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Impact of Apolipoprotein E ε4 and HIV on Cognition and Brain Atrophy: Antagonistic Pleiotropy and Premature Brain Aging

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Abstract

Objective—The apolipoprotein E (*APOE*) ε4 allele may accelerate the progression of HIV disease, and increase the risk for developing HIV-associated neurocognitive disorder (HAND). Whether *APOE*ε4 allele(s) and age may influence brain atrophy in HIV patients is unknown and was evaluated.

Methods—Automated morphometry on magnetic resonance images, using FreeSurfer analyses, neuropsychological testing and *APOE* genotyping were performed in 139 subjects [70 seronegative controls (SN); 69 clinically-stable HIV subjects].

Results—Compared to SN, HIV subjects had smaller volumes throughout the brain regardless of their HAND status. Compared to APOEε4− subjects, SN controls with *APOE*ε4 had better memory and larger global brain volumes (cerebral white matter and cortex) while HIV subjects with the *APOE*ε4 allele(s) had poorer cognition (verbal fluency, learning, executive function and memory) and smaller cerebral and cerebellar white matter and subcortical structures. Further stratification of age showed that younger (<50 years) *APOE*ε4+SN subjects had larger putamen and cerebral white matter, while younger *APOE*ε4+HIV subjects had poorer performance on verbal fluency and smaller brain volumes [3-way (HIV-status × *APOE*ε4 × Age) interaction-p-values=0.005 to 0.03].

Interpretation—These findings suggest that *APOE*ε4 allele(s) may show antagonistic pleiotropy on cognition and brain atrophy in SN controls, but may lead to premature aging with neurodegeneration in younger HIV patients prior to the development of HAND. Potential mechanisms for such interactions may include stronger neuro-inflammation or greater amyloid deposition in younger HIV subjects with *APOE*ε4 allele(s). Early screening for the *APOE*ε4 allele and brain atrophy with morphometry may guide neuroprotective intervention of cognitively

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normal HIV subjects prior to the development of HAND. Longitudinal follow-up studies and larger sample sizes are needed to validate these cross-sectional results.

Keywords

HIV; APOE; age; morphometry; brain

INTRODUCTION

Stable antiretroviral (ARV) treatments may control viral replication and partially restore immune function in HIV-infected individuals; however, 35–60% of patients continue to demonstrate mild to moderate cognitive deficits characterized as HIV-associated neurocognitive disorders (HAND) (Antinori et al., 2007). Many HIV-infected individuals also have co-morbid conditions, such as drug abuse and possible neurotoxic effects of antiretroviral medications (ARVs) that may contribute to cognitive deficits (Chang et al., 2011). Furthermore, genetic vulnerabilities, such as having the apolipoprotein-E (*APOE*) ε4 allele(s), a well-known risk factor for the development of Alzheimer's disease in older individuals (Chen et al., 2002; Jorm et al., 2007), may accelerate the progression of HIV disease (Burt et al., 2008) and double the risk for developing HAND (Corder et al., 1998; Spector et al., 2010). However, the relationship between *APOE*ε4 allele(s) and cognitive deficits in HIV-infected individuals remains controversial. One study found higher prevalence of HIV-associated dementia (HAD) only in older (≥50 years) HIV patients (Valcour et al., 2004), several found no relationship (Dunlop et al., 1997; Joska et al., 2010; Pomara et al., 2008), and one even found better cognitive performance on delayed memory in HIV subjects with the ε4 allele(s) (Pomara et al., 2008).

HIV-infected individuals, especially those with HAND, also consistently showed brain atrophy on structural MRI, using manual (Ances et al., 2006; Aylward et al., 1995; Aylward et al., 1993) or semi-automatic analyses (Cohen et al., 2010; Jernigan et al., 2005), as well as automated segmentation techniques (Chiang et al., 2007; Klunder et al., 2008; Thompson et al., 2005). Brain atrophy in HIV patients occurred in the basal ganglia, the hippocampus, various cortical regions, cerebral white matter as well as the cerebellum, and most studies found correlations between the severity of regional brain atrophy and cognitive deficits (Ances et al., 2006; Aylward et al., 1995; Aylward et al., 1993; Chiang et al., 2007; Cohen et al., 2010; Jernigan et al., 2005; Klunder et al., 2008; Thompson et al., 2005).

Given the persistently high prevalence of HAND despite antiretroviral treatments, identifying risk factors for these individuals is crucial for early intervention or neuroprotective therapy. Since the relationship between *APOE*ε4 genotype and brain atrophy is unknown in HIV patients, the aims of this study are to determine: 1) whether the presence of the ε4 allele(s) exacerbates brain atrophy or cognitive deficits in HIV patients stable on ARVs; 2) whether age interacts with HIV and ε4 allele(s) on brain atrophy and cognitive dysfunction. We hypothesized that HIV+ subjects with *APOE*ε4 allele(s) would show greater global cortical and subcortical brain atrophy and cognitive deficits, and these abnormalities would be more pronounced in older individuals.

MATERIALS AND METHODS

Research participants

139 participants [70 seronegative (SN) subjects ages: 45.9±1.5 years, 62(89%) men, 16(23%) *APOE*ε4+; and 69 HIV subjects ages: 47.4±1.2 years, 63(91%) men, 22(32%) *APOE*ε4+] were included in the study. Each subject signed an approved consent form and completed detailed clinical and neuropsychological evaluations. SN controls were included

based on the following criteria: 1) Fluent in English and able to provide informed consent; 2) ≥18 years of age; 3) HIV-seronegative. HIV subjects also had criteria 1 and 2 above; 3) HIV-positive; 4) stable on ARVs for >6 months or on no ARVs; 5) had nadir CD4≤500/mm^3. Exclusion criteria for both subject groups were: 1) Any illness known to alter brain structures; 2) history of illicit drug or alcohol dependence by DSM-IV criteria; 3) positive urine toxicology screen; 3) head trauma with loss of consciousness >30 minutes; 4) severe abnormalities on screening laboratory tests that might confound brain imaging measures or function; or 5) any contraindications for MRI.

Neuropsychological testing

Each participant performed a battery of neuropsychological tests sensitive for detecting HAND (Antinori et al., 2007), and included tests in seven cognitive domains: memory, learning, attention/working memory, executive function, speed of information processing, fluency and motor skills (Appendix Table A). For each subject, a z-score adjusted for age-and-education normalized to a database of 273 subjects was calculated for each cognitive domain, and for a global z-score (average of all domains). In addition, depressive symptoms, which might influence cognitive performance, were assessed using the Center for Epidemiological Studies–depression scale (CES-D).

Twenty-nine (42%) HIV subjects had HAND (Antinori et al., 2007) [13 Asymptomatic Neurocognitive Impairment, 15 Minor Neurocognitive Disorder, and 1 HAD]; the remaining 40 HIV subjects had normal cognition (HIV+NC). Conversely, only four SN controls (6%) had cognitive deficits comparable to HAND.

APOE Genotyping

Genomic DNA was extracted from stored and banked blood using Quick-gDNA™ MiniPrep (Zymo Research Corporation, Orange, CA). Extracted DNA samples were then used as templates for PCR-based assay (Zivelin et al., 1997) to amplify the polymorphic regions of the *APOE* gene. The PCR products were visualized on 2% agarose gels, agarose-extracted, cleaned, and prepared for DNA sequencing. *APOE* genotypes were identified based on the sequencing results.

MRI acquisition

Subjects were scanned on a Siemens 3.0 Tesla TIMTrio MR system using a 12-channel phased-array radiofrequency coil. Structural imaging sequences included: 1) Magnetization Prepared RApid Gradient Echo (MP-RAGE, TR/TE/TI=2200/4.48/1000ms; 160 slices, 1×1×1mm); and 2) axial T2-weighted fluid-attenuated inversion recovery (FLAIR) to assess for possible brain pathology.

Image analyses

FreeSurfer 4.3.1 {http://surfer.nmr.mgh.harvard.edu} was used for automated reconstruction and labeling of subcortical and cortical regions. Brain structures were extracted from the skull and registered to an existing brain template prior to segmentation of each structure. Regional volumes were determined automatically in each hemisphere for amygdala, caudate, hippocampus, globus pallidus, putamen, thalamus, and global cerebral and cerebellar cortex and white matter (Figure 1). All regions of interest (ROIs) were visually inspected to ensure accurate segmentation. Segmented volumes were manually edited only when they were identified as outliers and caused by program errors. An estimated total intracranial volume (eTIV) was also computed based on the determinant of the transformation matrix used to register the brain with the template (Buckner et al., 2004).

Statistical Analyses

Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC). One-way analysis of variance (ANOVA) was used to compare all clinical characteristics, cognitive domains and brain volumes between HIV and SN subjects, with and without APOEε4 allele(s), and contrasts were generated for pair-wise comparisons. All analyses of brain volumes included age and eTIV as covariates. Two way- or three-way ANOVA was used to test the independent and interactive effects of HIV status, *APOE*ε4 status [at least one *APOE*ε4+allele] and age (<50 vs. ≥50 years) on cognitive function and brain volumes. Relationships between age, clinical characteristics and brain volumes with significant group differences were examined with Pearson correlations. Parametric tests were used since the data were normally distributed. P-values<0.05 were considered significant and trends were noted for $0.05 \leq p \leq 0.15$. Simes correction for multiple comparisons (Simes, 1986) was used for correlation analyses.

RESULTS

Clinical characteristics (Table 1)

The SN and HIV subject groups were similar in age, years of education, sex proportion (predominantly male), and showed no difference in the proportions of APOEε4+carriers, younger and older subjects, or in their racial or ethnic distributions. Five subjects were *APOE*ε4 homozygous, and all were HIV-positive (3 had HAND and 2 had no cognitive deficits). Amongst the HIV subjects, those with *APOE*ε4 allele had lower CD4 counts, but similar nadir CD4 counts, and slightly lower HIV dementia scale, compared to those without the *APOE*ε4 allele. Other measures for HIV disease severity (viral load, duration of HIV diagnosis, Karnofsky score) were similar in both groups. However, regardless of *APOE*ε4 status, HIV subjects had more depressive symptoms on the CES-D than the two SN control groups, but were not clinically depressed.

The majority of HIV subjects (55, or 80%) were taking ARVs. Specific regimens were: 16 nucleoside reverse transcriptase inhibitors (NRTIs)+non-NRTIs (NNRTIs), 29 NRTIs +protease inhibitors (PIs), three NRTIs+NNRTIs+PIs, six NRTIs only, and one with NNRTIs+PIs. No difference was found in the proportion of HIV subjects who were on ARVs or NRTIs between the two groups with or without *APOE*ε4 allele.

HIV and HAND effects on brain morphometry

The HIV subjects with or without HAND had similar Karnofsky scores, duration of HIV, Log viral load, viral suppression, and CD4 counts. However, HAND subjects had slightly lower education, lower scores on the HIV dementia scale, lower cognitive domain scores (by definition), and lower nadir CD4 counts than SN and HIV+NC subjects (Appendix Table A). Despite these differences, the HIV subjects with or without HAND did not differ in terms of atrophy in most brain regions (Figure 1, Appendix Table B) and were combined for further analyses. Of note, the eTIV also did not differ amongst SN controls and the 2 HIV groups.

Compared to SN controls, HIV subjects as a group had significantly smaller (−4.4% to −7.4%) volumes in the caudate, hippocampus, pallidum, putamen, thalamus, amygdala, hippocampi, cerebellar cortex, cerebral white matter and cortex, even when co-varying for age and for eTIV (Figure 1, Appendix Table B). Hence, the global cerebral volume was smaller in HIV than SN subjects (−4.8% p<0.001).

Relationship between brain morphometry, clinical characteristics and cognition

Correlations were found between brain volumes and various clinical variables in the HIV subjects ($p<0.05$–0.001), but none were significant after Simes correction and are considered trends for such associations. Specifically, several regional brain volumes correlated positively with Karnofsky scale, HIV Dementia Scale, and nadir CD4 counts, but negatively with duration of HIV diagnosis or whether viral load was detectable (see Appendix).

When all subjects were combined, their global z-scores correlated with brain volumes in bilateral amygdalae, cerebral white matter, thalami, and hippocampi ($0.22\leq r\leq 0.28$ and $0.001\leq p\leq 0.01$, co-varied for eTIV, Figure 2). Since the subjects with similar reductions in brain volumes may show marked differences in cognitive function (e.g., HAND vs. HIV +NC or SN), further analyses were performed to evaluate the possible contribution of *APOE*ε4 allele(s), and its interaction with age, on cognitive function and brain atrophy.

Relationship between APOEε4, cognitive performance, and age

HIV status and *APOE*ε4 status showed interaction effects on several cognitive tasks and domains (interaction-$p<0.001$ to 0.04; Figure 3). Specifically, HIV+*APOE*ε4+ subjects had poorer performance on Animal fluency, FAS fluency, Trail Making B, and in four domains (fluency, executive function, learning and memory) compared to HIV+*APOE*ε4− subjects and SN controls. In contrast, *APOE*ε4+SN controls scored higher (better) than *APOE*ε4−SN controls on the Auditory Verbal Learning Test Immediate Recall ($p=0.01$), memory domain ($p=0.03$), and trends for better performance on the learning ($p=0.09$) and fluency ($p=0.10$) domains. There were no significant differences in cognitive performance between HIV and control subjects without the *APOE*ε4 allele.

When the subjects were divided into younger (<50 years) and older (≥50 years) groups, *APOE*ε4 affected the Ruff Figural Fluency test in younger but not older subjects (3-way interaction-$p=0.05$, significance remained after adjustments for education). Younger *APOE*ε4+SN controls performed better than younger *APOE*ε4−SN controls (115.0±6.0 vs. 97.7±3.5, $p=0.02$), whereas younger HIV+*APOE*ε4+ subjects performed worse than younger HIV+*APOE*ε4− (81.6±2.8 vs. 95.0±3.7, $p=0.006$). Conversely, there were no significant *APOE*ε4-associated differences in the older SN or HIV groups, and no other three-way interactions were seen on other neuropsychological tests.

Relationships between APOEε4, brain volumes and age (Table 2, Figures 4A & 4B)

Across the 4 subject groups, significant differences were observed for all subcortical and cerebral brain volumes (see effect size and p-values in the one-way ANCOVA, Table 2). Specifically, the HIV+*APOE*ε4+ subjects had the smallest brain volumes in all brain regions evaluated, whereas the *APOE*ε4+SN controls had the largest brain volumes in all regions, except for the right caudate. This opposite effect of APOEε4 on brain volumes in SN versus HIV subjects was significant (repeated measure ANCOVA-$p=0.02$), with significant HIV-status × APOEε4 interactions in the right amygdala, right pallidum, left thalamus, left cerebellar white matter, bilateral cerebral white matter, right cerebral cortex and the global cerebral volume (see Table 2, two-way ANCOVA effect size and p-values, and Figures 4A & 4B).

Further analyses to evaluate the additional effect of age (< or ≥50 years) showed significant 3-way interactions (age × HIV-status × APOEε4) in bilateral putamen and left cerebral white matter volumes, as well as trends for such three-way interactions in the pallidum, thalamus and global cerebral volumes (Table 2, three-way ANCOVA, Figure 5). Specifically, compared to *APOE*ε4− individuals, *APOE*ε4+ was generally associated with smaller brain volumes in older (≥50 years), but larger brain volumes in younger, SN

individuals. Conversely, the presence of the *APOE*ε4 allele(s) was associated with smaller brain volumes in younger (<50 years), but not older, HIV subjects. In both the putamen and the right cerebral white matter, significant atrophy was seen in the younger, but not older, HIV+*APOE*ε4+ subjects compared to the HIV+*APOE*ε4− subjects, while the *APOE*ε4+ SN younger subjects showed larger volumes than both the younger *APOE*ε4−SN and older *APOE*ε4+SN subjects (Figure 5).

On linear regression analyses, most regions studied showed age-related decreases in brain volumes. All HIV and SN subjects were combined in the correlation analysis, since there were no significant HIV status-by-age interactions. The greatest age-related declines were observed in the thalamus (left/right: −6/−5% per decade, r=−0.47/−0.41, p<0.001/<0.001) and cerebral cortex (−4% per decade, r=−0.43, p<0.001 for left and right). After Simes correction, regions that remained significant for age-related decreases include cerebellar cortex (left/right: r=−0.38/−0.39; −4% per decade and p<0.001 for both), putamen (left/right: −5/4% per decade, r=−0.38/−0.31, p<0.001 for both), pallidus (left/right: −5/−3% per decade, r=−0.37/−0.27, p<0.001/<0.002), hippocampus (left/right: −2/−3% per decade, r=−0.25/−0.34, p=0.003/<0.001), and amygdala (left/right: r=−0.22/−0.26, p=0.009/0.002; −3% per decade for both).

However, when the subject groups were evaluated for age-related volume decline and stratified by presence of *APOE*ε4 allele(s), differences in the slopes were observed bilaterally in the putamen and thalamus (Figure 6). While both SN groups and the HIV +*APOE*ε4− group showed age-related volume declines in these two structures, the HIV +*APOE*ε4+ subjects did not show age-related declines, possibly since these structures had already reached a lower set-point at a younger age.

DISCUSSION

This is the first study to characterize the impact of the *APOE* polymorphism on brain structures of HIV-infected subjects with and without HAND. In this group of relatively young subjects (average age < 50 years), the presence of *APOE*ε4 allele(s) was associated with poorer cognitive performance (verbal fluency, learning, executive function and memory) in HIV subjects but better memory in SN controls. Similarly, HIV+*APOE*ε4 carriers had smaller brain volumes while the SN controls had larger brain volumes than those without the ε4 allele. Stratifying the subjects into younger (<50 years) and older (≥50 years) age groups further confirmed that *APOE*ε4 was associated with larger putamen and cerebral white matter in younger SN subjects, but with poorer performance on verbal fluency and smaller volumes in the younger HIV subjects. These findings suggest the APOEε4 allele(s) demonstrate antagonistic pleiotropy, showing a positive effect only in younger but not older healthy SN individuals, but an earlier manifestation of the negative impact of the allele(s) on the brain function and structures of younger HIV subjects.

In this group of clinically stable and mostly ARV-treated HIV subjects, we found smaller brain volumes throughout regardless of their HAND status. This finding is somewhat consistent with recent reports regarding presymptomatic subcortical neurodegeneration in chronic HIV infection (Chao et al., 2003; Di Sclafani et al., 1997; Jernigan et al., 2005), smaller total white matter volumes in HIV subjects stable on ARVs (Cardenas et al., 2009), and smaller cerebellum in HIV patients (Elsheikh et al., 2010; Klunder et al., 2008). However, our findings are different from those reported earlier in the epidemic, when smaller whole brain (Aylward et al., 1995; Ge et al., 2003) and basal ganglia volumes (Aylward et al., 1993) were primarily observed in HIV subjects with dementia, but not in those without neurological or cognitive deficits. Possible etiologies for brain atrophy independent of HAND status include many common factors: 1) long duration of infection

(~12 years); 2) older age; 3) longer usage of potentially neurotoxic ARVs, especially NRTIs; 4) similar proportion of subjects with incomplete viral suppression in the CNS; 5) greater prevalence of recreational drug use compared to SN controls; and 6) ongoing neuroinflammation associated with all of these factors. However, these factors could not explain why subjects with or without HAND showed similarly atrophic brain structures.

The *APOE*ε4 genotype is another factor that might contribute to brain atrophy and cognitive deficits. HIV subjects with *APOE*ε4 allele(s) indeed performed poorer on several cognitive domains, including verbal fluency, learning, executive function and memory, and had smaller brain volumes, especially in terms of the global cerebral white matter, global brain volume, and subcortical regions. The cognitive test findings are consistent with prior reports of higher risks for HAND in those with the *APOE*ε4 allele (Corder et al., 1998; Spector et al., 2010), but opposite from a smaller study that found better immediate and delayed recall in non-demented HIV subjects with the *APOE*ε4 than those without this allele (Pomara et al., 2008). Furthermore, compared to the corresponding SN age groups, *APOE*ε4 was associated with poorer performance on the Ruff Figural Fluency test in our younger, but not older, HIV subjects.

Paradoxically, while poorer global z-scores correlated with smaller volumes in our study, many of these regions were atrophic even in HIV+NC subjects. This suggests that overall cognitive dysfunction is only marginally associated with brain atrophy (5–7% in terms of variance), and that development of HAND in HIV-subjects may be due to many other factors, such as dopaminergic deficits (Chang et al., 2008), lower nadir CD4 (Cohen et al., 2010; Valcour et al., 2006), and APOEε4, which may lead to compromised neuronal function. Moreover, HIV+NC subjects may have sufficient cognitive reserve to compensate and maintain performance despite brain atrophy. Functional MRI studies indeed demonstrated decreased brain activation in normal neural networks but increased brain activation in contralateral or adjacent brain regions of HIV-infected individuals who were able to maintain normal performance on a variety of cognitive tasks (Chang et al., 2001; Chang et al., 2004b; Ernst et al., 2009; Maki et al., 2009; Melrose et al., 2008; Tomasi et al., 2006).

Consistent with prior reports, we found that *APOE*ε4 allele has little or in fact a positive influence on cognition in younger SN individuals (Alexander et al., 2007; Han et al., 2007; Mondadori et al., 2007), but a negative effect in older SN individuals (Chen et al., 2002; Jorm et al., 2007). A recent functional MRI study also found over-activity of brain function in young ε4-carriers that was disproportionately reduced with advancing age even before the onset of measurable memory impairment (Filippini et al., 2011). These findings suggest that *APOE*ε4 has antagonistic pleiotropy, with a positive influence in younger individuals but a negative influence in older subjects. However, this antagonistic pleiotropic effect in healthy individuals remains controversial since a recent large study of 5,445 community dwelling individuals across three age groups (20–24 years, 40–44 years and 60–64 years) found no evidence of higher cognitive performance in the young ε4 carriers (Bunce et al., 2011). Conversely, *APOE*ε4 had negative effects on brain volumes, and on the Ruff fluency test, in our younger but not older HIV subjects. This negative influence of ε4 allele(s) in the younger HIV subjects suggests that the antagonistic pleiotropic effect of the ε4 allele(s) is manifested earlier, or the ε4 allele(s) may lead to premature aging in the setting of HIV. Several recent neuroimaging studies indeed reported lesser brain perfusion (Ances et al., 2010) and lower brain glutamate concentrations (Ernst et al., 2010) in HIV subjects, at levels that were equivalent to those in SN control subjects at least 10 years older.

One possible mechanism for the earlier expression of the gene may be due to a more robust neuro-inflammatory response in younger relative to older HIV individuals. This hypothesis

is consistent with our prior findings of greater elevations in the glial marker myoinositol and lower neuronal marker N-acetylaspartate-to-creatine ratios in younger than older HIV individuals (Chang et al., 2004a). Likewise, a stronger negative impact of *APOE*ε4 on learning and memory deficits was observed in younger patients with multiple sclerosis (MS) (Shi et al., 2008), who typically have significant neuroinflammation. Enhanced neuro-immune responses with increased production of microglial pro-inflammatory markers (NO, TNF-alpha, IL-12, and IL-6) in *APOEε4* genetic background also were reported in cell culture studies (Vitek et al., 2009). The enhanced neuro-inflammatory response in younger HIV or MS patients may be even stronger than the neuro-inflammatory changes that occur with normal aging, which in turn may interact with the *APOE*ε4 protein to reduce neuronal outgrowth and sprouting (Teter et al., 1999), as seen in transgenic mouse models (Buttini et al., 1999; Dumanis et al., 2009). *APOE* protein is predominantly expressed by astrocytes (Pitas et al., 1987) and involved in the delivery of cholesterol and lipids from astrocytes to repair injured neurons. However, glial activation associated with ongoing HIV infection may down-regulate the *APOE* expression, as found in rodent studies (Arora et al., 2009), while the decreased clearance of the neurotoxic *APOE*ε4-protein may lead to brain injury by enhancing β-amyloid induced oxidative damage (Lauderback et al., 2002) and by facilitating the aggregation and deposition of β-amyloid fibrils (Ma et al., 1994), as found in neuropathology studies of HIV patients (Green et al., 2005). All of these mechanisms may further enhance the neuro-inflammatory response.

There are some limitations to the current study. First, despite the use of a well-validated automated brain segmentation technique, FreeSurfer, to evaluate brain morphometric changes in of a group of HIV patients without other major co-morbid neurological conditions or illicit drug or alcohol dependence, we relied on an eTIV to normalize the brain volumes. However, the eTIV may not be as accurate as using a three-dimensional T2-weighted image to determine the ICV. Second, the lack of further decline in brain volumes in our older HIV subjects, and the inconsistent result with some of the studies regarding antagonistic pleiotropy on cognition in healthy subjects, with the ε4 allele(s) may be due to a sample bias since the sample sizes for these subgroups were relatively small, leading to trend effects only in many brain regions measured. Third, our findings are based on a cross-sectional study; a longitudinal study is needed to verify the age-dependent pleiotropic effect of *APOE*ε4 allele(s) on cognition and brain atrophy.

In conclusion, our finding of smaller brain volumes in HIV subjects with normal cognition suggests presymptomatic neurodegeneration in these individuals, who appeared to have compensatory processes or cognitive reserve to maintain normal cognitive performance. The brain atrophy seen in the younger HIV subjects with *APOE*ε4 allele(s), with no further atrophy in the older HIV subjects, suggests that the neurotoxic effect of *APOE*ε4 is manifested early in HIV subjects, which may be related to the more robust neuroinflammation in these younger subjects. Early screening for the *APOE*ε4 allele and brain atrophy with morphometry may guide neuroprotective intervention of cognitively normal HIV subjects prior to the development of HAND. Longitudinal studies and larger sample sizes for each subgroup are needed to validate these cross-sectional findings on the effects of *APOE*ε4 allele(s) on the progression of brain atrophy in these individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Research Highlights

- *APOE*ε4 allele is associated with greater brain atrophy in HIV+ individuals.
- HIV subjects with *APOE*ε4 allele(s) performed worse on most cognitive tests.
- Younger but not older HIV+ individuals are affected by having the ε4 allele(s).
- *APOE*ε4 allele(s) may contribute to neurodegeneration in younger HIV patients.

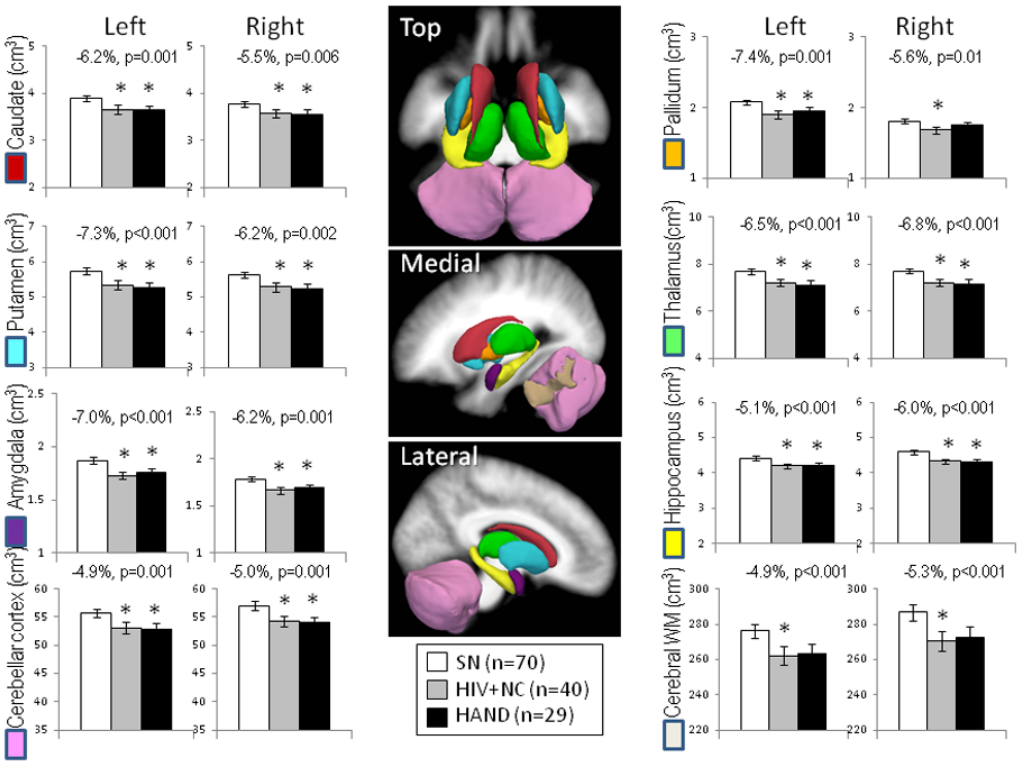

Figure 1.

Center panel: Top, medial and lateral views of averaged MRIs from all subjects, with an overlay of colorized, three-dimensionally rendered subcortical brain structures evaluated (see corresponding color blocks shown in the vertical axes of the bar-graphs). The bar-graphs show smaller subcortical volumes in both left and right hemispheres of the HIV subjects compared to seronegative (SN) controls in each of the 8 brain regions. P-values and % difference values above bar-graphs indicate differences between all HIV and SN subjects. The asterisks indicate differences ($p<0.05$) between SN subjects and either HIV-positive subjects with normal cognition (HIV+NC, gray bars) or with HIV-associated neurocognitive disorder (HAND, black bars). All p-values are corrected for eTIV and age.

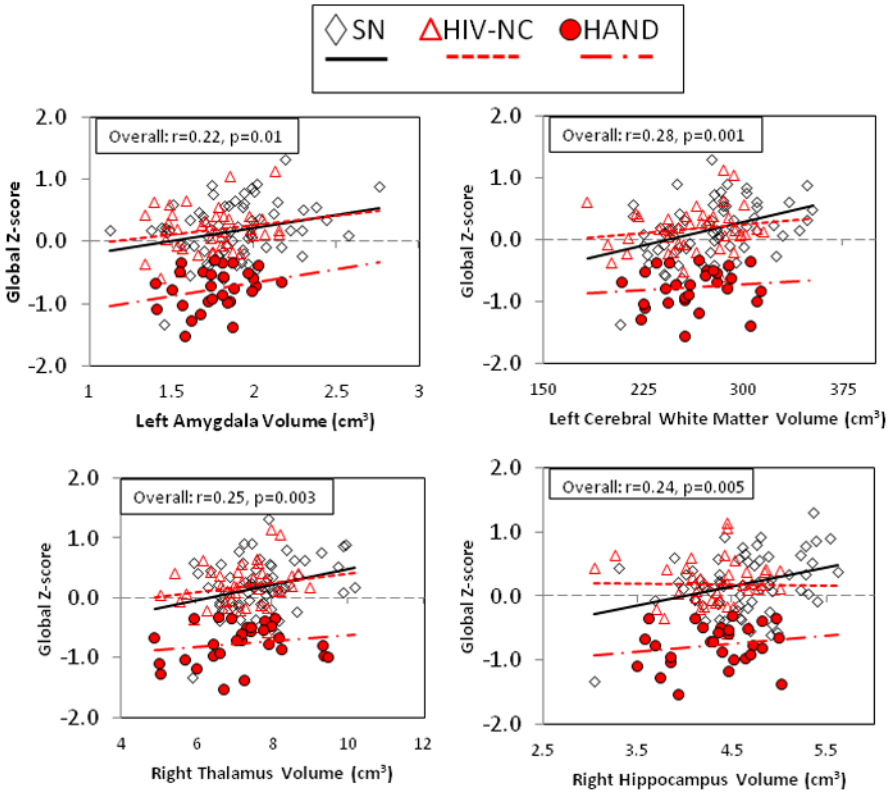

Figure 2.

Representative correlations between global cognitive z-score and subcortical brain volumes. Significant correlations were found in the amydala (left: r=0.22, p=0.01; right: r=0.23, p=0.01, not shown), cerebral white matter (left: r=0.28, p=0.001; right: r=0.24, p=0.005, not shown), thalamus (left: r=0.23, p=0.006, not shown; right: r=0.24, 0=0.005) and hippocampus (left: r=0.21, p=0.01, not shown; right: r=0.24, p=0.005). Note that HIV subjects with or without HAND had similarly smaller range of brain volumes relative to SN subjects, but only HAND subjects had poorer global z-scores than the other two groups. SN subjects had similar volumes and cognitive z-scores as the HIV+NC subjects. However, for each region, the slopes of the trend lines in relation to morphometric measures were similar across the 3 groups. Similar trends were observed in other brain structures. All p-values and partial correlations are adjusted for eTIV.

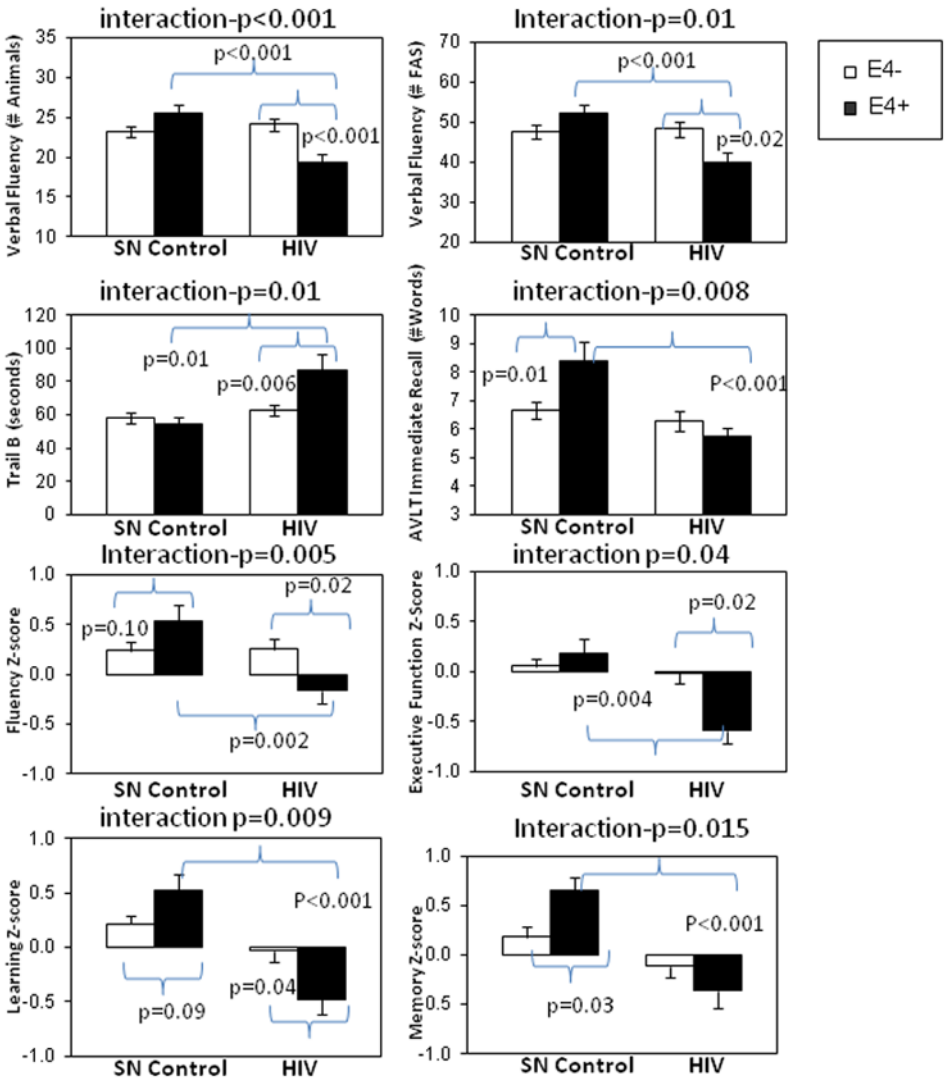

Figure 3.

Two-way interactions (*APOE*ε4 allele × HIV status) show that the presence of *APOE*ε4 is associated with poorer performance in HIV subjects but not in SN controls in each of 4 cognitive tasks (**top 4 bar-graphs**) and 4 cognitive domains (**bottom 4 bar-graphs**). **White bars**=*APOE*ε4−; **Black bars**=*APOE*ε4+

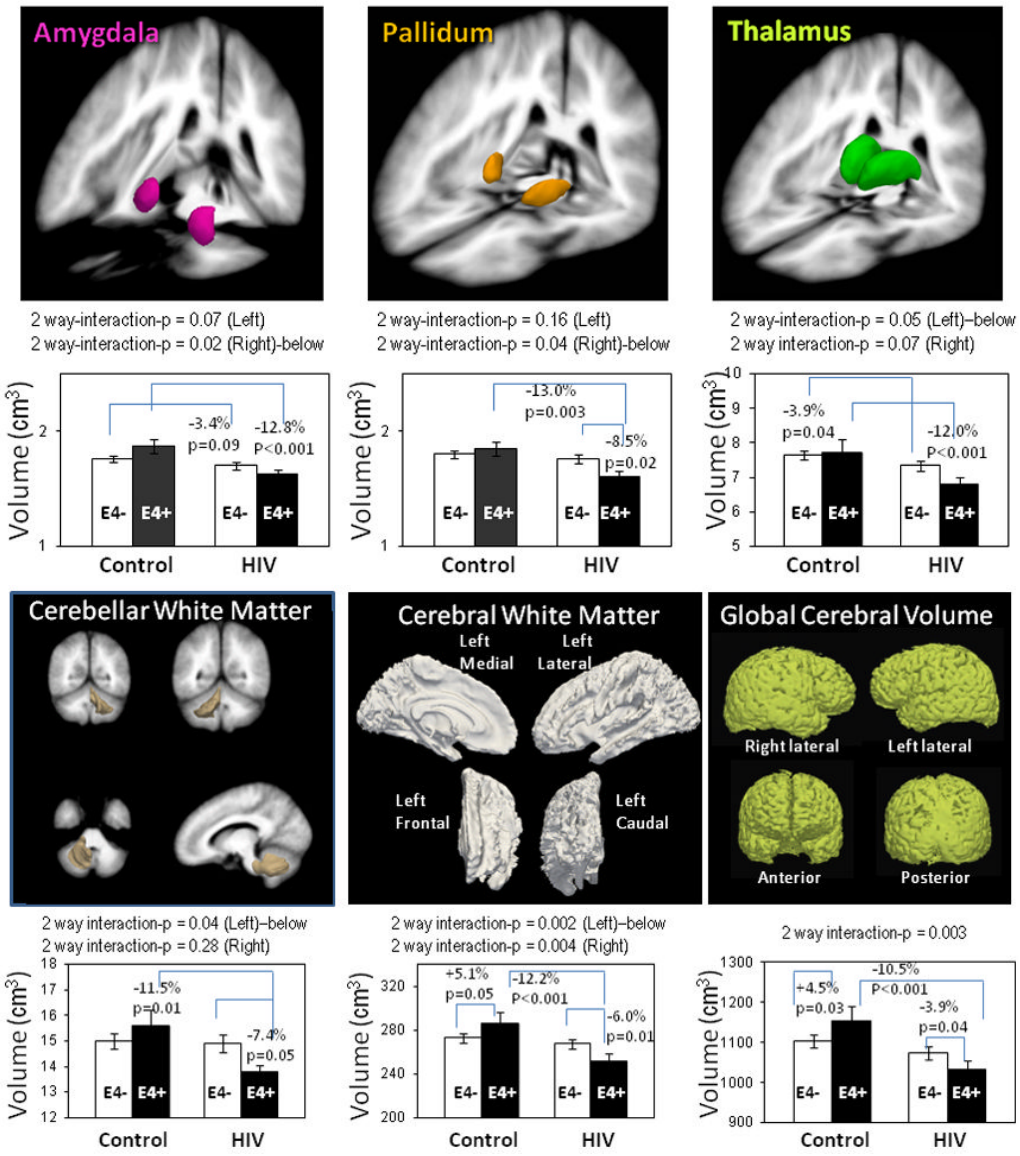

Figure 4.

Figure 4A & B. Two-way interactions (*APOE*ε4 allele × HIV status) show that SN subjects with APOEε4 allele(s) had similar volumes in the amydala, pallidum and thalamus (**4A**) or larger volumes in the cerebellar white matter, cerebral white matter and global brain volume (**4B**) compared to the SN subjects without the ε4 allele. However, HIV subjects with the ε4 allele(s) had smaller volumes than HIV subjects without the ε4 allele or the SN subjects. All p-values are adjusted for age and eTIV.

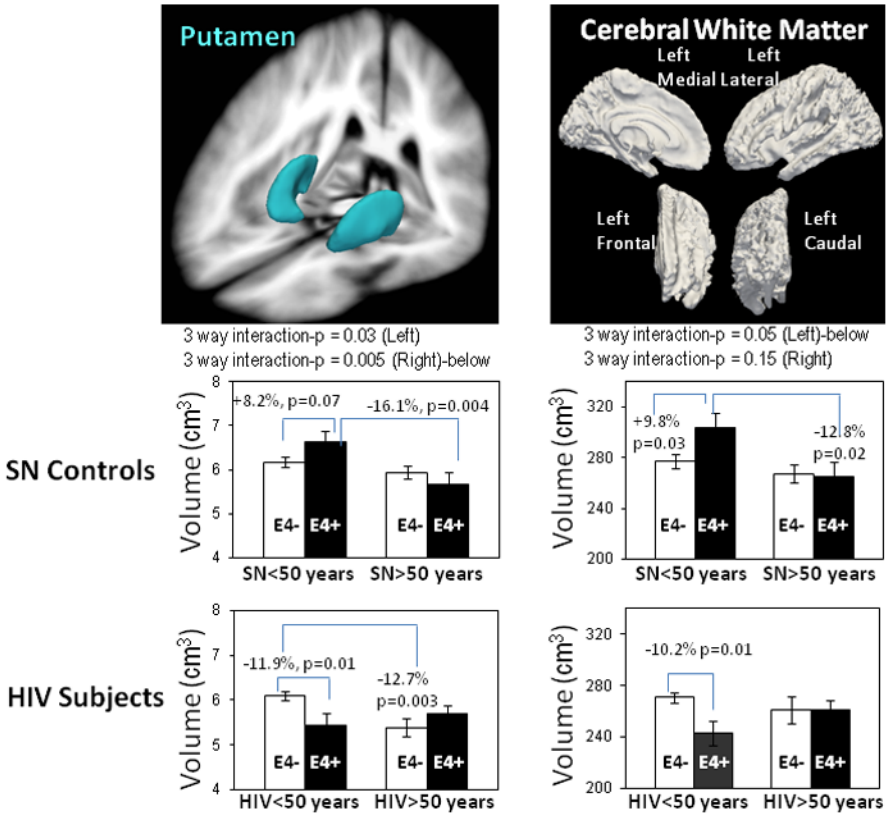

Figure 5.

Three-way interactions (*APOE*ε4 allele × Age × HIV status) show that the presence of *APOE*ε4 allele (black bars) has a positive effect on the putamen and left cerebral white matter in the younger (<50 years) *APOE*ε4+SN subjects, but a negative effect on these brain volumes in the younger HIV+*APOE*ε4+ subjects, with little or no further effect in the older (≥50 years) HIV+*APOE*ε4+ subjects. All p-values are adjusted for eTIV.

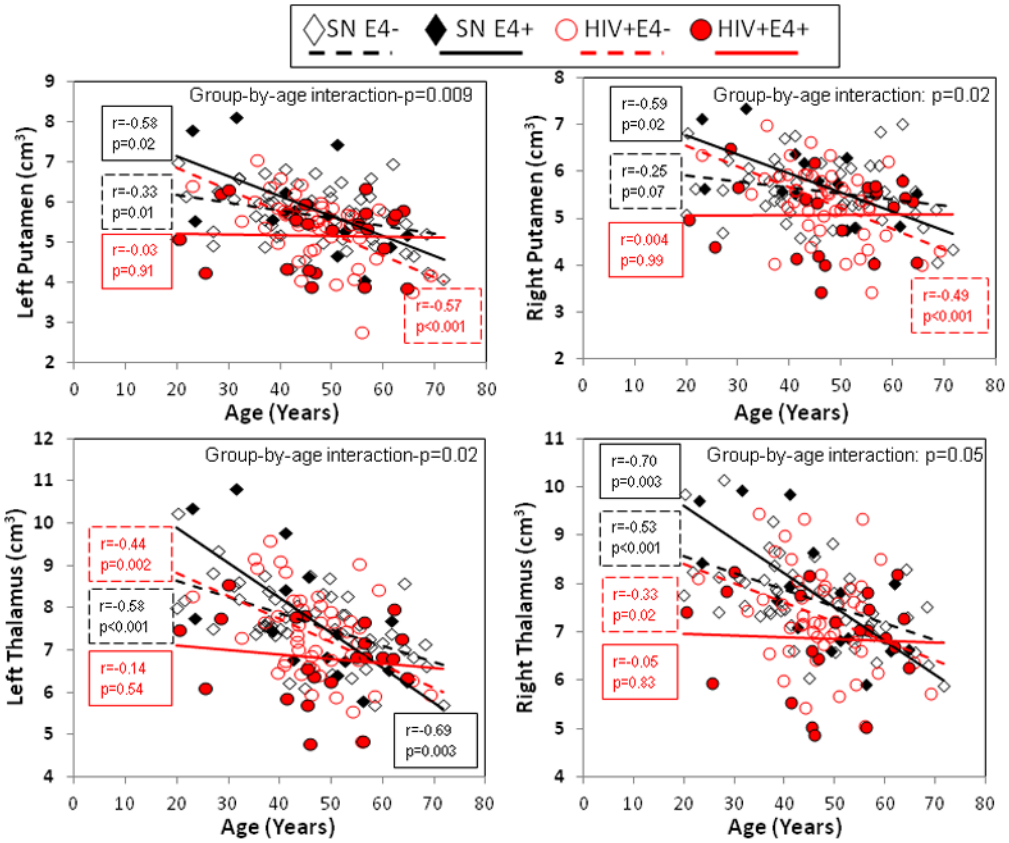

Figure 6.

Linear regressions showing age-related decline in bilateral putamen and thalamus volumes in HIV subjects without the ε4 allele, and in SN subjects with or without ε4 allele(s). The steepest decline is seen in the SN *APOE*ε4+ subjects. Conversely, little or no further age-related decline in these two brain structures is observed in the HIV+*APOE*ε4+ subjects (solid red line), possibly since their brain volumes are already smaller at younger ages, as a result of HIV infection.

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Table 1

Clinical Characteristics and Cognitive Performance of Research Participants (Mean±S.E.)

	SN Control *APOE*ε4− (n=54)	SN Control *APOE*ε4+ (n=16)	HIV+ *APOE*ε4− (n=47)	HIV+ *APOE*ε4+ (n=22)	ANOVA or Fisher's Exact p-value
Age (years)	45.8 ± 1.8	46.0 ± 3.2	47.0 ± 1.2	48.3 ± 2.7	0.85
Younger (50 years)/Older (≥50 years)	32 (59%)/22 (41%)	9 (56%)/7 (44%)	31 (66%)/16 (34%)	11 (50%)/11 (50%)	0.63
Education (years)	14.7 ± 0.3	15.8 ± 0.5	14.7 ± 0.4	14.5 ± 0.5	0.42
Male Sex (%)	49 (91%)	13 (81%)	44 (94%)	19 (86%)	0.50
Race: American Indian	1 (2%)	0 (0%)	3 (6%)	0 (0%)	0.94
Asian	9 (17%)	1 (6%)	6 (13%)	3 (14%)	
Black	2 (4%)	0 (0%)	2 (4%)	1 (5%)	
Native Hawaiian	1 (2%)	0 (0%)	1 (2%)	0 (0%)	
White	35 (65%)	14 (88%)	29 (62%)	13 (59%)	
Mixed	6 (11%)	1 (6%)	6 (13%)	5 (23%)	
Ethnicity (Hispanic/Non-Hispanic)	3 (6%)/51 (94%)	2 (13%)/14 (87%)	8 (17%)/39 (83%)	2 (9%)/20 (91%)	0.31
Nadir CD4 (#/mm^3)			210 ± 22.9	167 ± 35.9	0.31
CD4 (#/mm^3)			492 ± 32.6	348 ± 51.7	0.02 (Cohen's d=−0.63)
Log Viral Load (copies/mL)			2.5 ± 0.2	2.8 ± 0.3	0.43
# (%) with Detectable Viral Load			17 (36%)	9 (41%)	0.79
Karnofsky Score (0–100)			91.8 ± 1.3	90.0 ± 2.1	0.44
HIV Dementia Scale (0–16)			14.7 ± 0.2	13.8 ± 0.4	0.05 (Cohen's d=−0.50)
Duration HIV Diagnosis (months)			146 ± 12.7	147 ± 16.1	0.98
# (%) on ARVs/# (%) NRTIs			37 (79%)/36 (77%)	18 (82%)/18 (82%)	1.0 0.76
CES-Depression Score	6.9 ± 0.8	8.8 ± 1.1	12.8 ± 1.4	11.4 ± 2.4	0.005[a,b] (η^2=0.09)

[a] $p<0.05$ (Cohen's d=0.77) for HIV+*APOEε4*− > SN Control *APOEε4*−

[b] $p<0.05$ (Cohen's d=0.53) for HIV+*APOEε4*+ > SN Control *APOEε4*−

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Table 2

Automatically segmented brain volumes (cm^3, Mean ± S.E.) in the SN controls and HIV subject with and without APOEε4 allele

Brain Region		SN Control *APOE*ε4− (n=54)	SN Control *APOE*ε4+ (n=16)	HIV+ *APOE*ε4− (n=47)	HIV+ *APOE*ε4+ (n=22)	One-way ANCOVA Across 4 Groups	Two-way ANCOVA HIV × *APOE*ε4	Three-way ANCOVA HIV × *APOE* ε4 × age
						effect size (η^2) and (p-value)*§		
Amygdala	L	1.85 ± 0.04	1.94 ± 0.08 (+4.9%)	1.76 ± 0.03 (−4.9%)	1.69 ± 0.05 (−8.6%)	0.34 (0.001)*b, c, d, e*	0.02 (0.07)	0.006 (0.28)
	R	1.76 ± 0.03	1.87 ± 0.06 (+6.3%)	1.70 ± 0.03 (−3.4%)	1.63 ± 0.04 (−7.4%)	0.34 (0.001)*c, d, e*	0.03 (**0.02**)	0.001 (0.68)
Caudate	L	3.90 ± 0.07	3.91 ± 0.13 (+0.3%)	3.70 ± 0.08 (−5.1%)	3.57 ± 0.13 (−8.5%)	0.32 (0.01)*b, c, e*	0.002 (0.49)	0.01 (0.10)
	R	3.79 ± 0.07	3.77 ± 0.13 (−0.5%)	3.62 ± 0.08 (−4.5%)	3.49 ± 0.12 (−7.9%)	0.28 (0.03)*b, c*,	0.002 (0.59)	0.01 (0.21)
Hippocampus	L	4.41 ± 0.06	4.48 ± 0.13 (+1.6%)	4.24 ± 0.07 (−3.9%)	4.12 ± 0.10 (−6.6%)	0.37 (0.004)*b, c, d, e*	0.006 (0.27)	0.01 (0.17)
	R	4.56 ± 0.07	4.74 ± 0.12 (+3.9%)	4.36 ± 0.06 (−4.4%)	4.25 ± 0.09 (−6.8%)	0.43 (<0.001)*b, c, d, e*	0.01 (0.07)	0.001 (0.60)
Pallidum	L	2.07 ± 0.04	2.09 ± 0.09 (+1.0%)	1.97 ± 0.05 (−4.8%)	1.83 ± 0.07 (−11.6%)	0.35 (0.003)*b, c, e*	0.01 (0.16)	0.015 (0.09)
	R	1.80 ± 0.03	1.85 ± 0.06 (+2.8%)	1.76 ± 0.04 (−2.2%)	1.61 ± 0.05 (−10.6%)	0.25 (0.007)*c, e, f*	0.025 (**0.04**)	0.02 (0.08)
Putamen	L	5.69 ± 0.10	5.85 ± 0.28 (+2.8%)	5.38 ± 0.12 (−5.4%)	5.16 ± 0.17 (−9.3%)	0.35 (0.005)*b, c, d, e*	0.01 (0.21)	0.026 (**0.03**)
	R	5.58 ± 0.09	5.71 ± 0.22 (+2.3%)	5.36 ± 0.11 (−3.9%)	5.07 ± 0.17 (−9.1%)	0.32 (0.006)*c, e*	0.01 (0.11)	0.04 (**0.005**)
Thalamus	L	7.65 ± 0.12	7.74 ± 0.38 (+1.2%)	7.35 ± 0.15 (−3.9%)	6.81 ± 0.21 (−11.0%)	0.53 (<0.001)*b, c, e, f*	0.01 (**0.05**)	0.01 (0.08)
	R	7.68 ± 0.11	7.79 ± 0.31 (+1.4%)	7.33 ± 0.15 (−4.6%)	6.86 ± 0.22 (−10.7%)	0.51 (<0.001)*b − f*	0.01 (0.07)	0.01 (0.13)
Cerebellar white matter	L	15.0 ± 0.30	15.6 ± 0.63 (+4.0%)	14.9 ± 0.35 (−0.7%)	13.8 ± 0.27 (−8.0%)	0.19 (0.07)	0.03 (**0.04**)	0.004 (0.43)
	R	14.1 ± 0.27	14.2 ± 0.46 (+0.7%)	14.0 ± 0.28 (−0.7%)	13.2 ± 0.34 (−6.4%)	0.11 (0.23)	0.01 (0.28)	0.00 (0.83)
Cerebellar cortex	L	55.7 ± 0.88	56.0 ± 1.7 (+0.5%)	52.9 ± 0.88 (−5.0%)	53.3 ± 1.2 (−4.3%)	0.43 (0.01)*b, d*	0.00 (0.83)	0.001 (0.69)
	R	56.8 ± 0.93	57.6 ± 1.7 (+1.4%)	54.1 ± 0.86 (−4.8%)	54.2 ± 1.1 (−4.6%)	0.42 (0.01)*b, d*	0.00 (0.83)	0.00 (0.80)
Global cerebral white matter	L	273 ± 4.4	287 ± 9.5 (+5.1%)	268 ± 4.4 (−1.8%)	252 ± 6.5 (−7.7%)	0.53(<0.001)*a, c− f*	0.04 (**0.002**)	0.014 (**0.05**)
	R	284 ± 4.8	296 ± 10.6 (+4.2%)	277 ± 4.9 (−2.5%)	259 ± 7.1 (−8.8%)	0.55 (<0.001)*c − f*	0.03 (**0.004**)	0.007 (0.15)
Global cerebral cortex	L	248 ± 3.6	257 ± 9.1 (+3.6%)	240 ± 3.9 (−3.2%)	237 ± 5.3 (−4.4%)	0.60 (0.002)*b, c, d, e*	0.01 (0.11)	0.001 (0.67)
	R	245 ± 3.5	258 ± 8.5 (+5.3%)	238 ± 3.5 (−2.9%)	234 ± 5.2 (−4.5%)	0.62 (<0.001)*a− e*	0.02 (**0.02**)	0.001 (0.62)

Brain Region		SN Control *APOE*ε4− (n=54)	SN Control *APOE*ε4+ (n=16)	HIV+ *APOE*ε4− (n=47)	HIV+ *APOE*ε4+ (n=22)	One-way ANCOVA Across 4 Groups	Two-way ANCOVA HIV × *APOE*ε4	Three-way ANCOVA HIV × *APOE* ε4 × age
						effect size (η^2) and (p-value)*§		
Global cerebral volume		1,103 ± 15.7	1,153±38.0 (+4.5%)	1,074±16.2 (−2.6%)	1,032±22.9 (−6.4%)	0.63 (<0.001)*a–f*	0.02 (**0.003**)	0.006 (0.17)
Estimated TIV		1,274±24.5	1,281±43.4 (+0.5%)	1,291±24.3 (+1.3%)	1,293±43.1 (+1.5%)	0.00 (0.96)	0.00 (0.96)	-

*For One-way and two-way ANCOVA: After covarying for age and estimated total intracranial volume (eTIV).

§For three-way ANCOVA: After covarying for eTIV.

a p<0.05 for SN Control *APOE*ε4+ > SN Control *APOE*ε4−

b p<0.05 for HIV+*APOE*ε4− < SN Control *APOE*ε4−

c p<0.05 for HIV+*APOE*ε4+ < SN Control *APOE*ε4−

d p<0.05 for HIV+*APOE*ε4− < SN Control *APOE*ε4+

e p<0.05 for HIV+*APOE*ε4+ < SN Control *APOE*ε4+

f p<0.05 for HIV+*APOE*ε4+ < HIV+*APOE*ε4−

Table 3

Cognitive Domains Z-Score* (Mean ± S.E.) for SN and HIV subjects with and without APOEε4

	SN Control *APOE*ε4− (n=54)	SN Control *APOE*ε4+ (n=16)	HIV+ *APOE*ε4− (n=47)	HIV+ *APOE*ε4+ (n=22)	One-way ANOVA effect size (η^2)	One-way ANOVA p-value
Fluency	0.25 ± 0.09	0.54 ± 0.15	0.26 ± 0.10	−0.17 ± 0.14	0.08	0.01[c, e, f]
Executive Functions	0.06 ± 0.11	0.18 ± 0.19	−0.02 ± 0.15	−0.59 ± 0.16	0.07	0.02[c, e, f]
Speed of Information Processing	0.02 ± 0.09	0.18 ± 0.16	−0.18 ± 0.11	−0.49 ± 0.16	0.08	0.01[c, e]
Attention/Working Memory	0.15 ± 0.09	−0.08 ± 0.18	−0.45 ± 0.12	−0.76 ± 0.16	0.18	<0.001[b, c, e]
Learning	0.21 ± 0.09	0.52 ± 0.12	−0.03 ± 0.12	−0.48 ± 0.20	0.14	<0.001[c, d, e, f]
Memory	0.18 ± 0.11	0.65 ± 0.13	−0.11 ± 0.12	−0.36 ± 0.17	0.13	<0.001[a, c, d, e]
Motor Skills	−0.01 ± 0.12	0.33 ± 0.20	−0.23 ± 0.15	−0.27 ± 0.21	0.04	0.15
Global	0.12 ± 0.06	0.33 ± 0.10	−0.11 ± 0.08	−0.45 ± 0.11	0.18	<0.001[b, c, d, e, f]

*Z-scores are adjusted for age and education

[a] p<0.05 for SN Control *APOE*ε4+ > SN Control *APOE*ε4−

[b] p<0.05 for HIV+*APOE*ε4− < SN Control *APOE*ε4−

[c] p<0.05 for HIV+*APOE*ε4+ < SN Control *APOE*ε4−

[d] p<0.05 for HIV+*APOE*ε4− < SN Control *APOE*ε4+

[e] p<0.05 for HIV+*APOE*ε4+ < SN Control *APOE*ε4+

[f] p<0.05 for HIV+*APOE*ε4+ < HIV+*APOE*ε4−