Reduced Region-Specific Corpus Callosum Volumes Correlate with Low Nadir CD4 and Decreased Cognition in HIV-Infected Carriers of the ApoE4 Allele

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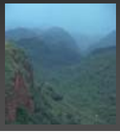


ABSTRACT

Background: Low nadir CD4 cell count and apolipoprotein E4 (ApoE4) allele are known risk factors for HIV-related neurocognitive impairment. ApoE4 is also predictive of development or progression of Alzheimer's disease (AD). The severity of dementia in HIV infection has been correlated to diffusion alterations within the corpus callosum, and in AD, with regionally specific atrophy of the same structure. We hypothesize that volumetric changes in callosal regions may correlate with nadir CD4, presence of an ApoE4 allele, and neurocognitive deficits.

Methods: Seventeen HIV-seropositive men over age 50 (8 with at least one ApoE4 allele; 9 without ApoE4) underwent T1-weighted structural magnetic resonance imaging at 3.0 Tesla, clinical and neuropsychological tests, and ApoE4 genotyping. Nadir CD4 count was determined by patient self-report and confirmed as possible. We utilized FreeSurfer software to parcellate corpus callosum into five segments. Analysis of covariance was used to study the effects of callosal volumes on cognitive domains where neuropsychological test performance differed significantly ($p<.05$) between ApoE4-positive and non-ApoE4 groups. Independent variables were age, education, ApoE4 group status, and callosal segment volume. Regional volumes that were associated with cognitive deficit were examined for correlation with nadir CD4.

Results: Education and viral load did not differ significantly between ApoE4-positive and non-ApoE4 groups. Reduction of normalized total and regional callosal volumes in ApoE4 carriers did not reach significance. Significant ApoE4-associated impairment included diminished visuospatial function and non-verbal memory, which showed effects at significant or trend ($p<.1$) levels of splenium (posterior), genu (anterior), and central callosal volume. Age and education did not contribute significantly to the model. Lower nadir CD4 correlated with decreased splenium (p=0.048, R=0.712) and genu (p=0.040, R=0.730) volume in ApoE4 carriers only.

Conclusions: HIV-seropositive individuals possessing at least one ApoE4 allele are more vulnerable to effects of low nadir CD4 count, which may contribute to region-specific corpus callosum atrophy and thereby to cognitive impairment. Early antiretroviral therapy may be more vital for these patients than for those without the allele.

BACKGROUND

Apolipoprotein E is essential to the metabolism and transport of serum lipids, and mediates cholesterol metabolism in the brain. The E4 isoform (ApoE4) has been linked to impaired cognitive function in the non-demented elderly[1], to vascular dementia[2], and to increased vulnerability to late-onset Alzheimer's Disease (AD)[3]. Inheritance of a single ApoE4 allele nearly doubles an individual's lifetime risk of developing AD[4]. Diffusion tensor imaging has revealed compromised white matter integrity in the posterior corpus callosum of healthy non-demented ApoE4 carriers[5]; and even in healthy, cognitively normal older individuals, presence of the allele has been shown to affect implicit learning[6].

Region-specific atrophy of the corpus callosum has been implicated in AD, mild neurocognitive impairment, and cognitive complaints of older adults with intact neuropsychological functioning[7]. When impairment is slight the posterior callosal region may be the most affected, with atrophy progressing to other regions as dementia grows more severe. Callosal atrophy has also been linked to impaired cognitive and motor function in healthy elderly subjects with age-related white matter changes[8].

As previously reported by our group, HIV-infected patients who are over 50 years of age and heterozygous for ApoE4 have a higher risk of developing HIV-associated dementia than do non-carriers of the allele[9]. Nadir CD4 count has been proposed as a predictor of current cognitive and neurological status[10]. A recent comparison of four nadir CD4 cutoff points (200 to 350 cell/ml) found a trend toward a higher prevalence of impairment as CD4 nadir decreased, along with significantly greater deficits in attention, working memory and executive function[11]. Relative to HIV-seronegative controls, non-demented AIDS patients show a trend toward lower total callosal volume as well as significantly reduced thickness throughout the region, with the thinning in anterior sections correlated to CD4 T-cell decline[12]. Diffusion abnormalities detected in the genu and splenium have been associated with cognitive and motor speed effects of HIV infection[13,14]. **The present study investigates the relationship among neurocognitive test performance, corpus callosum volume, nadir CD4 and presence of an ApoE4 allele.**

METHODS

- In a cross-sectional study, 17 older (age > 50 years) HIV-seropositive patients underwent MRI on a 3.0 Tesla Philips scanner. Pulse sequence parameters for T1-weighted images were T_R=6.7 ms, T_E=3.1 ms, and flip angle of 8°, with an image slice thickness of 1.2 mm and in-plane resolution of 1.0 mm^2.
- FreeSurfer software was utilized to process the T1-weighted images. The workflow included estimation of intracranial volume (ICV) and white matter hyperintensity (WMH) volume.
- StatView (v.5, SAS Institute, Inc.) was used to execute T-tests, analysis of covariance (ANCOVA), and regression for statistical analysis of callosal volumetric data and their possible link to nadir CD4 levels.
- **The corpus callosum was segmented into five labels: posterior, mid-posterior, central, mid-anterior and anterior**. Regional and total callosal volumes were divided by ICV to adjust for head or overall brain size. **Statistical analyses were done on normalized volumes**.
- Neuropsychological testing of patients was performed within 30 days of MRI.
- Determination of ApoE genotype involved[9]:
  - o Restriction enzyme of the 227 base pair PCR product of each specimen
  - o Digestions with /HhaI, HaeII, and AflIII; /followed by resolution by gel electrophoresis
- **Statistical significance is defined by $p < 0.05$, and trends by $0.05 \le p < 0.1$**. Due to small sample size and the exploratory nature of this work, p-values were not corrected for multiple comparisons.

Characteristics of Cohort

- The study population:
  - All participants were HIV-seropositive and on highly active antiretroviral therapy (HAART)
  - 8 patients (**ApoE4-positive group**) who had at least one E4 allele were selected from a larger cohort
  - 9 individuals (**ApoE4-negative or "control" group**) did not carry the ApoE4 allele
  - HIV RNA, or "viral load", was undetectable (< 50 copies/10^6 cells) in 15 patients, and borderline (52 copies/10^6 cells) or low (177 copies/10^6 cells) in 2 others, both non-carriers of the ApoE4 allele.
  - **ApoE4 carriers were significantly older than the non-carriers (p=0.006)** and possessed more years of education (p=0.092).
  - **Nadir CD4$^+$ T-cell count** (the lowest ever reached) **was significantly *higher* in ApoE4 carriers (p=0.031)** than in non-carriers after adjusting for age. Current CD4 count did not differ between groups (p=0.94). (CD4 data was missing for one ApoE4-negative subject.)
  - NART FSIQ (National Adult Reading Test Full-Scale Intelligence Quotient) did not differ between ApoE4 carriers and non-carriers (p=0.99)
  - Dementia status had not been assigned to many in our cohort; however, a quick review of z-transformed neuropsychological test scores indicates that very few, if any, would be classed as demented.

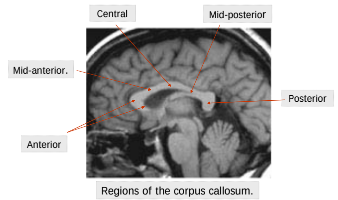
		Overall (N = 17)	ApoE4-positive (N=8)	ApoE4-negative (N=9)
Gender (%)	Male	17 (100)	8 (100)	9 (100)
Age (years)	Mean (S.D.)	55.8 (5.2)	63.1 (6.1)	53.9 (5.6)
Education (years)	Mean (S.D.)	15.1 (2.4)	16.5 (2.3)	14.7 (1.9)
WMH (%)	Present	6 (35.3)	4 (50)	2 (22.2)
Race/Ethnicity (%)	Caucasian	13 (76.5)	7 (87.5)	6 (67)
	Asian/Pacific Islander	2 (12)	0 (0)	2 (22)
	Hispanic	1 (6)	0 (0)	1 (11)
	Native American/Native Alaskan	1 (6)	1 (12.5)	0 (0)
Current CD4 (cells/mm^3)	Mean (S.D.)	414.4 (148.7)	388.8 (183.5)	396.3 (175.4)
Nadir CD4 (cells/mm^3)	Mean (S.D.)	104.9 (103.6)	206.5 (136.3)	112.1 (106.4)

Table 1. Selected demographic and clinical parameters.

RESULTS

ApoE4 and Corpus Callosum Volume

Total and regional corpus callosum volumes were normalized to ICV, which did not differ between ApoE4 carriers and non-carriers (p=0.64). ANOVA by ApoE4 group status showed **no significant differences in normalized callosal volumes between carriers and non-carriers of the ApoE4 allele** (e.g., total corpus callosum: p=0.86). When age, current CD4 count, education and WMH volume were each included separately as covariates, the last two variables had significant main effects but ApoE4 group differences in callosal volumes remained non-significant.



Regions of the corpus callosum.

ApoE4 and Cognitive Impairment

As expected, HIV-infected patients carrying an ApoE4 allele show neurocognitive deficits when compared with non-carriers.

Subjects underwent standard neuropsychological testing that included the Rey Complex Figure Test (Copy, Immediate Recall and Delayed Recall tasks). **ANOVA of test scores by ApoE4 category suggests that non-verbal memory and visuospatial ability are significantly diminished in HIV-infected patients carrying the ApoE4 allele. Presence of the ApoE4 allele was also associated with significantly poorer performance on the Rey Auditory Verbal Learning Test (List B), Grooved Pegboard Tests for dominant and non-dominant hand, Wechsler Adult Intelligence Scale (WAIS) Digit-Symbol Modalities (DSM), WAIS Letter-Number Sequencing, and Trail-Making Test B**. *These results are based on raw rather than on z-transformed test scores and must not be taken as conclusive.*

Cognitive domain	Test	ApoE4 (p-value)
Non-verbal memory	Rey IR	0.023
	Rey DR	0.028
Visuospatial function	Rey Copy	0.012

Table 2. ANOVA: effect of ApoE4 allele presence on selected raw test scores.

Performance on Rey Complex Figure Test

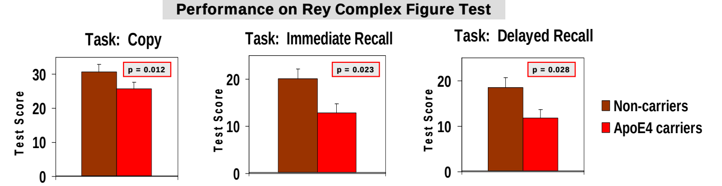


Figure 1. Mean of raw test scores. Error bars represent standard error of the mean.

Q. Do ApoE4 group status and regional corpus callosum volumes have a combined effect on cognition?

Right: **results of ANCOVA for neuropsychological test scores that showed effects of corpus callosum volume. Rey Copy and the mean of Rey IR and DR test scores were outcomes, ApoE4 group status the factor, and regional callosal volumes (separately) the independent variables**. We did not control for age or NART FSIQ, as they were not correlated with these test scores (and were verified to have no significant main effects). Education was not included as a covariate because it correlated with callosal volumes and because of small sample size.

(For RAVLT (List B), Grooved Pegboard Test, Trail-Making B, and WAIS DSM and LN-Sequencing test scores, similar ANCOVA was performed. Age correlated with test performance; after controlling for age, *no regional corpus callosum volume had a significant or trend-level effect on these test scores*.)

Cognitive domain	Test	p-value	
		ApoE4	Regional callosal volume
Non-verbal memory	Rey IR and DR (mean)	0.022	0.055 (anterior)
		0.011	0.088 (central)
		0.012	0.089 (posterior)
		0.012	0.058 (total)
Visuospatial ability	Rey Copy	0.003	**0.025 (posterior)**
		0.004	**0 .048 (mid-posterior)**
		0.005	0.075 (central)
		0.006	0.061 (total)

Table 3. ANCOVA of raw neuropsychological test scores by ApoE4 category. Independent variables: callosal volumes.

A. Regional callosal volumes (particularly posterior) affect test scores of non-verbal memory and visuospatial function, but presence of an ApoE4 allele is more significant. Interactions between ApoE4 group status and callosal volumes did not contribute here. Tests other than Rey Complex Figure Test (IR, DR and Copy) showed no significant effects of corpus callosum volume.

Corpus Callosum Volume Dependence on CD4 Nadir: Possible ApoE4 Effect

Q. Do ApoE4 group status and nadir CD4 count have a combined effect on regional corpus callosum volumes?

ANCOVA of callosal sectional volumes by ApoE4 category, with CD4 nadir as an independent variable, indicated a trend main effect of CD4 nadir (p=0.061) but not of ApoE4 group status on the volume of the mid-posterior region. Effects on callosal volumes of CD4 nadir interaction with ApoE4 allele presence were not significant: interaction p-values for anterior (genu), posterior (splenium), and total corpus callosum volumes were 0.11, 0.10 and 0.16, respectively. *However, the ANCOVA may be of questionable validity because nadir CD4 levels are associated with ApoE4 category in our study population* (Figure 2).

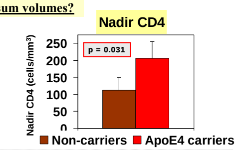


Figure 2. Mean nadir CD4 count is 84% higher in ApoE4-positive group than for ApoE4-negative patients. After controlling for age, the difference is significant (p=0.031).

Figure 3 shows the direct relationship of CD4 nadir to genu, splenium, mid-posterior and total corpus callosum volumes for subjects in both ApoE4 groups:

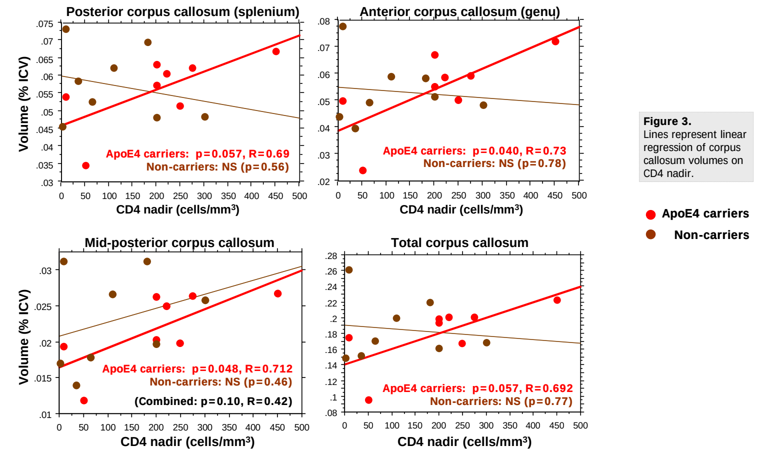


Figure 3. Lines represent linear regression of corpus callosum volumes on CD4 nadir.

A. Among ApoE4 carriers only, anterior and mid-posterior callosal volumes correlate significantly with nadir CD4, and posterior and total corpus callosum volumes show a trend toward such an association. Small sample size and unbalanced nadir CD4 count between the two ApoE4 groups are likely to be masking the true relationship among callosal volume, CD4 nadir and presence of the ApoE4 allele.

Summary

A connection between reduced corpus callosum volume and low nadir CD4 count was observed only in individuals possessing an ApoE4 allele; CD4 nadir correlated positively with genu (p=0.40), splenium (p=0.057), mid-posterior (p=0.048) and total (p=0.57) callosal volumes. These volumes were associated with visuospatial function and non-verbal memory test scores. No relation was found between callosal volume and nadir CD4 in ApoE4-negative patients. **HIV-infected patients who carry an ApoE4 allele may therefore be at increased risk of neurocognitive impairment stemming from corpus callosum atrophy**.

Prior research has linked both low nadir CD4 and the ApoE4 allele with diminished cognition. **The significantly higher CD4 nadir levels in our ApoE4-positive group compared to non-carriers (Figure 2) probably obscured the relationship between ApoE4 presence and nadir CD4 in this study.** For example, we found regional callosal volumes to be associated with performance on only three neuropsychological tests (Table 3) out of the nine that showed significant differences between the two ApoE4 groups. Furthermore, although test scores showed main effects of ApoE4 and callosal volumes, interactions between the volumes and ApoE4 presence were not significant. These results appear likely to change if ApoE4-positive and -negative study participants are well-matched for nadir CD4, particularly because regional callosal volumes did show ApoE4-nadir CD4 interaction effects (Figure 3).

Small sample size limited our ability to adjust for variables such as white matter lesion volume and duration of HIV infection. A larger study population, matched for CD4 nadir count, should be recruited for future work. Improved analytical techniques (e.g., use of normalized neuropsychological test scores, and more accurate estimates of ICV and WHM volume) and other imaging modalities (e.g., diffusion tensor imaging) will help to clarify associations among cognitive deficits, low nadir CD4, corpus callosum atrophy and presence of an ApoE4 allele.

CONCLUSIONS

In ApoE4 carriers, CD4 nadir correlated with reduced regional and total corpus callosum volumes. This relationship was not seen in non-carriers. Callosal volumes and ApoE4 presence are also associated with performance on certain neurocognitive tests. The findings related to cognitive performance are not surprising as these cases were selected from a cohort where ApoE4 risk had previously been described. Should these data be confirmed, research to determine if prompt initiation of HAART can modify the above-described relationships may be advisable. Further investigation using large ApoE4-positive and -negative groups matched for CD4 nadir is indicated.

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