subjected to *in vitro* aggregation testing as well as biochemical characterization (Supporting Information Table S1). Preliminary secondary structure and neuronal cytotoxicity analysis screened that GP-3-6 (aa.THGIRPVVSTQLLL) exhibited a transition to a typical $\beta$-fold conformation and neuronal damage effects upon induction (Supporting Information Fig. S6). This peptide is situated between two antiparallel $\beta$-sheets within the invariant region C2 flanked by V2−V3 loops of gp120 (Supporting Information Fig. S7A). Further characterization involving Congo red staining, ThT binding assays, circular dichroism (CD) spectroscopy, atomic force microscopy, and TEM analysis were conducted to elucidate the fibril formation process associated with GP-3-6 peptide behavior (Fig. 1F and G, Fig. S7B−S7G). Moreover, gp120

recognition towards fibrotic GP-3-6 could be observed through immunological assays conducted *in vitro* (Fig. S7H), and this recognition displayed concentration-dependent characteristics (Fig. S7I and S7J). Further, sequence conservation of GP-3-6 was confirmed by gp120 library matching in the UniProtKB library (https://www.uniprot.org/blast). Following this, we investigated the presence of such amyloid fibrils in HAND patients and explore underlying mechanisms causing nerve damage.

## 2. Identification of amyloid fibrils and GAPs in HAND patients

We further identified gp120-derived amyloid-like fibrils in HAND patients. CSF samples were collected from healthy donors ($n = 3$), $HIV^+$ subjects with ($n = 6$) or without HAND ($n = 3$) to confirm the presence of GAPs (Supporting Information Table S2). Initially, we performed TEM and immunogold staining. As shown in Fig. 1H and Supporting Informatin Fig. S8A, visualization of CSF samples confirmed the propensity of the fibrils-containing constructs in HAND patients, with the typical morphology of amyloids appearing in patients with severe HIV-associated dementia, with an average diameter of 5.66 nm (Fig. 1I). Moreover, CSF samples obtained from two groups of $HIV^+$ patients exhibited the $gp120^+$/$amyloid^+$ co-staining (Fig. S8B). However, we observed no significant elevation in gp120 levels or free $A\beta_{1-42}$ of all HAND patients compared to that of HIV-1 infections (Fig. S8C−S8E), but the CSF from HAND patients with identified fibrils demonstrated enhanced recognition by gp120 ($P < 0.05$) (Fig. 1J), indicating the recognition of amyloid fibrils by gp120. The corresponding CSF samples ($n = 2$) were then subjected to gp120-targeted LC−MS/MS analysis (Fig. S8F). We identified over 600 and 300 peptides derived from gp120 in the non-HAND and HAND patients, respectively (Fig. S8G). We found that peptides were consistent with the sequence of GP-3-6 or homologous to GP-3-6 were detected in the non-HAND and HAND patients (Fig. 1K, Supporting Information Table S3), suggesting that the occurrence pathway of GAPs is relatively conservative and persists in HAND patients.

Additionally, to enhance our understanding of the role of GAPs in the brain of HAND patients, neuropeptidomes was employed to analyze differentially expressed peptides derived from gp120 in the CSF of HAND patients and $HIV^+$ individuals without HAND (Supporting Information Table S4). The results showed that 32 of the 39 peptides identified were highly expressed in non-HAND individuals (Supporting Information Table S5), indicating their potential as soluble GAP precursors (Supporting Information Fig. S9A). The most notable difference was an increase in the levels of GP-18 (aa. LKAQFPNKTIIFNQ) in non-HAND patients (Fig. S9B). We observed that GP-18 exhibited a concentration-dependent binding to Congo red and could form aggregates with a typical $\beta$-fold structure as well as ThT-positive species (Fig. S9C−S9G). We further confirmed the secondary structure and fibrillar morphology of GP-18, which formed long and rigid amyloid fibrils recognized by gp120 (Fig. 1L and M, Fig. S9H−S9L). GP-18 was located before the CD4-binding loop within $\beta$-sheets of gp120 and its conservation across various HIV-1 strains (https://www.uniprot.org/blast). These findings highlight the presence of novel neurotoxic GAPs in patients with HAND and the potential utility of peptidomics profiling for identifying amyloid proteins associated with HAND.

## 3. CCR5 signaling contributes to GAPs-related neuronal deficits

We further investigated the neuropathological function of GAPs and found that mature fibrils, rather than soluble GP-3-6 monomers, were responsible for the neurotoxicity (Supporting Information Fig. S10A). We subsequently observed varying degrees of cytotoxicity in mouse primary mouse neurons, neuroblastoma cells, microglia, and astrocytes when exposed to fibrillar GP-3-6 and GP-18 (Fig. 2A and B, Fig. S10B−S10H), supporting the association of GAPs with HAND. To explore the mechanisms by which GAPs induce neural damage, we focused on the gp120-binding coreceptor CCR5, a negative regulator of CREB, which has been recognized as a key mechanism in neurological disease[5]. We examined CCR5 expression in *Tg-gp120* mice at different ages and observed varying levels of upregulation (Supporting Information Fig. S11A−S11C). Co-localization between amyloid fibrils and upregulated CCR5 expression was detected in *Tg-gp120* mice, suggesting that amyloid fibrils activate CCR5 (Fig. 2C). We found the differential CCR5 expression among cell types associated with HAND events and found no significant changes in microglia, but significantly enhanced in neurons and astrocytes (Fig. 2D, Fig. S11D). *In vitro* interventions with GP-3-6 and GP-18 confirmed the upregulation of neuronal CCR5 was accompanied by the downregulation of CREB and activation of p38 mitogen-activated protein kinase in *Tg-gp120* mice and neurons (Fig. 2E−I, Fig. S11E−S11I), suggesting that GAPs directly activate neuron-specific CCR5.

We then investigated the protective effects of CCR5 inhibition against HIV-1 gp120-or GAPs-induced neurotoxicity (Fig. 2J). MVC known for selectively antagonizing CCR5 function and brain tissue absorption, was used for *in vitro* treatment, which showed prevention of neuronal cell death induced by GAPs but had no impact on GAPs-amyloid formation (Fig. 2K and L, Supporting Information Fig. S12A). However, MVC administration reversed neuropathological damage in mice (Fig. S12B) and reduced amyloid accumulation (Fig. 2M), inconsistent with the earlier *in vitro* results. Further investigating the reason was that MVC treatment decreased proliferating GFAP levels (Fig. 2N, Fig. S12C), suggesting a neuroprotective effect of inhibiting host CCR5 against GAPs-induced neuronal toxicity. Subsequently, we observed a significant increase in neuropil density as indicated by MAP-2, SYP, $BrdU^+$-$NeuN^+$ double-labeled neurons, along with the inhibitory effects of MVC treatment on gp120-induced Iba1 activation compared with solvent-treated mice (Fig. 2N and O, Supporting Information Fig. S13A). The neuropathological changes resulting from CCR5 inhibition were manifested in the improvement of memory deficits and spatial learning in *Tg-gp120* mice (Fig. S13B). We further explored the key molecular CCR5-signaling pathways and validated that inhibiting CCR5 enhances CREB expression, revealing a significant decrease in p38 mitogen-activated protein kinase and pAKT levels compared to control *Tg-gp120* mice (Fig. 2P, Fig. S13C−S13G). Collectively, in this part, we confirmed CCR5 signaling as a neurotoxic target of gp120 and GAPs. Our findings provide insights into the etiology of HAND and suggest that targeting amyloidogenic segments of gp120 could be a promising approach for drug development, which also supports the potential use of MVC in clinical treatment for HAND.

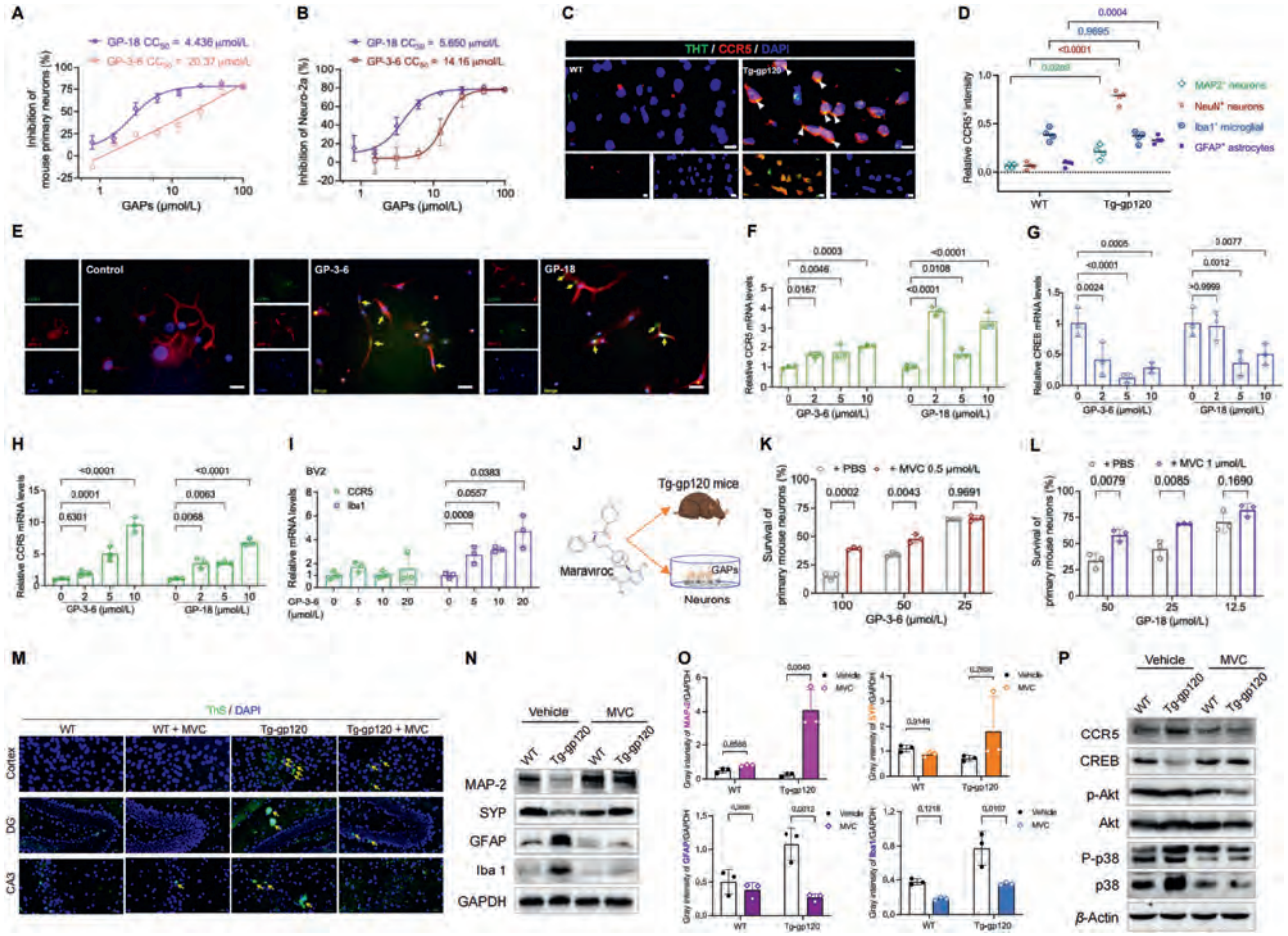


**Figure 2** CCR5 signaling contributes to GAPs-related neuronal deficits. (A, B) Cytotoxicity of fibrillar GP-3-6 and GP-18; (C) CCR5 (red) combined with amyloid fibrils staining (green) in the cortex of mice. Scale bar = 10 μm; (D) Compatible with CCR5 expression of 14-month-old mice, $n = 4$; (E) Expression of CCR5 (green) induced by GP-3-6 (5 μmol/L) or GP-18 (2 μmol/L) for 48 h. Scale bar, 20 μm; (F−I) Gene expression in mouse primary neurons (F, G), Neuro-2a (H), and BV2 (I) cells induced by fibrillar GAPs for 24 h; (J) Schematic diagram of MVC processing *in vitro* and *in vivo*; (K, L) 24 h cytotoxicity of MVC and GAPs treatments; (M) Amyloid fibrils staining by ThS, scale bar = 50 μm; (N−P) Western blots and densitometric analysis, $n = 3$.


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## Author contributions

Shuwen Liu, Tong Zhang, Suiyi Tan, Chan Yang: Investigation. Chan Yang, Ruyu Wang, Chen Cheng, Haobin Li, Kunyu Lu, Jinshen Wang, Hao Yang, Jianfu He, Hao Su and Qingping Zhan: Methodology, Validation, Formal analysis, Data Curation. Tong Zhang and Jiaqi Yu: Resources, Provided samples of clinical patients. Chan Yang, Ruyu Wang and Shuwen Liu: Writing -Original Draft. Shuwen Liu, Suiyi Tan, Guodong Hu and Chan Yang: Supervision, Funding acquisition

## Conflicts of interest

The authors declare no competing interests.

## Appendix A. Supporting information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2025.02.024.

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