APOE-ε4 associates with hippocampal volume, learning, and memory across the spectrum of Alzheimer's disease and dementia with Lewy bodies

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Word count:

Abstract 147; text 4,090

Numbers:

Figures 2; Tables 4; Supplementary Material (included); References 61

ABSTRACT

INTRODUCTION:

Although the apolipoprotein E ε4-allele (*APOE*-ε4) is a susceptibility factor for Alzheimer's disease (AD) and dementia with Lewy bodies (DLB), its relationship with imaging and cognitive measures across the AD/DLB spectrum remains unexplored.

METHODS:

We studied 298 patients (AD=250, DLB=48; 38 autopsy-confirmed; NCT01800214) using neuropsychological testing, volumetric MRI, and *APOE* genotyping to investigate the association of *APOE*-ε4 with hippocampal volume and learning/memory phenotypes, irrespective of diagnosis.

RESULTS:

Across the AD/DLB spectrum: (1) hippocampal volumes were smaller with increasing *APOE*-ε4 dosage (no genotype x diagnosis interaction observed), (2) learning performance as assessed by total recall scores was associated with hippocampal volumes only among *APOE*-ε4 carriers, and (3) *APOE*-ε4 carriers performed worse on long-delay free word recall.

DISCUSSION:

These findings provide evidence that *APOE*-ε4 is linked to hippocampal atrophy and learning/memory phenotypes across the AD/DLB spectrum, which could be useful as biomarkers of disease progression in therapeutic trials.

Keywords: *APOE*; MRI; hippocampus; Alzheimer's disease; dementia with Lewy bodies; learning; memory; endophenotype

1. INTRODUCTION

Pathological hallmarks of AD are extracellular β-amyloid (Aβ) plaques, and intracellular neurofibrillary tangles (NFT). DLB is characterized by intraneuronal α-synuclein inclusions of Lewy bodies (LBs) and Lewy neurites [1,2]. Clinically, AD and DLB are diagnosed almost exclusively using their respective international consensus diagnostic criteria [1,3,4]. While clinical criteria are generally adequate for providing an initial diagnosis and to inform use of symptomatic therapies, several issues are noteworthy. First, the hallmark proteinopathies of AD and DLB frequently coexist, even among patients diagnosed with a single specific form of dementia in life [5–7]. Second, clinical diagnoses do not always match with autopsy results, often revealing additional incidental co-pathologies, e.g. small vessel disease [8,9]. Third, concomitant pathologies contribute to substantial heterogeneity in disease presentation and progression [7]. These findings serve to challenge the classic neurodegenerative disease distinctions when relying on clinical diagnosis alone. Thus, the identification of common genotype-phenotype (endophenotypic) relationships across the AD/DLB spectrum may offer an objective approach to address these limitations [10]. Indeed, genotype in combination with morphometric measurements derived from structural imaging have emerged as important biomarkers in dementia, which have the potential to advance diagnostic accuracy and improve therapeutic end-points in disease-modifying trials (including of mixed disease).

Apolipoprotein E (*APOE*) is an important gene that may influence the expression of dementia across the AD to Parkinson's disease spectrum (Figure-S1) [11,12]. Human *APOE* has three allelic variants, resulting from two single nucleotide polymorphisms, which differ at one or two amino acid positions: ε2 (Cys-112/Cys-158), ε3 (Cys-112/Arg-158), and ε4 (Arg-112/Arg-158) [13]. *APOE*-ε4 is a well-recognized susceptibility factor for late-onset AD, while *APOE*-ε2 is considered protective against AD. Recent neuropathological studies demonstrate an overrepresentation of *APOE*-ε4 not only in AD but also α-synucleinopathies, specifically among patients showing LB pathology with coexisting “high-level” AD (mixed AD/DLB) and none/“low-level” AD (“pure” DLB, or Parkinson’s disease dementia [PDD]) [11,12]. Additionally, the associations between *APOE*-ε4 and cerebrovascular pathologies, including cerebral small vessel disease and amyloid angiopathy, have been reported in AD [14]. Given that *APOE*-ε4 is a shared susceptibility factor across the AD/DLB spectrum, its association with imaging and cognitive endophenotypes irrespective of specific clinical diagnosis may clarify its role in shared mechanisms of neurodegeneration.

One important brain structure that can be measured through imaging is the hippocampus, which undergoes early neurodegenerative changes in AD, while it is relatively preserved early in the course of DLB [1,3,15]. Hippocampal degeneration in DLB is typically related to the severity of NFT pathology, possibly via mechanisms similar to AD [16,17]. Deficits in learning and memory, which are linked independently to hippocampal integrity and neurogenesis, are also common features of both AD and DLB dementias [18]. No study to date has assessed the interrelationships among *APOE*-ε4, hippocampal volumes, and cognition across the AD/DLB spectrum.

Herein, we investigated the hypothesis that *APOE*-ε4 may be associated with magnetic resonance imaging (MRI)-derived hippocampal volumes, learning, and memory performance, across the AD/DLB spectrum.

2. METHODS

2.1 Participants:

We included 298 participants (AD=250, DLB=48), recruited from the Cognitive Neurology and Geriatric Psychiatry clinics and enrolled in the prospective Sunnybrook Dementia Study (SDS [19]; ClinicalTrials.gov: NCT01800214) at the Sunnybrook Health Sciences Centre, University of Toronto. The details of this study have been previously reported [19]. All participants underwent a detailed neurological evaluation, including standardized MRI, comprehensive neuropsychological battery [20], and *APOE* genotyping [21]. Upon recruitment, AD was diagnosed using the Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria [22], while DLB was diagnosed using the Third Report of DLB Consortium criteria [1]. All cases were retrospectively re-assessed using the current diagnostic criteria for possible/probable AD [3], and possible/probable DLB [4]. Probable AD included those with amnestic (N=174) and non-amnestic (N=16) presentations, while possible AD allowed for the inclusion of those with a high burden of white matter hyperintensities (WMHs) of presumed vascular origin (>10 cm^3)(N=60) [3]. Probable DLB was diagnosed if two or more of the core clinical features of cognitive fluctuations, visual hallucinations, parkinsonism, or REM behaviour disorder were present (N=32), while possible DLB was diagnosed when only one of these core features was present (N=16). Diagnostic consensus was achieved through review by at least two physicians (MM, NH, and SEB) with expertise in dementia diagnosis. Neuropathologic confirmation was available on 38 patients, assessed using standardized techniques. The study was approved by the Sunnybrook Research Ethics Board. All participants (or surrogate caregivers) provided informed consent as per the Declaration of Helsinki.

Details of participant selection and categorization are shown in Figure-1.

2.2 MRI Acquisition:

MRIs were acquired on a 1.5 T Signa system (GE Healthcare) as per standardized protocols compatible with the Alzheimer's Disease Neuroimaging Initiative (ADNI). We used three sets of structural MRI sequences to obtain T1, T2, and proton-density (PD) weighted images. T1-weighted images were acquired using an axial three-dimensional spoiled gradient echo sequence. PD/T2-weighted images were obtained using an interleaved axial dual-echo spin-echo sequence. MRI parameters are provided in Methods-S1.

2.3 MRI Processing:

MRIs were processed using the Semi-Automated Brain Region Extraction and Lesion Explorer processing pipeline (SABRE-LE, http://imaging.brainlab.ca), as published previously [23,24]. Briefly, a tri-featured approach using T1, T2, and PD-weighted images was employed to extract the supratentorial total intracranial volume (sTIV), followed by volumetric quantification of cerebrospinal fluid (CSF), white matter (WM), and gray matter (GM) [24] (Methods-S1). To compare the general atrophy patterns in AD versus DLB, the volumes for the following regions-of-interest were also obtained: whole brain (WM+GM), ventricular CSF, and GM of the frontal, parietal, occipital, and temporal lobes. Visual inspection with appropriate manual interventions was implemented by trained operators to ensure accuracy. Processed skull-stripped T1-weighted MRIs were carried forward for all further segmentations.

2.4 Hippocampal Segmentation:

Hippocampal volumes were obtained using an automated, multi-atlas segmentation procedure, validated on our representative hospital-based SDS sample with cross-validation in the widely-available ADNI-1, demonstrating excellent inter/intra-rater reliabilities (Methods-S1) [25]. Briefly, each participant's processed T1-weighted MRI was matched to a multi-atlas template library. A voxel-wise voting strategy was applied to combine the best templates into the target image space, followed by template-to-target registration, label mapping, and hippocampal segmentation [25]. Normalized hippocampal volume ratios (HVa) were calculated as: [(raw hippocampal volume/sTIV)×10^6].

2.5 White Matter Hyperintensity Quantification:

WMHs on MRI are radiological markers of cerebral small vessel disease, which contribute to significant cognitive decline [26]. We quantified global WMH volumes to assess their confounding influence on HVa, using PD/T2-weighted images [24]. Several quality control procedures were completed to minimize false-positive classifications, including manual checks by trained operators. Normalized WMH volume ratios (WMHa) were calculated as: [(raw WMH volume/sTIV)×10^6].

2.6 Neuropsychological Assessments:

Neuropsychological tests were administered within three months of MRI acquisitions. The following evaluations were administered as part of a comprehensive battery: 1) Mini-Mental State Examination (MMSE) as a global measure of cognitive function [27], 2) Mattis Dementia Rating Scale (DRS) for dementia severity [28], and 3) California Verbal Learning Test (CVLT) for episodic verbal learning and memory performance [29]. We used total recall learning scores, and long-delay and short-delay recalls of CVLT (details provided in Methods-S1). The DRS-memory subscore was included as an additional measure of global memory function. Trained psychometrists blinded to neuroimaging, diagnosis, and genotype administered all assessments.

2.7 Neuropathology:

AD was neuropathologically assessed using tau (AT8) and Aβ immunohistochemistry via the National Institute of Aging–Reagan guidelines, which incorporates NFT staging [2]. LB pathology was documented and staged using α-synuclein immunohistochemistry via the Third Report of DLB Consortium, as either amygdala, brainstem, limbic, or diffuse neocortical type [1]. Mesial temporal sclerosis was determined on hematoxylin-eosin/Luxol-fast-blue (H&E/LFB) staining. Table-S1 lists further details on immunostaining.

2.8 Statistical Analysis:

Participants were categorized by *APOE*-ε4 dose into non-carriers (ε4−/−), heterozygotes (ε4+/−), and homozygotes (ε4+/+). Demographic and clinical data were compared using one-way ANOVA for normally-distributed data, or Kruskal-Wallis H test (H) for non-parametric distributions. Categorical data were analyzed using chi-squared (χ^2) or Fisher's Exact tests. The atrophy patterns in AD versus DLB were compared using ANOVA, adjusting for age at scan, sex, sTIV, formal education, and dementia severity (DRS total score).

To test the association between *APOE*-ε4 dose (independent variable) and HVa (dependent variable) in the pooled cohort of AD and DLB patients, we used multiple linear regressions adjusting for clinical diagnosis, age at scan, and formal education (Model-1)

(Methods-S1). The dose-dependent relationship of *APOE*-ε4 on HVa was modeled by treating *APOE*-ε4 as a numeric continuous variable (i.e., 0, 1, or 2 ε4 alleles).

To assess whether the association of *APOE*-ε4 with HVa was stable across the diagnostic categories, we tested an interaction between *APOE*-ε4 dose and clinical diagnosis. If the interaction was non-significant, it was removed from the models to interpret the main effects. Subsequently, the following sensitivity analyses were performed: 1) further adjusting the model for sex and WMHa (Model-2), 2) repeating the analyses after excluding *APOE*-ε2-carriers due to *APOE*-ε2's protective influence against AD, 3) separately evaluating the association within the AD and DLB samples, and 4) evaluating the GM volumes of frontal, parietal, occipital, and temporal lobes across the AD/DLB spectrum to determine the specificity of our findings to the hippocampus.

To assess whether HVa differ in its association with CVLT total recall scores based on *APOE*-ε4 status, hierarchical multiple linear regressions were performed separately among *APOE*-ε4-carriers (ε4+) and non-carriers (ε4–). In step-1, a block of variables known to influence learning ability (i.e., age at scan, formal education, and DRS total score) were first entered, followed by HVa in step-2. This analysis was repeated in AD and DLB stratified groups. As other CVLT and DRS-memory indices failed to meet linear model assumptions, Mann-Whitney U-tests were employed to compare performance in ε4+ versus ε4–.

Finally, while restricting the sample to those with pathological confirmation, we tested the association between *APOE*-ε4 and antemortem HVa using multiple linear regressions, adjusting for pathology-based groups (defined as per [1]), age at scan, and formal education.

All analyses were performed in SPSS (V22.0, IBM). As our analyses were pre-planned, informed by scientific literature, and the cognitive variables are inter-related, statistical significance was maintained at $P<0.05$, two-sided.

3. RESULTS

3.1 Demographic, Clinical, and Neuroimaging Characteristics:

Of the 298 participants (48% men, 52% women), 124 were ε4–/–, 133 ε4+/–, and 41 ε4+/+, including 8 ε2/4 and 13 ε2/3 cases. Demographic and clinical characteristics were not different among these groups (Table-1).

The DLB group had a higher male prevalence versus female (67% vs. 33%), while the AD group had a higher female prevalence (56% vs. 44%)($\chi^2_{1,298}$=8.00, P=0.0047), as consistent with the existing literature [11] (Table-S2). No statistically-significant differences were present in other demographic and clinical variables analyzed between AD and DLB. *APOE* genotype frequencies in AD and DLB were in Hardy-Weinberg equilibrium.

Compared to AD, the DLB group showed less ventricular CSF volume (P=0.0198), and relative preservation of the whole brain (P=0.0011) and temporal GM volume (P=0.0065) (Figure-S2). These patterns are in line with the general atrophy profiles of AD and DLB patients [30].

3.2 Hippocampal Volumes:

The left and right HVa were highly correlated (Pearson's r=0.79, P<0.0001). Thus, bilateral HVa were assessed in all analyses. Table-2 presents the normalized volumetric data.

In Model-1, *APOE*-ε4 dose and age at scan emerged as significant predictors of HVa (Table-3). *APOE*-ε4 was inversely related to HVa in a dose-dependent manner: genotype ε4+/+ was associated with smaller, ε4+/– with intermediate, and ε4–/– with larger HVa, on average (β=-0.20, P<0.0001). No interaction between *APOE* dosage and clinical diagnosis was observed

(P=0.9422), suggesting that the association of *APOE*-ε4 on HVa was stable across the AD/DLB spectrum. Age at scan was inversely (β=-0.42, P<0.0001) related to HVa.

In models stratified for clinical diagnosis, *APOE*-ε4 was also inversely associated with HVa within the AD (β=-0.20, P=0.0006) and DLB (β=-0.28, P=0.0428) subgroups in a dose-dependent fashion (Table-S3; Figure-2).

The dose-dependent association of *APOE*-ε4 with HVa remained unchanged after additionally adjusting for sex and WMHa (Model-2, Table-3); as well as in other sensitivity analyses performed upon excluding *APOE*-ε2 carriers (β=-0.20, P<0.0001) or cases with genotype ε2/4 only (β=-0.21, P<0.0001). No significant association of *APOE*-ε4 with other GM regions-of-interest was observed.

3.3 Learning and Memory Performance:

All models in the hierarchical multiple linear regressions showed a statistically significant overall fit for the data (Table-4). In the pooled analysis, after including age at scan, formal education and DRS scores, the inclusion of HVa into the regression contributed significantly to the model in ε4+, but not ε4– subgroups. Specifically, HVa associated significantly with CVLT total recall performance accounting for an additional 3% of variance in scores among ε4+, while the relationship was non-significant among those who were ε4–.

This analysis within the AD and DLB stratified groups was confirmatory, where the inclusion of HVa contributed significantly to the models only in AD ε4+ and DLB ε4+ subgroups (but not in AD ε4– and DLB ε4–) explaining additional variances in CVLT total recall scores.

The ε4+ individuals performed more poorly on DRS-memory and CVLT long-delay recall measures. The CVLT long-delay (20-minute) free recall performance was significantly worse in ε4+ versus ε4– across all participants (U_{256}=6715.0, P=0.0320) (Table-S4). The DRS-memory findings serve as additional validation of the CVLT results.

3.4 Neuropathology:

Of the 38 patients with neuropathological examination, 25 were clinically-diagnosed as AD (8 possible/17 probable) and 13 as DLB (5 possible/8 probable). All AD cases were pathologically confirmed to have AD upon autopsy (Braak: 1 I/II, 7 III/IV, 17 V/VI), including 4 cases with coexisting LBs (2 as diffuse, 1 amygdala, 1 brainstem only). All DLB cases had LB pathology upon autopsy (12 as diffuse, 1 limbic), with varying degrees of concomitant NFT pathology (Braak: 2 I/II, 4 III/IV, 7 V/VI). Demographic data, antemortem HVa, and details of each pathology-confirmed case are provided (Table-S5; Table-S6).

In our pathology-confirmed sub-sample, the ε4+ showed smaller antemortem HVa versus ε4– (β=-0.32, P=0.0495), with non-significant interaction between *APOE*-ε4 × pathology-defined groups (P=0.2616) (Table-S7). When analyzed based on *APOE*-ε4 dose, a trend-level relationship was observed (β=-0.29, P=0.0839). The antemortem HVa correlated with post-mortem Braak NFT stages (Spearman’s ρ=-0.32, P=0.0476) (Figure-S4).

4. DISCUSSION

We found that across the AD/DLB dementia spectrum: 1) *APOE*-ε4 was associated with smaller hippocampal volumes in a dose-dependent manner, 2) global learning performance was uniquely related to hippocampal volumes among ε4+ but not ε4– individuals, and 3) the ε4+ participants were significantly impaired on long-delay free recall of words. The *APOE*-ε4 dose was also inversely related to hippocampal volumes in the AD and DLB subgroups. Likewise, global learning performance was associated with hippocampal volumes only among the ε4+ of AD and DLB but not among the respective ε4– individuals. Furthermore, we found an inverse relationship between *APOE*-ε4 dosage and hippocampal volumes in our pathology-confirmed sub-sample.

A prior study using ADNI-1 data has reported an association between *APOE*-ε4 and smaller hippocampal volumes in AD [31], although there are contradictory reports [32–34]. ADNI-1 is a multi-centre clinical trial population representing relatively well-educated and comorbidity-free individuals, which may limit the generalizability of those results. To our knowledge, no study has explored an association of *APOE*-ε4 with hippocampal volumes in DLB, or across the AD/DLB spectrum including quantification of small vessel disease, or has examined this relationship using pathology-confirmed cases. This is important, given the frequent occurrence of mixed pathologies not only in the clinic but also community-based samples [9,35]. We incorporated all cases meeting the clinical diagnostic criteria for AD or DLB, irrespective of the extent of small vessel disease on MRI, knowing that several cases will also have mixed AD/DLB on autopsy; indeed, we showed this to be the case in our autopsy-confirmed sub-sample. Compared to ADNI, we have previously shown our sample to be more generalizable than ADNI to the real world where co-pathologies such as white matter disease are highly prevalent [36].

We demonstrate that this association appears to be stable across the AD/DLB spectrum as well as in the AD and DLB groups, irrespective of the specific clinical diagnosis. We found support for this finding by systematically adjusting for pertinent confounders including WMHs, which can independently impact hippocampal volumes directly, or indirectly via vascular damage [19,37]. Although the precise mechanisms remain unclear, it is likely that *APOE*'s allele-specific structural and biochemical properties play a significant role. Two types of mechanisms for *APOE* have been highlighted using animal and *in vitro* investigations, with supportive evidence accumulating from human studies: Aβ-dependent and Aβ-independent mechanisms. These mechanisms reflect altered properties for *APOE*-ε4 compared to the *APOE*-ε3 and *APOE*-ε2 alleles [13], which may be operational across the AD/DLB spectrum, as discussed below.

The Aβ-dependent mechanisms postulate increased deposition and impaired clearance of Aβ in ε4+, as shown in mouse models expressing human *APOE*-ε4 [38]. This hypothesis is supported in humans, with findings of more extensive Aβ deposition in ε4+ of AD in temporoparietal regions, as detected by Pittsburgh Compound-B (PIB) [39]. Similarly, a significantly lower CSF level of Aβ-42, an indirect measure of increased cerebral retention, is demonstrated in AD ε4+ [40]. Patients clinically-diagnosed with DLB are also commonly PIB-positive (~60%), and elevated Aβ deposition in the cortex is reported in ε4+ across the LB disease spectrum [41]. Such augmented *APOE*-ε4-associated Aβ deposition in the brain including neocortical regions can disrupt cortico-hippocampal networks and may indirectly lead to hippocampal atrophy via cortical denervation. In fact, an association between elevated Aβ deposition and smaller hippocampal volumes has been reported with modulatory effects on episodic memory in AD [42]. This sequential progression of events, from Aβ deposition to hippocampal atrophy to memory dysfunction, may be exacerbated in the context of much heavier Aβ burden in ε4+ of AD and DLB. Hence, such *APOE*-ε4-associated hippocampal loss as observed in our study, along with greater impairment in delayed memory recall, could have been predicted.

Hypothesized Aβ-independent mechanisms include an increased tendency of *APOE*-ε4 to induce tau hyperphosphorylation and undergo proteolysis, leading to ineffective repair capacity, cytoskeletal abnormalities, and mitochondrial energy disruptions [13,43]. Tau hyperphosphorylation and accumulation of C-terminal truncated fragments have been detected in the hippocampus of transgenic mice expressing human *APOE*-ε4 [44]. Likewise in humans, greater levels of hyperphosphorylated tau, along with lower expression of proteins associated with neuronal transport and synaptic health were observed in post-mortem hippocampal tissues from *APOE*-ε4-homozygous versus *APOE*-ε3-homozygous AD patients [45]. Such functional abnormalities can adversely impact synaptodendritic health, especially in highly dynamic structures like the hippocampus, which undergoes extensive neuroplastic changes crucial to its role in learning/memory [13,45]. Indeed, healthy ε4+ individuals show abnormal task-based activation and resting-state connectivity patterns, as well as impaired glucose metabolism in the hippocampus [46,47]. Hence, hippocampal volume loss associated with *APOE*-ε4 may also be a consequence of functional abnormalities in neuronal metabolism and synaptodendritic maintenance, commencing decades before clinically-overt dementia.

As AD pathology frequently coexists with LB pathology [5,35], it is possible that *APOE*-ε4 may contribute to hippocampal degeneration in DLB cases by exacerbating AD-type tauopathy. In fact, the odds of carrying *APOE*-ε4 also progressively decrease across the AD to LB disease spectrum [11], much like the severity of tauopathy. Previous work by Kantarci and colleagues have found antemortem hippocampal volumes to decrease from high to intermediate to low “likelihood” of DLB, as per the Third Report of DLB Consortium scheme [16]. Moreover, like AD, antemortem hippocampal volumes in DLB relate to the severity of NFT pathology [16,17]. These observations are consistent with our pathology data. Thus, the *APOE*-ε4 isoform may affect hippocampal volume by aggravating or independently instigating tauopathy across the AD/DLB spectrum, perhaps via modulation of tau hyperphosphorylation. Future work incorporating multimodal indices that correlate with AD-type patterns (e.g., SPARE-AD [49,50]) would be exciting avenues to explore these hypotheses.

The estimates obtained for the association of *APOE*-ε4 with antemortem hippocampal volume in our relatively small pathology-confirmed sub-sample were comparable to those of our clinical sample, suggesting a well-characterized cohort. *APOE*-ε4 has also been associated with poor prognosis in DLB, especially in those with smaller hippocampal volumes [48]. Altogether, these observations suggest an important link between *APOE*-ε4 and hippocampal degeneration across the AD/DLB spectrum. While DLB is characterized by relative preservation of the medial temporal lobe (MTL) structures on MRI versus AD in early stages, several DLB patients may present with hippocampal/MTL atrophy, as observed in our study. Given the immense relevance of this observation in clinical and research settings and for patient recruitment in clinical trials, further characterization of this heterogeneity in DLB cases is desirable, as we move closer to precision medicine approaches.

Some evidence links AD and LB pathologies together, suggesting possible synergistic interactions among tau, Aβ, and α-synuclein in promoting fibrillization and subsequent neurodegeneration, especially in mixed dementia. For example, α-synuclein and tau co-fibrillization in LB aggregates have been observed [51]. Likewise, enhanced accumulation of α-synuclein associated with Aβ in animal models, as well as increased coexistence of α-synuclein lesions in human brains with Aβ deposits (versus those without) have been reported [52,53]. This suggests that in ε4+, exacerbation of Aβ burden and/or tauopathy may also impact α-synuclein aggregation, contributing to hippocampal loss in the context of mixed disease (Figure-S5).

APOE-ε4 may also have distinct roles in α-synuclein aggregation [54], and the underlying mechanisms may be different from AD-type proteinopathies. A more extensive pattern of atrophy associated with *APOE*-ε4 in regions beyond the hippocampus in DLB/PDD and in “mixed” versus “pure” DLB cases may highlight such mechanisms in the context of pure α-synucleinopathies. This approach may ideally be pursued in the future using voxel-based morphometry which allows for the elucidation of focal anatomical changes irrespective of predefined regional or structural boundaries.

We also found the association of hippocampal volumes with global learning performance uniquely among ε4+ in the overall sample, and in AD and DLB subgroups. This reinforces *APOE*-ε4's involvement as an important moderating factor in hippocampal degeneration across the AD/DLB spectrum with measurable cognitive effects among ε4+ carriers. Such association was not observed among ε4–, indicating that other factors are probably more important in these individuals in determining hippocampal and associated cognitive phenotypes. Learning deficits in ε4+ have previously been reported in both human and animal studies [13,55]. Recently, a large study confirmed learning impairments in ε4+ of Parkinson's disease and Parkinson's disease dementia [56]; however, unlike the current study, specific associations with hippocampal volumes were not investigated.

Finally, patients with ε4+ also performed poorly on delayed recall measures in our pooled sample, as consistent with the previous AD and PDD literature [55,56]. Indeed, learning and memory also depend upon hippocampal synaptodendritic plasticity, constant neurogenesis, and communications within the brain's memory networks. It is thus conceivable that *APOE*-ε4 may contribute to cognitive deficits secondary to injury to these pathways, including hippocampal degeneration.

The strengths of our study include a well-characterized cohort, validated through standardized instruments and expert consensus, pathological confirmation in a subset of patients demonstrating a high degree of concordance between clinical and post-mortem diagnoses, rigorous image-processing methods including a hippocampal segmentation scheme validated specifically for older adults and mixed dementia applications, and adjustments for pertinent confounders including WMH volumes.

There are certain limitations. First, as this was a cross-sectional study, causal inferences could not be drawn. Second, concomitant AD-type pathology is common in clinically-diagnosed DLB, and even evident in cognitively-normal elderly and “pure” DLB [57]. Given that “pure” DLB cases are relatively uncommon as consistent with our pathology data and were underrepresented in our study, results should be extended to these cases with caution. Third, the pathology of transactive response DNA-binding protein-43 (TDP-43) is known to influence cognitive and hippocampal phenotypes [6]. As only a few participants (N=13) underwent TDP-43 immunohistochemistry, its influence on our results could not be assessed. Finally, our DLB group was limited by a relatively small sample-size, although comparable to other single-centered investigations [58–60]. This nevertheless prevented us from analyzing cognitive differences within the DLB group in detail (i.e., CVLT long- and short-delay recalls). Likewise, our pathology-confirmed sample was also small, precluding us from examining relationships separately within the pathology-defined groups. Therefore, it is important to validate our findings in studies such as ONDRI and CCNA, which are examining patients covering the full spectrum of neurodegenerative diseases using a comprehensive and standardized research platform.

4.1 Conclusions:

Our study identifies hippocampal volume and performance in learning/memory as important endophenotypes of *APOE*-ε4 across the AD/DLB spectrum. A subset of these patients may be candidates for therapeutic interventions targeting *APOE*-ε4 using hippocampal volume as an outcome measure. If *APOE*-ε4 indeed operates through similar mechanisms, interventions that prevent hippocampal neurodegeneration or stimulate neurogenesis in AD ε4+ (e.g. exercise [61]) may also be beneficial for DLB ε4+ cases. Slowing of this disproportionate degeneration could be a viable end-point in clinical trials, to facilitate precision medicine approaches in the future, especially for late-onset sporadic forms of dementia, which often includes mixed pathologies.

5. ACKNOWLEDGMENTS

The authors thank the participants, their relatives, psychometric assessors, and examiners who contributed to the Sunnybrook Dementia Study since its inception. The authors are grateful to Melissa Holmes and Christopher Scott for their assistance in imaging database queries and technical support. The authors are also thankful to Alicia McNeely and Courtney Berezuk for assistance in image processing, Isabel Lam for helping with clinical database queries, Dr. Fuqiang Gao for providing radiological expertise for the identification and exclusion of strokes, and Dr. Fadi Frankul for help in compiling the autopsy results.

The authors report no conflicts of interest with the work presented in this study. This work was supported by Canadian Institutes of Health Research grant (MOP13129) to M.M. and S.E.B, and an Early Researcher Award to M.M. from the Ministry of Research, Innovation, and Science (MRIS; Ontario). U.S. was supported by Ontario Graduate Scholarship, Margaret & Howard Gamble Research Grant, and Scace Graduate Fellowship in Alzheimer's Research, University of Toronto. The authors also gratefully acknowledge financial support from the following sources: M.M. receives salary support from the Department of Medicine at Sunnybrook Health Sciences Centre and the University of Toronto, as well as the Sunnybrook Research Institute. WS reports support from the Alzheimer’s Association (US) and Brain Canada (AARG501466).

6. FIGURE CAPTIONS

Figure 1: Flowchart of participant selection and categorization.

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; DLB, dementia with Lewy bodies; ε4+, carriers of at least one *APOE*-ε4 allele; ε4– or ε4−/−, *APOE*-ε4 non-carriers; ε4+/+, *APOE*-ε4 homozygotes; ε4+/−, *APOE*-ε4 heterozygotes.

Figure 2: The association of *APOE*-ε4 with hippocampal volumes across the spectrum of AD and DLB.

Boxplots presenting the normalized hippocampal volume ratios for the pooled sample of AD and DLB (A), and within the clinical diagnostic categories of AD and DLB (B), along with *P*-values showing significant relationships. The AD/DLB spectrum can be conceptualized as representing a continuum, with amyloidopathy and tauopathy at one extreme and α-synucleinopathy at the other extreme, with varying degrees of the three proteinopathies in the middle (C). *APOE*-ε4 has been identified as a risk factor across this spectrum [11]. Our study identifies a link between *APOE*-ε4 and hippocampal volumes in AD, DLB, as well as across the AD/DLB spectrum.

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Figure 1. Flowchart of participant selection and categorization.

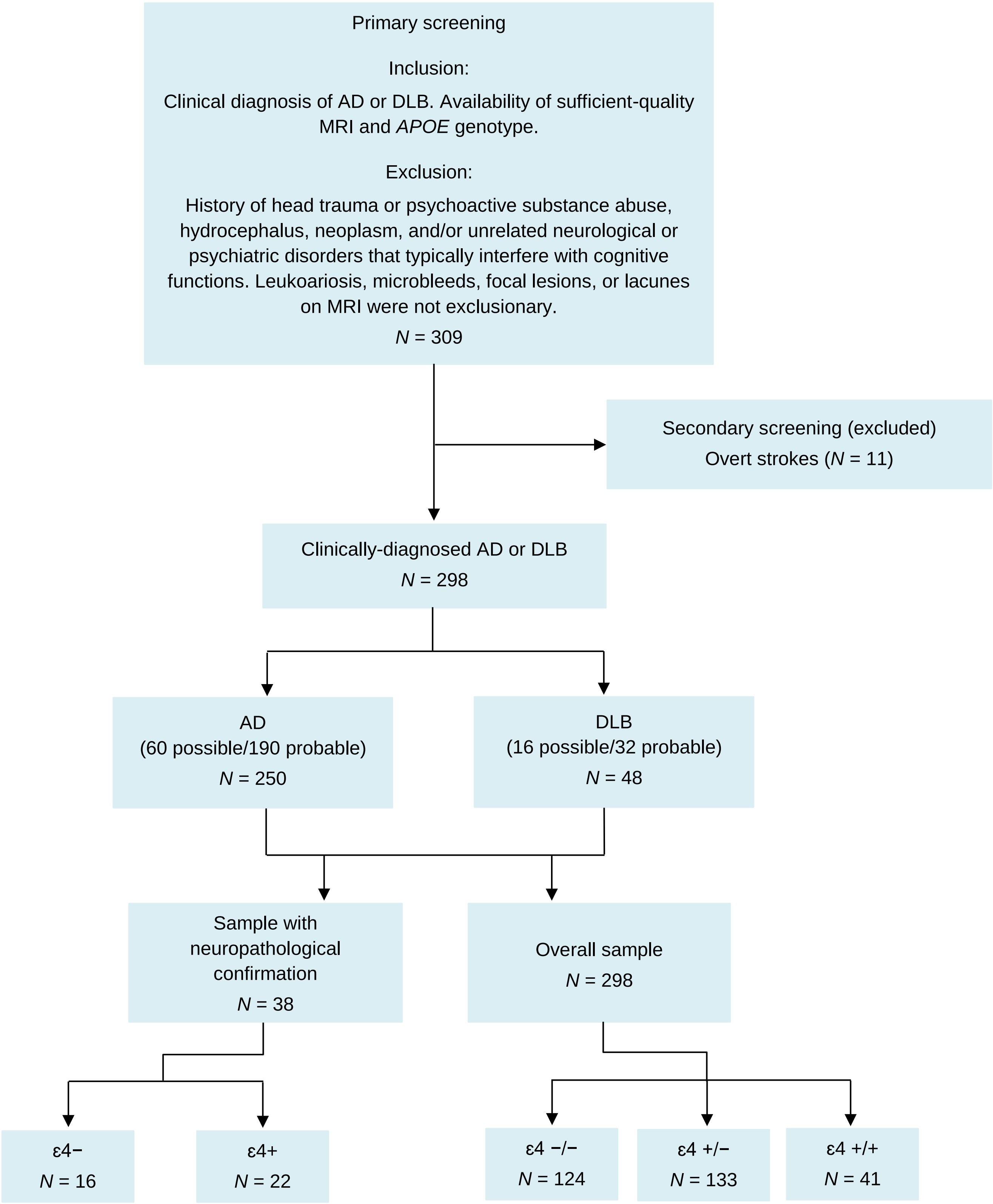


Table 1. Demographic and general clinical characteristics by *APOE* groups.

Characteristics	*APOE* groups ε4 –/– [*N* = 124]	 ε4 +/– [*N* = 133]	 ε4 +/+ [*N* = 41]	*P* value
Age, mean (SD), y				
At onset	68.5 (9.8)	66.6 (10.9)	66.2 (8.4)	0.24 ¶
At scan	72.5 (9.4)	71.2 (8.9)	69.9 (7.9)	0.22 ¶
Disease duration, mean (SD), y	4.0 (2.7)	4.6 (6.0)	3.7 (2.2)	0.63 ‡
Sex, No. (%)				
Male	64 (52)	61 (46)	18 (44)	0.56 §
Female	60 (48)	72 (54)	23 (56)	
Formal education, mean (SD), y	14.0 (3.6)	14.0 (3.6)	13.0 (3.8)	0.24 ¶
MMSE total, mean (SD) *	23.5 (4.3)	23.7 (3.8)	22.6 (5.5)	0.37 ¶
DRS total, mean (SD) †	118.3 (14.7)	118.3 (12.8)	120.0 (12.1)	0.78 ¶
Clinical diagnosis, No. (%)				
AD	103 (83)	113 (85)	34 (83)	0.90 §
DLB	21 (17)	20 (15)	7 (17)	

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; DLB, dementia with Lewy bodies; DRS, dementia rating scale; MMSE, mini-mental status examination; * Score out of 30; † Score out of 144; ‡ Kruskal-Wallis H test; § Chi-squared test; ¶ One-way ANOVA.

Table 2. The volumetric data for hippocampal volumes and white matter hyperintensities by *APOE* genotype.

	APOE genotype				
Characteristics	ε4 –/–	ε4 +/–	ε4 +/+	ε3/3	ε3/4
HVa, mean (SD)	4054.0 (635.0)	3926.4 (559.4)	3778.9 (660.2)	4029.2 (627.1)	3906.8 (547.8)
AD	4053.8 (651.3)	3904.0 (583.5)	3803.3 (668.5)	4023.5 (640.4)	3883.6 (566.9)
DLB	4054.6 (562.4)	4045.8 (375.3)	3660.2 (654.4)	4055.3 (577.0)	4044.3 (402.7)
WMHa, median (IQR)	3289.6 (6848.5)	3616.1 (7583.3)	2305.3 (5371.0)	3652.2 (7604.3)	3434.0 (7218.5)
AD	3403.4 (7653.4)	3434.0 (7218.5)	1984.7 (6419.6)	3670.3 (7665.0)	3434.0 (7156.8)
DLB	3104.6 (6624.1)	3899.6 (11208.9)	2827.2 (2101.9)	3173.0 (6898.7)	3343.1 (9234.3)

Abbreviations: APOE, apolipoprotein E; HVa, normalized hippocampal volume ratios (×10^6); IQR, interquartile range; SD, standard deviation; WMHa, normalized white matter hyperintensities volume ratios (×10^6).

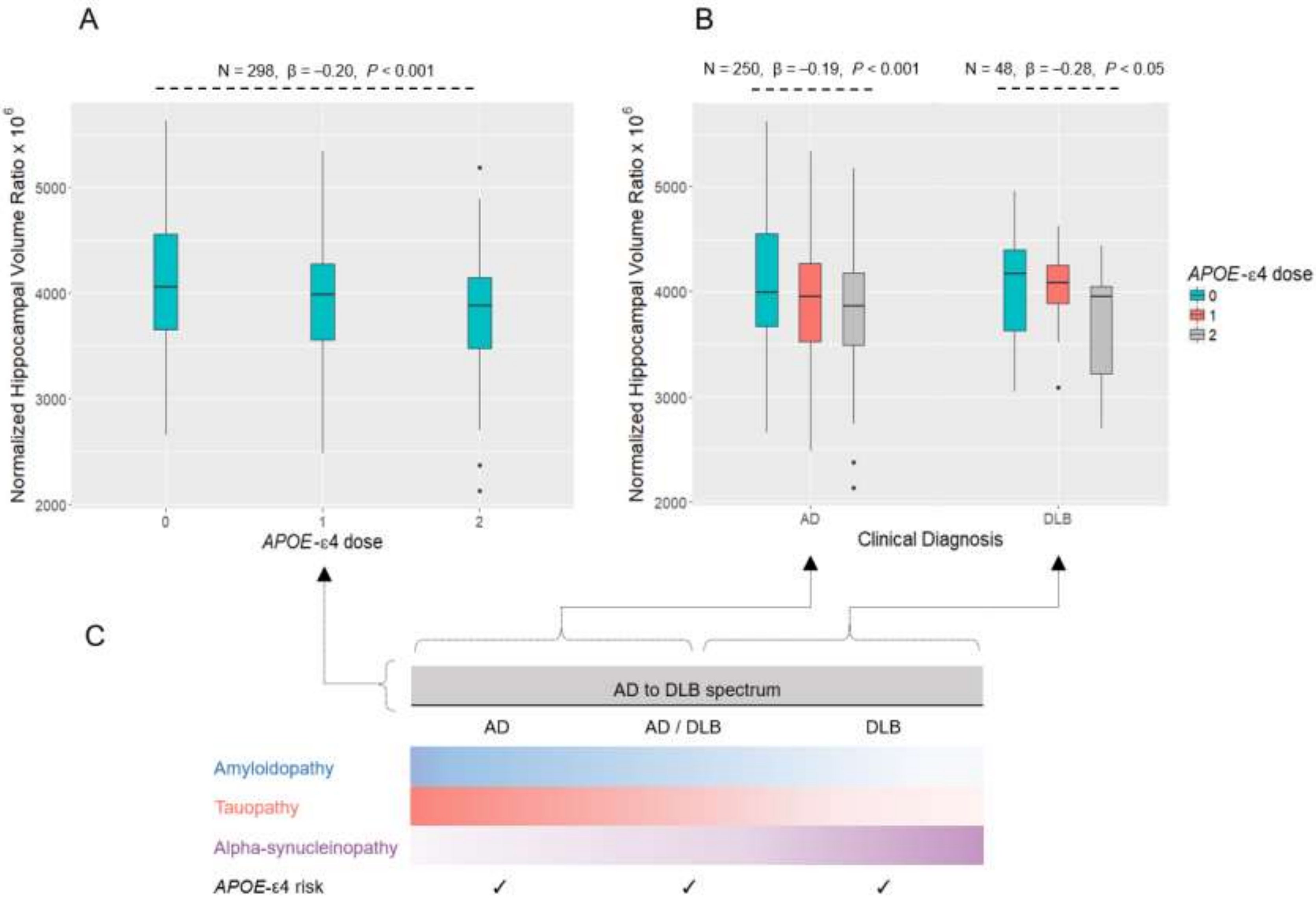
Table 3. Multiple linear regressions presenting effects of independent variables on hippocampal volume: *APOE*-ε4 dose-dependent models.

Predictors	HVa, difference							
	Model 1†				Model 2†			
	b	95% CI	β	*P* value	b	95% CI	β	*P* value
APOE-ε4 dose	-176.64	-268.52, -84.76	-0.20	< 0.0001	-180.44	-272.37, -88.51	-0.20	< 0.0001
Clinical diagnosis (DLB)	41.15	-130.13, 212.43	0.03	0.64	64.86	-108.91, 238.63	0.04	0.46
Age at scan, y	-28.22	-35.27, -21.17	-0.42	< 0.0001	-26.30	-34.10, -18.51	-0.39	< 0.0001
Formal education, y	-12.14	-29.53, 5.25	-0.07	0.17	-11.65	-29.05, 5.75	-0.07	0.19
Sex (females)		–			87.22	-42.53, 216.98	0.07	0.19
WMHa *		–			-69.43	-182.99, 44.13	-0.07	0.23
R squared	0.197				0.204			

APOE-ε4 was treated as a continuous variable to assess dose-dependency. Unstandardized coefficients (b) with 95% confidence intervals (CI), standardized coefficients (β), and *P* values are presented, along with each model's R-squared statistic.

Abbreviations: APOE, apolipoprotein E; HVa, normalized hippocampal volume ratios ($\times 10^6$); WMHa, normalized white matter hyperintensities volume ratios ($\times 10^6$); * log-transformed values were analyzed; † *APOE*-ε4 dose × clinical diagnosis interaction was non-significant ($P > 0.88$) and removed from the model to assess the main effects.

Figure 2. The association of *APOE*-ε4 with hippocampal volumes across the spectrum of AD and DLB.



Boxplots presenting the normalized hippocampal volume ratios for the pooled sample of AD and DLB (A), and within the clinical diagnostic categories of AD and DLB (B), along with *P*-values showing significant relationships. The AD/DLB spectrum can be conceptualized as representing a continuum, with amyloidopathy and tauopathy at one extreme and α-synucleinopathy at the other extreme, with varying degrees of the three proteinopathies in the middle (C). *APOE*-ε4 has been identified as a risk factor across this spectrum [11]. Our study identifies a link between *APOE*-ε4 and hippocampal volumes in AD, DLB, as well as across the AD/DLB spectrum.

Table 4. Hierarchical multiple linear regressions relating CVLT total recall scores with hippocampal volume in ε4+ versus ε4–.

Total	ε4+ [*N* = 158]		ε4– [*N* = 110]	
	Step 1	Step 2	Step 1	Step 2
Age at scan	-0.15 *	-0.10	-0.06	0.01
Formal education	0.05	0.07	-0.02	-0.01
DRS total	0.61 †	0.59 †	0.61 †	0.60 †
HVa	–	0.17 *	–	0.14
R squared	0.42	0.45 ‡	0.37	0.38

AD	ε4+ [*N* = 133]		ε4– [*N* = 92]	
	Step 1	Step 2	Step 1	Step 2
Age at scan	-0.16 *	-0.11	-0.08	-0.01
Formal education	0.02	0.04	0.01	0.02
DRS total	0.61 †	0.59 †	0.58 †	0.57 †
HVa	–	0.16 *	–	0.15
R squared	0.41	0.43 ‡	0.34	0.35

DLB	ε4+ [*N* = 25]		ε4– [*N* = 18]	
	Step 1	Step 2	Step 1	Step 2
Age at scan	-0.06	-0.01	-0.05	-0.05
Formal education	0.26	0.36 *	-0.18	-0.18
DRS total	0.55 *	0.40 *	0.84 †	0.84 †

HVa	–	0.35 *	–	-0.01
R squared	0.50	0.60 ‡	0.72	0.72

Standardized beta (β) are presented, unless otherwise stated. In Step 1, age at scan, formal education, and DRS total score variables were entered, followed by HVa in Step 2.

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; CVLT, California verbal learning test; DLB, dementia with Lewy bodies; DRS, dementia rating scale; HVa, normalized hippocampal volume ratios ($\times 10^6$); ε4+, carriers of at least one *APOE*-ε4 allele; ε4–, *APOE*-ε4 non-carriers.

* $P < 0.05$; † $P < 0.001$; ‡ Statistically-significant F change (versus model 1) at $P < 0.05$.