RESEARCH ARTICLE

Regionally specific changes in the hippocampal circuitry accompany progression of cerebrospinal fluid biomarkers in preclinical Alzheimer's disease

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Abstract

Neuropathological and in vivo brain imaging studies agree that the *cornu ammonis* 1 and subiculum subfields of the hippocampus are most vulnerable to atrophy in the prodromal phases of Alzheimer's disease (AD). However, there has been limited investigation of the structural integrity of the components of the hippocampal circuit, including subfields and extra-hippocampal white matter structure, in relation to the progression of well-accepted cerebrospinal fluid (CSF) biomarkers of AD, amyloid- β 1-42 ($A\beta$) and total- τ (τ). We investigated these relationships in 88 aging asymptomatic individuals with a parental or multiple-sibling familial history of AD. Apolipoprotein (APOE) ϵ 4 risk allele carriers were identified, and all participants underwent cognitive testing, structural magnetic resonance imaging, and lumbar puncture for CSF assays of τ , phosphorylated- τ (p- τ) and $A\beta$. Individuals with a reduction in CSF $A\beta$ levels (an indicator of amyloid accretion into neuritic plaques) as well as evident τ pathology (believed to be linked to neurodegeneration) exhibited lower subiculum volume, lower fornix microstructural integrity, and a trend towards lower cognitive score than individuals who showed only reduction in CSF $A\beta$. In contrast, persons with normal levels of τ showed an increase in structural MR markers in relation to declining levels of CSF $A\beta$. These results suggest that hippocampal subfield volume and extra-hippocampal white matter microstructure demonstrate a complex pattern where an initial volume increase is followed by decline among asymptomatic individuals who, in some instances, may be a decade or more away from onset of cognitive or functional impairment.

KEYWORDS

Alzheimer's disease, amyloid- β , APOE ϵ 4, cerebrospinal fluid biomarkers, fimbria, fornix, hippocampal subfields, preclinical, structural magnetic resonance imaging, τ

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1 | INTRODUCTION

The clinical symptoms of Alzheimer's disease (AD) are preceded by well over a decade of brain changes including deposition of amyloid- β ($A\beta$) into plaques, appearance of neurofibrillary tangles, and synaptic and neuronal loss. These pre-symptomatic manifestations of AD identify stages of disease progression at which effective intervention might delay its evolution and appearance of symptoms. Hippocampal volume loss or atrophy, estimated from magnetic resonance images (MRI), as well as cerebrospinal fluid (CSF) measures of *tau* pathology have both been noted as biomarkers of neurodegeneration that may be used for staging of pre-clinical AD (Sperling et al., 2011). However, a recent longitudinal study demonstrated that baseline pre-clinical classification based on hippocampal volume and CSF *tau* yielded different longitudinal outcomes (Gordon et al., 2016). Given the urgent need to facilitate discovery of effective preventive strategies, we wished to investigate the specific importance (beyond total hippocampal volume) of detailed image analyses of sensitive and critical components of the hippocampal circuitry believed to be involved early in the evolution of AD toward symptom onset.

Several studies have investigated the relationship between hippocampal volume and $A\beta$ pathology, as measured by CSF or positron emission tomography (PET), in cognitively healthy individuals and yielded discrepant results (Villemagne and Chetelat, 2016): $A\beta$ accumulation has been associated with hippocampal volume decrease (Bourgeat et al., 2010; Hsu et al., 2015; Jack et al., 2014; Mormino et al., 2009; Storandt, Mintun, Head, & Morris, 2009) and increase (Chetelat et al., 2010). A lack of any association has also been reported (Bourgeat et al., 2010; Dickerson et al., 2009). It is thus still under debate whether $A\beta$ accumulation is sufficient, in and of itself, for AD-related hippocampal atrophy, or whether the concurrent accumulation of *tau* is required. Several studies show that the accumulation of both $A\beta$ and *tau* pathology enhances hippocampal atrophy (Gomar, Conejero-Goldberg, Davies, & Goldberg, 2016; Gordon et al., 2016; Jack et al., 2014; Wang et al., 2016), while others support that *tau*-related atrophy of the hippocampus can occur independently of $A\beta$ (Jack et al., 2013b; Wirth et al., 2013).

These variable findings may, in part, be a reflection of differential subfield-specific vulnerability and sensitivity to AD pathology. Postmortem staging of AD-related neurofibrillary pathology (Braak and Braak, 1991) and in vivo brain imaging studies (Amaral et al., 2016; de Flores, La Joie, & Chtelat, 2015a; La Joie et al., 2013; Wang et al., 2012) agree that the *cornu ammonis* (CA) 1 and subiculum are most vulnerable to atrophy in the face of AD-related pathology. A more thorough examination of the hippocampal subfields in the context of CSF biomarkers could improve our understanding of the accumulation of AD pathology and the maintenance and degeneration of vulnerable neural circuitry.

Beyond the architecture of the hippocampus itself, white matter microstructural differences, measured using diffusion-weighted MRI, have recently been associated to AD pathology and have raised the importance of integrating these white matter integrity measures in models of AD progression (see Amlien and Fjell, 2014 for a review). Although the instability of the fornix is well-described in AD, it is

unclear if alterations at the microstructural level are observable in relation to pathological burden in cognitively healthy individuals. Contradictory results have been reported in relation to $A\beta$: both a lower (Brown et al., 2017; Gold et al., 2014) and greater (Racine et al., 2014) fractional anisotropy in the fornix have been associated with $A\beta$ burden. CSF *tau* concentration has been associated with lower mean diffusivity in white matter adjacent to the hippocampus (Bendlin et al., 2012). Similarly to hippocampal gray matter, the early relationship between extra-hippocampal white matter microstructure and the accrual of both $A\beta$ and *tau* pathology is not well understood. The extent to which white matter and gray matter structures of the hippocampal circuit are related to or affected by independent pathological processes is still unclear (Kantarci, 2014).

The objective of this work is to study the relationship between the structure of critical components of the hippocampal circuit and CSF measures of $A\beta$ and *tau* pathology. We use novel technologies to identify the subfields of the hippocampus and the extra-hippocampal white matter (Amaral et al., 2016; Chakravarty et al., 2013; Winterburn et al., 2013) in 88 aging asymptomatic individuals with a familial history of AD. We evaluate the ratio of T1-weighted and FLAIR images as a high-resolution index of tissue microstructure within these regions of interest. We hypothesize that the structure of critical circuit components can be predicted by the conditional relationship between $A\beta$ and *tau* in the preclinical stage of the disease. Using a statistical model for this interaction, we investigate the presence of a tipping point where the structural integrity of the hippocampal circuit begins to degenerate, demonstrating possible progression towards AD.

2 | METHODS

Data used in the preparation of this article were obtained from the Pre-symptomatic Evaluation of Novel or Experimental Treatments for Alzheimer's Disease (PREVENT-AD) program (<http://www.douglas.qc.ca/page/prevent-alzheimer>) data release 3.0 (November 30, 2016). The PREVENT-AD program is described in more detail in the Supporting Information.

2.1 | Subjects

In total 217 cognitively healthy individuals with a history of a parent or multiple first degree relatives with AD were enrolled in INTREPAD (NCT no. 02702817), a two-year randomized placebo-controlled trial of the nonsteroidal anti-inflammatory drug naproxen sodium in over-the-counter dosage (Breitner et al., 2016). All participants were aged 60 years or older (55–59-years old if the subject was within 15 years of earliest-affected relative's onset of dementia), in good general health and with no diagnosable cognitive disorder. Participants provided signed informed consent to participate in this study, which was approved by the Research Ethics Board of the Douglas Mental Health University Institute.

The data were collected during the first three trial visits: (a) the enrollment visit consisted of a general health assessment and an MRI scan; (b) the baseline visit consisted of a detailed cognitive assessment

and an MRI scan; (c) a subset of participants returned for a lumbar puncture on a third visit. Of 217 participants initially enrolled, six were subsequently excluded owing to evidence of mild cognitive impairment (MCI), six subjects were not imaged due to claustrophobia, 16 MR images were discarded due to artifacts, and 90 participants underwent the lumbar puncture. The current analyses rely on 88 subjects who have all requisite data. The time interval between visits for these 88 subjects was 32 ± 31 days for enrollment and baseline, and 25 ± 24 days for baseline and lumbar puncture.

2.2 | Cognitive assessment

At the enrollment visit, the cognitive status of the participants was evaluated using CDR (Clinical Dementia Rating) and MoCA (Montreal Cognitive Assessment). Subjects with a CDR score of 0 and a MoCA score equal or $>26/30$ were considered eligible. At the subsequent baseline visit, participants were evaluated using an available Canadian French edition of the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS, Version A) (Randolph, Tierney, Mohr, & Chase, 1998). This 45-min battery yields Index Scores in five cognitive domains: language, immediate memory, delayed memory, attention, and visuospatial abilities, as well as a Total Score. We used scoring norms for the age decade 60–69 years for all participants regardless of age. Subjects were referred for a detailed cognitive evaluation to determine eligibility in the study if: (a) they had a score below 85 in two of the five RBANS cognitive domains, (b) their scores were subjectively considered to be low given their profession and education, (c) their scores deteriorated during the course of the 2-year study by more than one standard deviation, and (d) they expressed concern regarding cognitive changes. Following detailed cognitive evaluation (see Supporting Information), six subjects considered eligible at enrollment were subsequently excluded from the study.

2.3 | MRI acquisition

Subjects were imaged on a Siemens Trio 3 Tesla MRI system (Siemens Healthcare, Erlangen, Germany) with the 12-channel head coil at the Cerebral Imaging Centre of the Douglas Mental Health University Institute. T1-weighted structural brain images at 1 mm³ voxel resolution were acquired using the Alzheimer's Disease Neuroimaging Initiative protocol for magnetization prepared rapid acquisition by gradient echo with 3D sagittal acquisition, TR = 2.3 s, TE = 2.98 ms, TI = 0.9 s, $\alpha = 9^\circ$, bandwidth = 238 Hz/pixel, $256 \times 240 \times 176$ matrix, GRAPPA = 2, scan time 5:12 (Jack et al., 2008; Mugler & Brookeman, 1991). These T1w images were acquired at both the enrollment and baseline visits. Separately we acquired 1mm³ T2-weighted fluid attenuated inversion recovery (FLAIR) turbo spin echo images at the enrollment visit only using 3D sagittal acquisition with TR = 5 s, TE = 389 ms, TI = 1.8 s, $\alpha = 120^\circ$, bandwidth = 781 Hz/pixel, $225 \times 177 \times 176$ matrix, GRAPPA = 2, and scan time 6:27 (Hajnal et al., 1992).

2.4 | MRI analysis

All images were visually inspected for artifacts and 16 datasets were discarded. The remaining baseline T1-weighted images were pre-processed

using the minc-bpipe-library¹. The pre-processing pipeline of the T1-weighted images consisted of image denoising (noise = rician, beta = 0.5) (Coupe et al., 2008) and N4 nonuniformity correction (Tustison et al., 2010). Brain extraction was performed using BEaST (Eskildsen et al., 2012) following an affine registration to MNI space (Avants, Epstein, Grossman, & Gee, 2008). The mask was subsequently transformed back into native space and used to calculate whole brain volume. The images in native space were cropped, without resampling, to a smaller field-of-view that includes the whole brain.

The pre-processed T1-weighted images were segmented using MAGeT brain, a multi-atlas registration-based segmentation tool (Chakravarty et al., 2013; Pipitone et al., 2014). Five high-resolution T1-weighted MRI atlases of the hippocampal subfields were used (Winterburn et al., 2013). These atlases include labels for the right and left CA1, CA2/CA3, CA4/dentate gyrus (DG), stratum radiatum lacunum moleculare (SR/SL/SM), and subiculum. The same atlases have also recently labeled for the extra-hippocampal white matter structures including the alveus, fimbria, and fornix (Amaral et al., 2016). Structure volumes were extracted from the resulting discrete label images. An example of a pre-processed and segmented T1-weighted image is shown in Figure 1. All segmentations passed quality control by visual inspection.

In addition to hippocampal morphology, we evaluated the T1w/FLAIR signal intensity ratio to probe differences in extra-hippocampal white matter microstructure. A similar contrast (T1w/T2w) has been recently used to map the myelin content of the cerebral cortex (Glasser and Van Essen, 2011), and study the effects of ageing (Grydeland, Walhovd, Tamnes, Westlye, & Fjell, 2013) and disease (Ganzetti, Wenderoth, & Mantini, 2015; Iwatani et al., 2015). T1-weighted and FLAIR images include spatial biases that vary between subjects due to imperfections in the image acquisition techniques, which confound the analysis of T1-weighted and FLAIR signal intensities. By evaluating the T1w/FLAIR signal ratio we minimize these methodological confounds (primarily due to B1 field nonuniformity). Furthermore, the T1w/FLAIR contrast is enhanced in comparison to T1-weighted or FLAIR individually when a microstructural feature (or pathological process) shortens or lengthens both the T1 and T2 relaxation times. T1 and T2 relaxation times would be lengthened, causing a decrease in T1w/FLAIR ratio, by a partial loss of myelin sheaths, axons, and oligodendrocytes—microstructural alterations that have been previously observed in AD (Brun and Englund, 1986; Englund and Brun, 1990; Ihara et al., 2010; Sjobeck and Englund, 2003).

For each subject, the enrollment T1-weighted image was linearly aligned using a rigid transformation to the baseline T1-weighted image (Collins, Neelin, Peters, & Evans, 1994). The FLAIR image was first rigidly aligned to enrollment T1-weighted image. The two transformations were concatenated and applied to the FLAIR image to transform it directly into the subject's native baseline space for analysis, minimizing interpolation effects. The T1-weighted and FLAIR image intensities were averaged within each hippocampal label, including the white and gray matter structures.

¹<https://github.com/CobraLab/minc-bpipe-library>

FIGURE 1 Hippocampal subfield segmentation. Zoomed-in coronal views of pre-processed T1-weighted images with (bottom row) and without (top row) hippocampal subfield and extra-hippocampal white matter labels overlaid [Color figure can be viewed at wileyonlinelibrary.com]

2.5 | Apolipoprotein genotyping

We analyzed participants' Apolipoprotein (APOE) to identify individuals carrying the $\epsilon 4$ allele, a well-known risk factor for AD (Poirier et al., 1993; Saunders et al., 1993). Genomic DNA was extracted from buffy coat using the Qiagen EZ1DNAKit. Profiling of APOE 112/158 single nucleotide polymorphisms (which determine the $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ alleles) was performed using PCR followed by pyrosequencing. Detailed methods for these well-established procedures are described in the Supporting Information.

2.6 | Lumbar puncture and CSF assays

Participants underwent lumbar puncture in the L3/L4 or L4/L5 interspaces and CSF was collected following procedures specified by the European Union's BIOMARK-APD program (Lelental et al., 2016). The first 12 mL (out of 20) of CSF was collected in plastic (polypropylene) tubes to avoid absorbance of A β 42 by the tube wall. All samples were mixed gently to avoid possible gradient effects. No sample contained >500 erythrocytes/L. The samples were centrifuged at 2,000 g at 4°C for 10 min to eliminate cells and other insoluble material, and were then immediately frozen and stored at -80°C pending biochemical analyses, without being thawed or refrozen. Serum samples were collected at the same time.

The CSF samples were analyzed by ELISA assays for A β_{1-42} (IBL International 207 GmbH, Hamburg, Germany, Fujirebio Europe, formerly Innogenetics, Ghent, Belgium), phosphorylated tau 181 (p-tau) and total tau (tau) (Fujirebio 211 Europe) according to the instruction of the manufacturers and a standardized protocol developed by the BIOMARKAPD consortium (Lelental et al., 2016).

2.7 | Statistical analysis

Statistical analysis of subfield volumes was performed using general linear models in R (<https://www.r-project.org/>), with A β , tau, and A β -by-tau

interaction in the model, and brain volume, sex, age, years until parental onset, education and APOE $\epsilon 4$ status as covariates (Equation 1). We hypothesized that we would detect a relationship between CSF biomarkers of AD progression and the volumes of CA1, subiculum, and the molecular layers, as well as the entire hippocampus in both hemispheres. The model was applied individually to each of these structures. Using two-tailed $\alpha = 0.05$ throughout, we used the false discovery rate (FDR) method to adjust the p -values for the eight comparisons.

$$\begin{aligned} \text{volume} = & \beta_{\text{intercept}} + \beta_{\text{brainVolume}}(\text{Brain volume}) + \beta_{\text{gender}}(\text{Gender}) \\ & + \beta_{\text{age}}(\text{Age}) + \beta_{\text{years2parental}}(\text{Years2ParentalOnset}) \\ & + \beta_{\text{education}}(\text{Education}) + \beta_{\text{APOE}\epsilon 4}(\text{APOE}\epsilon 4\text{status}) \\ & + \beta_{\text{tau}}(\text{CSF tau}) + \beta_{\text{A}\beta}(\text{CSF A}\beta) \\ & + \beta_{\text{tau:A}\beta}(\text{CSF tau} \times \text{CSF A}\beta) + \text{error} \end{aligned} \quad (1)$$

To examine the direction of the conditional effects of A β and tau on the volumes, we separated the cohort into high/low CSF tau and high/low CSF A β groups. The A β cutoff was empirically determined by plotting the conditional tau coefficient as a function of A β using the Interplot package² (shown in Supporting Information Figure 2a, Solt & Hu, 2015), where the zero-crossing of the conditional tau coefficient occurs at A β_{cutoff} of 1,180 pg/mL. We used this cutoff to plot the linear relationship between volume and tau separately for the high- and low-A β groups. Similarly, we evaluated the tau cutoff to be 350 pg/mL (Supporting Information Figure S2B).

We used the same linear model as for the volumetry analysis (Equation 1) to relate the T1w/FLAIR ratio in the extra-hippocampal white matter tracts to the CSF biomarkers, omitting the total brain volume from the covariates. We hypothesized that we would detect a relationship between CSF markers and the microstructure of the fornix. Our investigation of the alveus, fimbria and total extra-hippocampal

²<https://CRAN.R-project.org/package=interplot>

TABLE 1 Cohort description

	All subjects	tau-A β -	tau+A β -	tau-A β +	tau+A β +
Preclinical stage	-	Stage 0	SNAP	Stage 1	Stage 2
Gender, men/women	27/61	5/23	3/7	14/28	5/3
Age (SD), years	62.8 (5.6)	61.8 (5.0)	64.0 (8.5)	62.8 (5.4)	65.2 (4.6)
Years until parental onset of AD (SD)	12.4 (6.9)	13.4 (6.8)	11.4 (8.3)	12.6 (6.3)	9.3 (9.3)
Education (SD), years	14.9 (3.0)	15.5 (2.8)	14.9 (1.7)	14.8 (3.3)	13.4 (2.7)
APOE ϵ 4 status, carriers/noncarriers	30/58	9/19	2/8	12/30	7/1
MoCA total score (sd)	27.8 (1.6)	28.1 (1.3)	26.5 (2.1)	27.9 (1.6)	27.6 (1.5)
RBANS total score (sd)	100.2 (11.6)	101.4 (9.2)	95.4 (9.5)	102.2 (12.9)	92.3 (10.8)

Summary statistics of the 88 subjects included in the analysis. The subjects were categorized into four groups using CSF *tau* and A β cutoffs of 350 and 1,180 pg/mL, respectively.

white matter is exploratory. We used the FDR method to adjust the *p*-values for the eight comparisons.

To examine whether the structural differences related to AD pathology in the hippocampal subfields and extra-hippocampal white matter are related or independent processes, we performed a linear model relating the white matter T1w/FLAIR ratio to the gray matter volume, where both MR metrics were residualized for nuisance variables. The analysis was limited to the hippocampal structures that were significantly associated with A β and *tau* pathology.

Using the empirically derived *tau* and A β cutoffs from the subfield volume analysis, we categorized the subjects into four groups putatively corresponding to pre-clinical stages of AD (Sperling et al., 2011) and suspected nonAD pathophysiology (SNAP, Jack et al., 2012). The number of subjects varied per group: 29 *tau*-A β - (Stage 0), 45 *tau*-A β (Stage 1), 10 *tau*+A β (Stage2), and 11 *tau*+A β - (SNAP). We compared the structural MRI metrics of the hippocampal circuit and RBANS total score between the four groups using six pairwise *t*-tests adjusted for multiple comparisons using FDR.

3 | RESULTS

3.1 | Distribution of the CSF *tau* and A β concentrations

The cohort of 88 subjects is characterized in Table 1 and Supporting Information Tables S1 and S2. There are no significant differences in age and education by sex or APOE ϵ 4 status. The distribution of the CSF measures is shown in Figure 2, where CSF levels of A β are inversely related to levels of A β in brain tissue (Grimmer et al., 2009). Figure 2 shows a "U" shape distribution, where participants with higher levels of *tau* appear at opposite ends of the CSF A β axis. There is a distinct pattern of APOE ϵ 4 carriers in the left branch of the "U" corresponding to subjects with low CSF A β and high *tau*, whereas noncarriers are predominant in the right hand side with high A β and high *tau*, also referred to as SNAP.

Overall, APOE ϵ 4 carriers have lower CSF A β ($p = .001$, $q = 0.004$) and a higher *tau* ($p = .031$, $q = 0.046$) (see Supporting Information

Figure S1A,B), but the genotype differences in *p*-*tau* were similar but only at a trend level ($p = .088$, $q = 0.088$). The observations with *tau* are limited by the small number of *tau*-positive subjects in this asymptomatic sample. Not surprisingly, *p*-*tau* and *tau* levels were strongly correlated (Pearson $r = 0.94$, Supporting Information Figure S1C). We therefore present analyses on A β and *tau*, noting here that similar but less robust results were observed with *p*-*tau* (data not shown).

3.2 | Hippocampal subfield volumes are differentially sensitive to preclinical AD pathology

The statistical results of the linear models described in Equation 1 are represented in Figure 3a. The interaction between the CSF concentrations for A β and *tau* predict ($q < 0.05$) the subiculum volume bilaterally.

FIGURE 2 Distribution of CSF A β and *tau* levels. Distribution of CSF A β and CSF *tau* levels color-coded for APOE ϵ 4 status. The horizontal and vertical dashed lines correspond to the CSF *tau* and A β cutoffs of 350 and 1,180 pg/mL, respectively, determined from the linear model relating the CSF markers to hippocampal subfield volumes [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 3 Relationship between CSF biomarkers and hippocampal subfield volumes. (a) Statistical results for the linear models relating CSF biomarkers to subfield volumes. Each line of the matrix represents one linear model corresponding to a different HC subfield. The size and color mapping of the circles represents the t-statistic, where positive t-statistics are in blue and negative in red. Due to the A β :tau interaction term, the direction of the A β and tau effects cannot be directly deduced from these results. All significant effects ($q < 0.05$, corrected for multiple comparisons using FDR) are labeled with 1-q. (b) Linear model of the right subiculum volume as a function of tau for the high- and low-CSF A β groups. (c) Linear model of the right subiculum volume as a function of A β for the high- and low-tau groups. The subiculum volumes in (b) and (d) were residualized to remove the effect of covariates (brain volume, sex, age, years until parental onset, education and APOE $\epsilon 4$ status). A similar plot for the true subiculum volumes (in mm³) is included in Supporting Information Figure S3 [Color figure can be viewed at wileyonlinelibrary.com]

A β levels predict the volumes of the right CA1, molecular layers and total HC. We also performed an exploratory analysis of the remaining subfields (CA4-DG and CA2-CA3) and no statistically significant results were observed (Supporting Information Table S3).

Figure 3b shows the relationship between the right subiculum volume and tau for the high- and low-A β groups. Although the high-A β group has greater volumes with tau accumulation, the low-A β group has smaller volumes with tau accumulation. However, the low-A β group has greater subiculum volumes than the high-A β group at low tau. On the other hand, Figure 3c shows that high- and low-tau groups have a similar subiculum volume at high CSF A β levels. The subiculum volume of the high-tau group is smaller for higher A β burden; whereas for the low-tau group, the subiculum volume is greater for higher A β burden. These diverging relationships hold when we remove potential outliers from our analysis (see Supporting Information Figure S4). We

analyzed a second model that included the CSF tau/A β ratio, a marker of increased risk of conversion to MCI (Li et al., 2007). A higher tau/A β ratio was related to a lower volume of the left subiculum ($p = .02$), though this effect did not survive multiple comparisons. tau/A β ratio was not significantly related to the other subfield volumes, nor total hippocampal volume.

APOE $\epsilon 4$ status is also a statistically significant predictor of the right and left subiculum volumes, but not the other subfields, where APOE $\epsilon 4$ carriers have a greater volume than noncarriers (Supporting Information Figure S7). Similarly to Figure 4, we plotted the linear relationship between the right subiculum volume and CSF markers for the two genotypes (shown in Supporting Information Figure S8) and show similar results.

For comparison, we also report the results for the right hippocampus in Supporting Information Figure S5. The CSF tau and A β

FIGURE 4 Relationship between CSF biomarkers and extra-hippocampal white matter T1w/FLAIR ratio. (a) Statistical results for the linear models relating CSF biomarkers to T1w/FLAIR signal ratios in the alveus, fimbria and fornix. Each column of the matrix represents one linear model corresponding to a different white matter structure. The size and color mapping of the circles represents the t-statistic. All matrix cells with significant effects ($q < 0.05$, corrected for multiple comparisons using FDR) are labeled with $1-q$. (b) Linear model of the left fornix T1w/FLAIR ratio as a function of τ for the high- and low-A β groups ($A\beta_{\text{cutoff}} = 950$ pg/mL). (c) Linear model of the left fornix T1w/FLAIR ratio as a function of A β for the high- and low- τ groups ($\tau_{\text{cutoff}} = 290$ pg/mL). The T1w/FLAIR ratios were residualized against the following covariates: Sex, age, years until parental onset, education, and APOE $\epsilon 4$ status [Color figure can be viewed at wileyonlinelibrary.com]

thresholds for the right hippocampus are 425 and 1,050 pg/mL, respectively, in comparison to 350 and 1,180 pg/mL for the right subiculum. A similar diverging relationship between high/low τ and A β is observed, although using cutoffs corresponding to a higher pathological burden for both τ and A β .

3.3 | T1w/FLAIR ratio is sensitive to AD pathology in the extra-hippocampal white matter

The statistical results for the linear models relating the T1w/FLAIR ratio of the extra-hippocampal white matter structures to τ and A β are shown in Figure 4a (complete statistical results are provided in Supporting Information Table S4). The interaction between τ and A β predicts the T1w/FLAIR ratio in the left fornix, right fimbria, and in the total extra-hippocampal white matter bilaterally. Similar to the subfield volumetry analysis, we determined τ and A β cutoffs of 290 and 950 pg/mL, respectively. These cutoffs correspond to lower and higher burden of τ and A β pathology respectively in comparison to the subfield volume analysis, which may reflect the differential sensitivity of the

respective tissue compartment and/or MR metric to τ and A β CSF biomarkers. Overall, a similar relationship between the CSF markers and structure is found in the white matter as for the gray matter. For the low-A β subgroup, T1w/FLAIR ratio of the left fornix is lower at high τ (Figure 4b). Whereas for the high-A β group, T1w/FLAIR ratio is greater at high τ . Opposite slopes are also shown for the high/low- τ subgroups in Figure 4c: the low- τ subgroup has a slightly positive slope as a function of A β pathology accumulation, whereas the high- τ subgroup has a slight negative slope. Similar trends occur for the right fimbria, as shown in Supporting Information Figure 9.

In contrast to the subfield volume analysis, APOE $\epsilon 4$ status was not a significant predictor of the T1w/FLAIR ratio for any of the extra-hippocampal white matter structures.

3.4 | No significant relationship between white and gray matter hippocampal structure in preclinical AD

In the right hemisphere, we compared the T1w/FLAIR ratio of the fimbria to the volumes of the CA1, subiculum and SR/SL/SM subfields

FIGURE 5 Structural and cognitive characterization of four CSF subgroups. The cohort was divided into four subgroups based on the *tau* and A β cutoffs of 350 and 1,180 pg/mL, respectively. *tau*-A β - refers to the subgroup of individuals with low CSF *tau* and high CSF A β concentrations, and *tau*+A β + refers to the subgroup with high CSF *tau* and low CSF A β concentrations. (a) The residualized subiculum volume is smaller in the *tau*+A β + subgroup than the *tau*-A β + ($p < .001$, $q = 0.004$), *tau*+A β - ($p = .034$; $q = 0.068$), and *tau*-A β - ($p = .019$; $q = 0.057$) subgroups. (b) The residualized T1w/FLAIR ratio of the left fornix is lower in the *tau*+A β + subgroup than the *tau*-A β + ($p = .006$; $q = 0.035$). The subiculum volume and fornix T1w/FLAIR ratio were residualized for brain volume, sex, age, years until parental onset, education and APOE $\epsilon 4$ status. (c) A trend towards decreased RBANS total score was observed in the *tau*+A β + subgroup in comparison to the *tau*-A β - ($p = .017$; $q = 0.052$) and *tau*-A β + ($p = .014$; $q = 0.052$) subgroups [Color figure can be viewed at wileyonlinelibrary.com]

individually. In the left hemisphere, we compared the T1w/FLAIR ratio of the fornix to the subiculum volume. No significant relationship ($p > 0.05$, uncorrected) was found between the white and gray matter structural MR measures. The Pearson's correlation between the right fimbria T1w/FLAIR ratio and subfield volumes was $r = 0.033$, 0.195, and -0.001 for the CA1, subiculum and SR/SL/SM, respectively. The correlation between the left fornix and left subiculum was $r = 0.103$.

3.5 | Structural metrics and cognitive performance of subgroups with different levels of AD-related pathology

Boxplots of the residualized right subiculum volume and left fornix T1w/FLAIR ratio for the four groups are shown in Figure 5a,b, respectively (un-residualized data is shown in Supporting Information Figure S10). There is a significant difference in the structural metrics between the *tau*-A β + and *tau*+A β + groups, where we observe a decrease in volume ($p < .001$, $q = 0.004$) and T1w/FLAIR ratio ($p = .006$; $q = 0.035$) as *tau* increases. The subiculum volumes of the *tau*+A β + group also tend to be smaller than the *tau*-A β - ($p = .034$; $q = 0.068$) and *tau*+A β - ($p = 0.034$; $q = 0.068$) groups, although these trends are not statistically significant.

We also evaluated the difference in residualized right hippocampal volume between the four groups. There are no significant differences in volume between the four groups. Using the hippocampal CSF cutoffs of 425 and 1,050 pg/mL for *tau* and A β , respectively, there is a trend towards a higher volume for *tau*-A β + with respect to *tau*+A β + ($p = .040$, $q = 0.149$) and *tau*-A β - ($p = .050$, $q = 0.149$). However the number of data points in the *tau*+A β - and *tau*+A β + groups using these cutoffs is very low, 4 and 5, respectively (Supporting Information Figure S6).

The differences in cognitive performance between the four subgroups mimic the structural differences: there is a trend toward lower RBANS Total Scale Score in the *tau*+A β + group (Stage 2) than in the *tau*-A β + ($p = .014$, $q = 0.052$) group (Stage 1) and *tau*-A β - ($p = .017$, $q = 0.052$) group (Stage 0), as shown in Figure 5c. If we instead dichotomized the group into high/low-*tau*, the high-*tau* group had a lower RBANS total score than the low-*tau* group ($p = .007$).

We also investigated whether the RBANS scores could be predicted by either the CSF or structural MRI markers of disease progression, controlling for nuisance variables (brain volume, age, gender, years to parental onset, education, and APOE $\epsilon 4$ status), and did not detect any significant relationship.

4 | DISCUSSION

This study investigates the relationship between MR-based indices of hippocampal subfield volume and extra-hippocampal white matter microstructure with CSF biomarkers of AD pathology (*tau* and A β) in cognitively normal older adults with a familial history of AD that may be in the presymptomatic phase of the disease. Our results extend previous work further downstream in the disease progression, showing that advanced MR-based descriptors of hippocampal circuit neuroanatomy and microstructure are sensitive to AD-related pathology putatively over a decade before symptom onset. The accumulation of both *tau* and A β pathology are related to a decrease in volume of critical hippocampal sub-compartments (CA1 and subiculum) previously demonstrated to be maintained through the course of healthy ageing and as being vulnerable at the earliest stages of AD-prodromes (Figure 5a) (Amaral et al., 2016; de Flores et al., 2015a, 2015b; Voineskos et al., 2015; Wang et al., 2012). The accumulation of AD-related pathology is

also related to an increase in T1w/FLAIR ratio in extra-hippocampal white matter (Figure 5b), more specifically in the fimbria and fornix, which have been shown to be vulnerable to ageing as well as AD-prodromes using volumetry and diffusion-weighted imaging (Amaral et al., 2016; Amlie and Fjell, 2014). Importantly, our findings demonstrate that the accumulation of both *tau* and A β results in degeneration within the hippocampal circuit, and that those carrying a high burden of *tau* or A β in the absence of the other do not necessarily demonstrate degeneration. We observed increases in volume and T1w/FLAIR ratio with the accumulation of pathology in these subgroups.

4.1 | Neuroanatomical signature related to AD pathology in the hippocampal subfields of asymptomatic subjects

The spatial signature of hippocampal subfield volume we observed is consistent with previous work in cognitively normal older adults (see Amaral et al., 2016; de Flores et al., 2015a for a review; Sankar et al., 2017). The bilateral subiculum demonstrate the strongest relationship with CSF measures, although we also detected a relationship with the volume of the CA1, molecular layers and total hippocampus in the right hemisphere. This spatial signature is in agreement with the postmortem staging of *tau* pathology where neurofibrillary tangles are first encountered in the entorhinal cortex, then spread to CA1 and subiculum (in particular the extremity of CA1 superimposing the subiculum) before affecting the whole hippocampus (Braak and Braak, 1991). A β deposition precedes the formation of tangles in the brain, but the spatial onset of A β deposition is different: while in the earlier stages the hippocampus is devoid of A β , in later stages the hippocampus shows a limited pattern of A β deposition. Although similar trends are observed bilaterally, the relationship between A β and subfield volume is only significant for the right subfields. Left-right asymmetries have been previously reported for A β (Frings et al., 2015) and *tau* (Ossenkoppele et al., 2016) pathology in AD, and shown to mirror clinical and neuroanatomical patterns.

4.2 | A tipping point in the accumulation of AD-related pathology

The concurrent presence of both *tau* and A β pathology was associated with a smaller hippocampal volume (Figures 3 and 5a). Total hippocampal volume and atrophy has been associated with the accumulation of both A β and neurofibrillary pathology in previous studies of asymptomatic individuals (Gomar et al., 2016; Gordon et al., 2016; Jack et al., 2014; Wang et al., 2016). A similar relationship has also been observed with other markers of neurodegeneration in cognitively healthy individuals, including cortical thickness (Fortea et al., 2014) and metabolic decline assessed using [18 F]FDG PET (Pascoal et al., 2017). To the best of our knowledge, this is the first report of this relationship at the level of the hippocampal subfields. Due to the differential vulnerability of the subfields to the disease progression (in particular the accumulation of *tau*), this greater anatomical specificity may help clarify the relationship between hippocampal volume and AD-related pathology.

Interestingly, we observed greater volumes at lower CSF A β levels in the absence of *tau*, which corresponds to preclinical Stage 1 (Sperling et al., 2011). We also observed larger volumes at greater *tau* levels in the absence of A β , potentially corresponding to SNAP (Jack et al., 2012). A positive correlation between hippocampal volume and A β pathology (measured using PET) has previously been reported in cognitively healthy subjects (Chetelat et al., 2010). An MRI study of healthy controls and subjects with subjective memory complaints reported greater cortical thickness at intermediate levels of A β pathology (measured using CSF) followed by thinner cortex in subjects with higher levels of A β (Fortea et al., 2011). The same group showed that the cortex was thicker in healthy controls with low CSF A β levels in the absence of abnormal p-*tau* levels; whereas the cortex was thinner in subjects with abnormal CSF levels of both A β and p-*tau* (Fortea et al., 2014). In a wider study of the AD continuum (including cognitively intact subjects, subjects with MCI and dementia), a transient increase in hippocampal volume was reported for subjects with low composite AD-CSF indices (defined as the sum of the normalized CSF concentrations of A β and *tau* reflecting the level of pathology and position along the AD continuum) followed by decline at higher indices (Gispert et al., 2015). These recent studies and our own results suggest that pathological burden is not necessarily associated with universal volumetric decline as described in several models of AD (Jack et al., 2013a).

Although all subjects included in this study are cognitively healthy, the lower volumes and lower T1w/FLAIR ratio of the *tau*+A β + group in comparison to *tau*-A β + group is mirrored by the decrease in cognitive performance of the *tau*+A β + group (Figure 5). The CSF cutoffs used in this study may thus correspond to an early tipping point in the structural integrity of the most vulnerable hippocampal subfields, accompanied by cognitive decline. It is not possible to determine from these data whether the positive correlations between volume and AD-related pathology reflect an adaptive remodeling mechanism, which may fail as pathology accumulates, or whether it is itself an early space occupying pathological accumulation (Jack et al., 2012).

An important difference between our study and those discussed above is the choice of CSF cutoffs used to categorize the cohort into groups of high/low A β and *tau* pathology. Our cutoffs are not based on clinical data since our cohort consists of cognitive healthy individuals, nor did we use published clinical thresholds. The cutoffs were derived from the statistical analysis of the relationship between the CSF and structural MRI markers of our cohort. Although the cutoff for *tau* ($\tau_{\text{cutoff}} = 350$ pg/mL) corresponds approximately to previously published clinical thresholds, the cutoff for A β ($\beta_{\text{cutoff}} = 1,180$ pg/mL) is much higher than in previous work ($\beta_{\text{cutoff}} = 530$ pg/mL) that used the Luminex technology (Hansson et al., 2006). Indeed it has been argued previously that clinical CSF cutoffs may not be appropriate for distinguishing preclinical groups (Mattsson et al., 2015).

A different approach would be to define CSF biomarker cutoffs to categorize the cohort into preclinical stages (Jack et al., 2012; Sperling et al., 2011), as done by our colleagues to investigate the association between CSF immune/inflammatory markers and CSF AD biomarkers in this same PREVENT-AD cohort (Meyer et al., under review).

Structural metrics and cognitive performance of these preclinical stages are shown in the Supporting Information Figures S10–S13.

4.3 | Effect of APOE genotype on the hippocampal circuit

APOE $\epsilon 4$ genotype is the strongest genetic risk factor for sporadic AD and the second most important risk factor after age (Strittmatter and Roses, 1995). The majority of τ + A β + (Stage 2) subjects are APOE $\epsilon 4$ carriers, whereas the majority of τ + A β – (SNAP) subjects are not APOE $\epsilon 4$ carriers. APOE $\epsilon 4$ carriers have previously been reported to be less common in SNAP than in preclinical AD and healthy controls (Jack et al., 2016), which may be a reflection of APOE $\epsilon 4$ as a major risk factor for A β pathology (Morris et al., 2010; Vemuri et al., 2010).

The relationship between APOE $\epsilon 4$ status and hippocampal morphology in cognitively healthy individuals is controversial (de Flores et al., 2015a; Fouquet, Besson, Gonneaud, La Joie, & Chetelat, 2014). The APOE $\epsilon 4$ carrier group in our cohort has lower A β and higher τ concentrations, and greater subiculum volumes than noncarriers. However, if we do not take into account the effect of the CSF markers, the subiculum volume is no longer significantly different between genotypes (Supporting Information Figure S7), and we observe a trend towards different linear relationships between volume and CSF markers for the two APOE groups (Supporting Information Figure S8). These results are consistent with studies that found increased rates of hippocampal atrophy over time in APOE $\epsilon 4$ carriers (Crivello et al., 2010; Moffat, Szekely, Zonderman, Kabani, & Resnick, 2000) and in individuals with family history of AD (Okonkwo et al., 2012). The strong relationship between APOE $\epsilon 4$ status and the accumulation of AD-related pathology in our cognitively healthy subjects suggests that studies relating APOE genotype to brain structure and cognition should be interpreted with caution in the absence of CSF (or PET) markers of pathology.

4.4 | Early microstructural differences in the fimbria and fornix associated with AD pathology

We detected a significant relationship between the CSF markers and microstructure (measured using the T1w/FLAIR ratio) of the left fornix, right fimbria, and both left and right total extra-hippocampal white matter. Similarly to the hippocampal subfield results discussed previously, the interplay between CSF levels of A β and τ predicts T1w/FLAIR ratio. The T1w/FLAIR ratio correlates with A β pathology in subjects with low τ . A recent diffusion imaging study of healthy adults with a familial history of AD reported a higher fractional anisotropy in the lateral fornix of the A β -positive group, determined using PET (Racine et al., 2014). On the other hand, in the presence of higher levels of A β pathology, the T1w/FLAIR ratio is lower at higher τ levels, which likely reflects a combination of demyelination, axonal degeneration and edema (Brun and Englund, 1986; Englund and Brun, 1990; Ihara et al., 2010; Sjoberg and Englund, 2003). As the latter become more prominent, these white matter tracts will likely atrophy as well. We performed an exploratory investigation of extra-hippocampal volumes and

did not detect any significant results in relation to τ and A β . Our group recently reported bilateral decreases in the fornix volume in healthy ageing across the course of the adult lifespan, and decreases in fimbria and fornix volumes in MCI and AD in comparison to healthy controls (Amaral et al., 2016). These results suggest that our MR index of white matter microstructure (T1w/FLAIR) may be sensitive to AD-related pathology before the onset of atrophy.

This is, to the best of our knowledge, the first investigation of the microstructure of the fimbria and alveus in the AD continuum, probably due to the limited resolution of diffusion-weighted imaging studies (~ 2 mm isotropic) and resulting partial volume effects for these small structures. A main advantage of the T1-weighted and FLAIR images is their higher resolution (1 mm isotropic), allowing us to sample the T1-weighted and FLAIR intensities within these thin extra-hippocampal white matter structures.

4.5 | White and gray matter structure of the hippocampal circuit are differentially vulnerable to AD pathology

A majority of the axonal projections in the fornix originate from the bilateral subicula and join to form the post-commissural fornix carrying axons to the mammillary bodies. Due to this structural connectivity, it has been hypothesized that the integrity of the fornix is, at least in part, linked to the integrity of the bilateral hippocampi. Multi-modal imaging studies have indeed shown correlations between the fractional anisotropy of the fornix and hippocampal atrophy in AD (see Kantarci, 2014 for a review), and between fornix diffusivity and volumes of CA1 and the anteromedial subiculum (Lee et al., 2012). The relationship appears to be weakened in the earlier stages of the disease. Hippocampal atrophy was associated with a reduction in fornix fractional anisotropy in cognitively normal individuals, however no fractional anisotropy alterations were observed in A β -positive subjects without atrophy (Kantarci et al., 2014). Although microstructural white matter damage in the fornix (assessed using several diffusion tensor metrics) was unrelated to hippocampal atrophy in early MCI, white matter changes in the fornix correlated with hippocampal atrophy in subjects that had carried MCI longer (Zhuang et al., 2013).

In our cross-sectional study of cognitively intact individuals, the extra-hippocampal white matter microstructure measures are not related to the subfield volumes estimated. This relationship may change as pathology accumulates and becomes more apparent.

4.6 | Limitations

One of the novel aspects of this work is the use of advanced MRI analysis techniques and high-resolution atlases of the hippocampal circuit. Nevertheless, our study has some methodological limitations. The first limitation regarding our data is the resolution of the MR images (1 mm³), which may be too coarse to capture subtle differences in the size and shape of thin structures within the hippocampal circuit. It is now possible to acquire sub-millimeter resolution images in clinical scan times, in particular at ultra-high magnetic fields (7 Tesla), which

could enhance sensitivity to minute structural differences in the pre-clinical phase of AD (Giuliano et al., 2017; McKiernan & O'Brien, 2017).

A second limitation for the subfield volumetry analysis is the current variability in segmentation protocols used in the literature, in particular the variability regarding the position of the boundary between CA1 and the subiculum due to limited contrast and resolution of MRI (Yushkevich et al., 2015). Furthermore, the molecular layers, including the SR/SL/SM, are rarely labeled separately from CA1, as in our atlases. These inconsistencies are of particular importance for research in pre-clinical AD since the initial target of *tau* pathology is at this boundary between CA1 and the subiculum (Braak & Braak, 1991), and thus most likely explains why volume differences in CA1 are most commonly first reported as a function of diagnosis progression, although decreases in subiculum volume have also been commonly reported, as in our study. There is an on-going effort to create a unified hippocampal segmentation protocol (Wisse et al., 2017), which will improve the consistency across studies and to gain a better understanding of AD risk and progression in vivo.

Another limitation is the lack of biological specificity of the structural MRI metrics: volume and T1w/FLAIR ratio. Complementary image contrasts and/or imaging modalities could help improve our understanding of the underlying microstructural changes. For instance, elevated concentration of CSF biomarkers have recently been associated with decreased myelin water fraction, measured using MRI, in several brain regions preferentially affected in AD (Dean et al., 2017), providing strong support for the early role of myelin in preclinical AD (Bartzokis, 2011).

Last, a limitation of our work is the lack of regional A β and *tau* measures in the brain. The on-going development of novel PET ligands for *tau* and A β imaging will allow us to gain a better view of the relationship between MR biomarkers and local/distant AD-related pathology, and how this relationship spreads throughout the brain.

5 | CONCLUSION

The results of our investigation of the structural integrity of vulnerable components of the hippocampal circuit in relation to the progression of CSF biomarkers of AD in asymptomatic older adults has three main implications for future AD research, including preventative and disease modifying therapies. First, our observation that smaller subiculum volumes were associated with the accumulation of both *tau* and A β is in agreement with prominent models of AD (Jack, 2014). How these two forms of pathology interact to result in hippocampal subfield atrophy is an on-going subject of research. Second, we detected a significant association between hippocampal white matter microstructure and the CSF markers, an association that appeared independent of hippocampal subfield volume. Although previous work using different image contrasts has shown that the microstructure of the fornix is associated with AD pathology in cognitively normal subjects, this is the first report of an association with microstructural differences in the fimbria. The advantage of our T1w/FLAIR marker is that T1-weighted and FLAIR images are currently included in many imaging protocols to investigate

brain morphometry and white matter lesions in ageing and AD. Our work highlights not only the need but also the possibility of adding sensitive biomarkers of early white matter pathology in the presymptomatic phase of AD, which is currently lacking in prominent models (Jack et al., 2013a). Finally, our results suggest an early transient phase in asymptomatic individuals where increases in the subiculum volume and T1w/FLAIR ratio of the fornix and fimbria are associated with AD pathology (Jack et al., 2013a). If longitudinal data confirm this multi-phased disease progression, future work could focus on whether the tipping point in these structural metrics reflects the beginning of irreversible degeneration of the hippocampal circuit and inevitable cognitive decline.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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