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Cerebrospinal fluid amyloid β_{42}, phosphorylated tau_{181}, and resting state functional connectivity

Liang Wang, MD[1], **Matthew R. Brier, BS**[1], **Abraham Z. Snyder, MD, PhD**[1,2], **Jewell B. Thomas, BA**[1], **Anne M. Fagan, PhD**[1,3,4], **Chengjie Xiong, PhD**[3,5], **Tammie L. Benzinger, MD, PhD**[2,3,4], **David M. Holtzman, MD**[1,3,4], **John C. Morris, MD**[1,3,4], and **Beau M. Ances, MD, PhD**[1,3,4]

[1]Department of Neurology, Washington University in Saint Louis, Saint Louis, MO

[2]Department of Radiology, Washington University in Saint Louis, Saint Louis, MO

[3]The Charles F. and Joanne Knight Alzheimer's Disease Research Center, Washington University in Saint Louis, Saint Louis, MO

[4]The Hope Center, Washington University in Saint Louis, Saint Louis, MO

[5]Division of Biostatistics, Washington University in Saint Louis, Saint Louis, MO USA

Abstract

Importance—Resting state functional connectivity magnetic resonance imaging (rs-fcMRI) has great potential for characterizing pathophysiological changes during the preclinical phase of Alzheimer's disease (AD).

Objective—To assess the relationship between default mode network (DMN) integrity and cerebrospinal fluid (CSF) biomarkers of AD pathology in cognitively normal older individuals

Design—Cross-sectional cohort study

Setting—Knight Alzheimer's Disease Research Center at Washington University in St Louis, Missouri.

Participants—207 older adults with normal cognition (Clinical Dementia Rating of 0).

Main Outcome measures—rs-fcMRI measures of DMN integrity.

Results—Decreased CSF $A\beta_{42}$ or increased CSF phosphorylated tau_{181} ($ptau_{181}$) were independently associated with reduced DMN integrity, with the most prominent decreases in functional connectivity observed between the posterior cingulate and medial temporal regions. Observed reductions in functional connectivity were not attributable to age or structural atrophy in the posterior cingulate and medial temporal areas. Similar rs-fcMRI findings in relation to CSF biomarkers were obtained using region-of-interest analyses and voxel-wise correlation mapping.

Conclusions—Both Aβ and tau pathology affect DMN integrity prior to clinical onset of AD.

Corresponding author: Beau M. Ances MD, PhD, Box 8111, 660 South Euclid Ave, Saint Louis, MO 63110, Telephone: (314) 747-8423, Fax: (314) 747-8427, bances@wustl.edu.

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Introduction

Accumulation of amyloid β (Aβ) and tau proteins, the pathologic hallmarks of Alzheimer's disease (AD), starts years before clinical onset[1–4]. Pathophysiological abnormalities in the preclinical phase of AD may be detected using cerebrospinal fluid (CSF) and/or neuroimaging biomarkers. CSF biomarkers have been recognized as key elements of research criteria for the preclinical phases of AD[5, 6]. Resting state functional connectivity magnetic resonance imaging (rs-fcMRI)[7], a non-invasive measure of brain integrity, has considerable potential in studies of preclinical AD[5, 8,9].

CSF $A\beta_{42}$ and amyloid imaging tracers such as Pittsburgh Compound B (PiB) measure amyloid burden in the brain[10, 11]. Both CSF tau and phosphorylated forms of tau (ptau) are hypothesized to reflect neurodegeneration and/or tau pathology[12, 13]. Symptomatic AD patients typically have a characteristic biomarker profile consisting of reduced CSF $A\beta_{42}$, increased PiB binding in the brain, and elevated CSF tau and ptau[11, 14]. Cognitively normal individuals can also exhibit biomarker evidence of AD pathology, with Aβ abnormalities more prevalent than alterations in tau or ptau[15–17].

rs-fcMRI abnormalities have been consistently observed in the default mode network (DMN) in symptomatic AD patients (for review see Greicius[18]). Aβ preferentially deposits in cortical association areas that prominently include nodes of the DMN[19]. Tau accumulation initially occurs in the limbic system[20], a sub-component of the DMN[21]. More recent rs-fcMRI investigations have detected DMN changes in asymptomatic individuals with increased amyloid deposition using PiB[22–24]. However, the association between functional connectivity in the DMN and CSF biomarker abnormalities requires further study[25, 26].

We investigated the relationship between CSF biomarkers (e.g., $A\beta_{42}$ and $ptau_{181}$) and rs-fcMRI in a large sample of cognitively normal individuals (N=207). We hypothesized that decreased CSF $A\beta_{42}$ and increased $ptau_{181}$levels would be associated with reduced DMN functional connectivity.

Materials and Methods

Participants

Participants were community-dwelling volunteers enrolled in aging and memory studies at the Charles F. and Joanne Knight Alzheimer's Disease Research Center at Washington University in Saint Louis. Detailed information regarding recruitment has been previously published[27]. Inclusion criteria were: 1) completion of MRI scans and CSF collection within 12 months of clinical assessment, 2) normal cognition, determined by a Clinical Dementia Rating (CDR) of 0[28], at the assessments closest to the time of MRI scanning and CSF collection. Individuals were excluded if they had a medical or psychiatric illness that could affect longitudinal follow-up or adversely affect cognitive performance. All studies were approved by the Human Research Protection Office with written informed consent obtained from all participants.

Clinical assessment

An experienced clinician conducted separate semi-structured interviews with the participant and an informant, and determined the presence or absence of dementia based on the principle of intra-individual cognitive decline relative to prior functional level[29]. Only CDR 0 subjects (i.e., cognitively normal) were included in the primary analysis. 207 cognitively normal participants had both MRI scans and CSF collection within 12 months of clinical assessment. Demographic information and CSF biomarker profiles are provided in Table 1.

Genotyping

DNA was extracted from peripheral blood samples. Genotyping for apolipoprotein E (*APOE*) was performed using procedures previously described[30].

CSF collection and analysis

CSF (20–30 mL) was collected at 8:00 AM after overnight fasting as previously described[10]. CSF samples were analyzed for $A\beta_{42}$, tau, and $ptau_{181}$ by plate-based enzyme-linked immunosorbent assay (INNOTEST; Innogenetics, Ghent, Belgium). Since CSF tau was highly correlated with CSF $ptau_{181}$ in the present cohort (Spearman rho = 0.836, $p<0.001$), we report only CSF $ptau_{181}$ in relation to functional connectivity. In addition, neither CSF tau nor $ptau_{181}$ was correlated with CSF $A\beta_{42}$ (both $p \geq 0.469$).

Image acquisition and pre-processing of rs-fcMRI data

Participants were scanned using a Siemens Trio 3T Trio scanner (Siemens Medical Systems, Erlangen, Germany). Two high-resolution structural scans were obtained with T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) sequence (echo time [TE] = 16 msec, repetition time [TR] = 2,400 msec, inversion time [TI] = 1,000 msec, flip angle = 8°, 256 × 256 acquisition matrix, 1 × 1 × 1 mm voxels). High-resolution T2-weighted fast spin echo (FSE) images were acquired (TE = 455 msec, TR = 3,200 msec, 256 × 256 acquisition matrix, 1 × 1 × 1 mm voxels). Two resting rs-fcMRI scans (164 volumes each) were acquired using a gradient echo sequence (TE = 27 msec, TR = 2.2 sec, 64 × 64 acquisition matrix, flip angle = 90°). Thirty-six axial slices with no gap parallel to the anterior–posterior commissure line with approximately 4.0 mm cubic voxels provided whole-brain coverage. Participants were instructed to fixate on a visual cross-hair, remain still, and not fall asleep during scanning. Details concerning rs-fcMRI preprocessing are provided in eMethod.

Quality assurance (QA) of rs-fcMRI data

The QA procedures for rs-fcMRI data have been previously described[31, 32]. Briefly, fMRI data quality was assessed by computing voxelwise root mean squared (rms) temporal variance (sd) averaged over the whole brain. Individuals with a mean preprocessed fMRI signal sd > 2.5% (before nuisance regression) or rms head motion > 1.25 mm were excluded. In addition, frames (volumes) with high variance were identified and removed[33, 34]. The number of frames excluded due to high variance did not correlate with any CSF variable (eMethod).

Definition of regions of interest

Generation of regions of interest (ROIs) within the DMN have been previously described[32]. Briefly, rs-fcMRI data were analyzed from a separate cohort of eight participants with mild AD dementia (CDR 1) and eight cognitively normal participants (CDR 0). A 6-mm-radius sphere centered on the PCC (MNI coordinates: −2, −54, 16) was used as a seed. Correlation maps using this PCC seed were obtained for each participant and averaged separately for the mild AD and cognitively normal groups. A group difference map was produced by subtracting the averaged map of mild AD participants from cognitively normal individuals. Participants with mild AD dementia showed reduced correlation between the PCC and other DMN nodes including the retrosplenial cortex extending to the precuneus, the left and right inferior parietal lobules (IPL), the left and right medial temporal lobe (MTL), and medial prefrontal cortex (MPFC) (see eTable 1). 6-mm-radius spheres centered on peak voxels from each region were subsequently used in the present ROI-based analyses.

ROI-based investigation of relationships between CSF biomarker levels and DMN integrity

Treating each of the CSF biomarkers as continuous variables, Spearman partial correlation was used to assess the relationships between CSF biomarkers and DMN integrity. ROI-based functional connectivity measures of the DMN (i.e., PCC-RSC, PCC-LIPL, PCC-RIPL, PCC-MPFC, PCC-LMTL and PCC-RMTL) were analyzed separately for each CSF biomarker controlling for potential confounding effects. Since prior work has suggested that *APOE* genotype (the presence or absence of ε4 allele) might impact CSF $A\beta_{42}$[35] and DMN functional connectivity[36, 37], we first assessed whether*APOE* ε4 status modulated the relationship between CSF $A\beta_{42}$ and DMN functional connectivity. The entire cohort of cognitively normal participants was divided into two sub-groups according to the presence or absence of at least one *APOE* ε4 allele. The correlations (Spearman's rho) between CSF $A\beta_{42}$ and functional connectivity were computed separately for the two sub-groups. For a given CSF $A\beta_{42}$-functional connectivity relationship, we compared the correlations obtained from *APOE* ε4 non-carriers to *APOE* ε4 carriers, and reported these correlations if a significant difference was found. Otherwise, the relationship between CSF $A\beta_{42}$ and functional connectivity was assessed within the entire cohort after adjusting for age, PCC and MTL volumes separately, and CSF tau/$ptau_{181}$ levels. Details of the comparisons of correlation coefficients between *APOE* ε4 non-carriers and carriers are provided in eMethod. Moreover, the associations between CSF $ptau_{181}$ and functional connectivity were examined after adjusting for age, PCC and MTL volumes separately, and CSF $A\beta_{42}$ levels. Computation was implemented using R (Version 2.15.1)[38] with a statistical threshold for significance of $p < 0.05$, uncorrected for multiple comparisons.

Voxel-wise whole-brain investigation of relationships between CSF biomarker levels and DMN integrity

Participants were classified as CSF $A\beta_{42}$ negative (>500 pg/ml) or positive (≤500 pg/ml), and CSF $ptau_{181}$ negative (<80 pg/ml) or positive (≥80 pg/ml)[35]. Correlation maps were generated for each participant using the PCC as a seed region. Fisher z-transformed subject-level correlation maps were submitted to second-level random effects analyses to identify voxels within a grey matter mask showing significant group contrast effects. These second-level analyses were conducted using two-sample *t*-tests implemented in Statistical Parametric Mapping 8 (SPM8) (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/); the resulting t-maps were thresholded at a cluster-level significance of $p < 0.05$.

Effects of PCC and MTL volumes on rs-fcMRI changes

To assess the likelihood that observed functional connectivity changes were attributed to PCC and MTL atrophy, high-resolution structural scans were processed using FreeSurfer (Version 5.10) (http://surfer.nmr.mgh.harvard.edu) to obtain regional volumes from the isthmus cingulate gyrus (i.e., PCC) and the hippocampus, entorhinal cortex and parahippocampal gyrus (combined to form MTL volume) (see eMethod). Each PCC and MTL volume was correlated separately for CSF $A\beta_{42}$ and $ptau_{181}$ using a Spearman correlation. Specifically, CSF $A\beta_{42}$-volumetric relationships were assessed after adjustment for age and CSF $ptau_{181}$, and CSF $ptau_{181}$-volumetric relationships were evaluated after adjustment for age and CSF $A\beta_{42}$.

Results

ROI-based measures of functional connectivity correlated with CSF biomarker levels

Functional connectivity between the PCC and the 6 independently defined DMN ROIs was computed using standard methodology[31]. Spearman partial correlations were computed between the functional connectivity measures and each CSF biomarker with adjustment for

potential confounding factors (see Table 2 for results). Correlations (Spearman's rho) between CSF $A\beta_{42}$ and functional connectivity were not significantly different between *APOE* ε4 non-carriers and carriers (all $p \geq 0.154$)(eTable 2). We therefore pooled *APOE* ε4 carriers and non-carriers in subsequent analyses. Decreased CSF $A\beta_{42}$ was associated with reduced functional connectivity between the PCC:left MTL (rho = 0.155, $p = 0.026$) and the PCC:right MTL (rho = 0.231, $p < 0.001$) after adjusting for age, PCC and MTL volumes separately, and CSF $ptau_{181}$. Increased CSF $ptau_{181}$ levels were associated with reduced functional connectivity between the PCC:left MTL (rho = −0.182, $p = 0.008$), and a trend level decrease for the PCC:right MTL (rho = −0.122, $p = 0.081$), and the PCC-MPFC (rho = −0.115, $p = 0.100$) after adjusting for age, PCC and MTL volumes separately, and CSF $A\beta_{42}$ levels. All other associations between functional connectivity and CSF $A\beta_{42}$ or CSF $ptau_{181}$ levels were not significant (all $p \geq 0.174$).

Topographies of altered functional connectivity as a function of CSF biomarker

In the voxel-wise analyses, each CSF biomarker was treated as a dichotomous variable. Group-contrast correlation maps are shown in Figure 1. CSF $A\beta_{42}$-positive pariticipants (≤500 pg/ml) exhibited lower positive correlations (reduced functional connectivity) between the PCC and left MTL compared to CSF $A\beta_{42}$-negative participants (Figure 1A, and eTable 3). The same group contrast revealed a reduction in the magnitude of anticorrelations between the PCC and left supramarginal gyrus, and between the PCC and right inferior frontal gyrus (at a trend-level). Parallel effects of CSF biomarkers on positive correlations and anticorrelations are consistent with previous rs-fcMRI results obtained in symptomatic (CDR 0.5 and CDR 1) individuals[31]. Relative to CSF $ptau_{181}$-negative participants, CSF $ptau_{181}$-positive (≥80 pg/ml) participants exhibited lower positive correlations between the PCC and left angular gyrus, and between the PCC and left MTL (at a trend-level). A reduction in the magnitude of anticorrelations was also observed between the PCC and right postcentral gyrus (Figure 1B, and eTable 3). Other functional connectivity changes that did not reach cluster-level significance ($p < 0.05$) are listed in eTable 3.

Relationship between CSF biomarkers and PCC and MTL volumetrics

We computed PCC and MTL volumes using FreeSurfer-defined ROIs. CSF $A\beta_{42}$ was correlated with MTL (Spearman rho = 0.172, $p = 0.012$) but not PCC volume (Spearman rho = −0.044, $p = 0.530$) after adjustment for age and CSF $ptau_{181}$. CSF ptau181 was not correlated with MTL or PCC volumes (all $p \geq 0.100$).

Comment

We assessed the relationship between neuroimaging indices of brain functional network integrity and well-validated CSF biomarkers of AD pathology in cognitively normal old individuals. We demonstrated that decreased CSF $A\beta_{42}$ and increased CSF $ptau_{181}$ were associated with reduced magnitude of correlations in the DMN and within areas normally anti-correlated to the DMN. The most prominent decreases in functional connectivity were seen between the PCC and MTL regions. The effects of CSF $A\beta_{42}$, CSF tau and $ptau_{181}$, each of which independently affected DMN functional connectivity, were not attributable to age or structural atrophy in the PCC and MTL.

Amyloid plaques preferentially form within DMN regions including the PCC and precuneus, anterior prefrontal, lateral parietal and temporal regions[11, 19]. Reduced functional connectivity among these regions[22–24, 39] is well documented in cognitively normal elderly with high amyloid burden[22–24, 39]. However, the MTL is not an early site of plaque formation. Therefore, the link between reduced PCC-MTL functional connectivity and lower CSF $A\beta_{42}$ may be related to other mechanims. One possibility is that functional

connectivity changes are more related to soluble than fibrillary forms of Aβ. Animal studies have demonstrated that oligomeric Aβ directly impairs synaptic function or causes synaptic loss, particularly within the MTL[40, 41]. We observed that reduced CSF $A\beta_{42}$ was associated with volume loss in the MTL but not PCC, which is consistent with the oligomere toxicity hypothesis. Further work examining CSF $A\beta_{42}$ in relation to soluble forms of Aβ are warranted. Another possibility is that the preferential involvement of PCC-MTL functional connectivity is related to the experimental findings demonstrating that regional Aβ deposition causes aberrant electrophysiological activity within spatially distributed functional networks[4243]. Given that Aβ preferentially accumulates in the PCC, we speculate that reduced PCC-MTL functional connectivity could be a consequence of aberrant activity in extensive anatomical connections between the PCC and MTL[44].

The association between CSF ptau and functional connectivity may be related to the progression of tangle pathology in the brain. Neurofibrillary tangles composed of ptau initially form in the trans-entorhinal cortex and spread in a topographically stereotypical manner, possibly via anatomic connections[20]. In our data, increased CSF ptau levels were associated with reduced functional connectivity within the anatomic pathways through which neurofibrillary tangles spread[45]. We suspect that the inverse relationship of CSF ptau and functional connectivity may be driven by the progression of tangle pathology. In cognitively normal individuals, tangles are largely confined to the MTL[1] and these tangles are associated with little or no neuronal loss[46]. In contrast, patients with very mild AD dementia (CDR0.5) have substantial neuronal loss (30–50%) in the entorhinal cortex[46, 47]. It is possible that elevated CSF ptau may be associated with neuronal and synaptic loss in the MTL that is not yet sufficent to produce overt clinical symptoms and not detectable by the present volumetric measure but is detectable at the group level using rs-fcMRI.

The convergent effects of decreased CSF $A\beta_{42}$ and increased CSF $ptau_{181}$ on the DMN provides insights into the early pathophysiology of AD. The PCC and MTL are two critical nodes of a larger network supporting episodic memory[48]. Stronger PCC-MTL functional connectivity is associated with better performance on memory tasks[49]. Structural atrophy of the PCC-MTL pathway is consistent with commonly recognized memory impairments in AD[50]. Thus, the available data suggest that memory impairment in the early phases of AD may be attributable to the convergent effects of both amyloid and tau pathology.

The present study has several limitations. Using both hypothesis-driven ROI-based analysis and voxel-wise whole-brain exploration, we observed that abnormal levels of CSF $A\beta_{42}$ and $ptau_{181}$ were associated with reduced functional connectivity within nodes of the DMN, most prominently in PCC:MTL measures. However, the effect sizes were modest, most likely because we studied pre-symptomatic individuals. Replication of our findings is needed in additional independent samples. Although we found no evidence that rs-fcMRI changes were attributable to PCC or MTL atrophy, we note that volumetric measurements were derived from FreeSurfer-defined regions that did not completely overlap with ROIs used in the rs-fcMRI analysis. In addition, volumetric changes in other DMN regions (besides the PCC and MTL) were not included in our analysis. Further studies using more rigorous approaches to control for the effects of structural brain changes are warranted. Patients with the mild symptomatic AD exhibit changes in multiple resting state networks (RSNs)[31]. Further work is warranted to examine the effects of CSF biomarker abnormalities in RSNs other than the DMN.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Dr. Ances has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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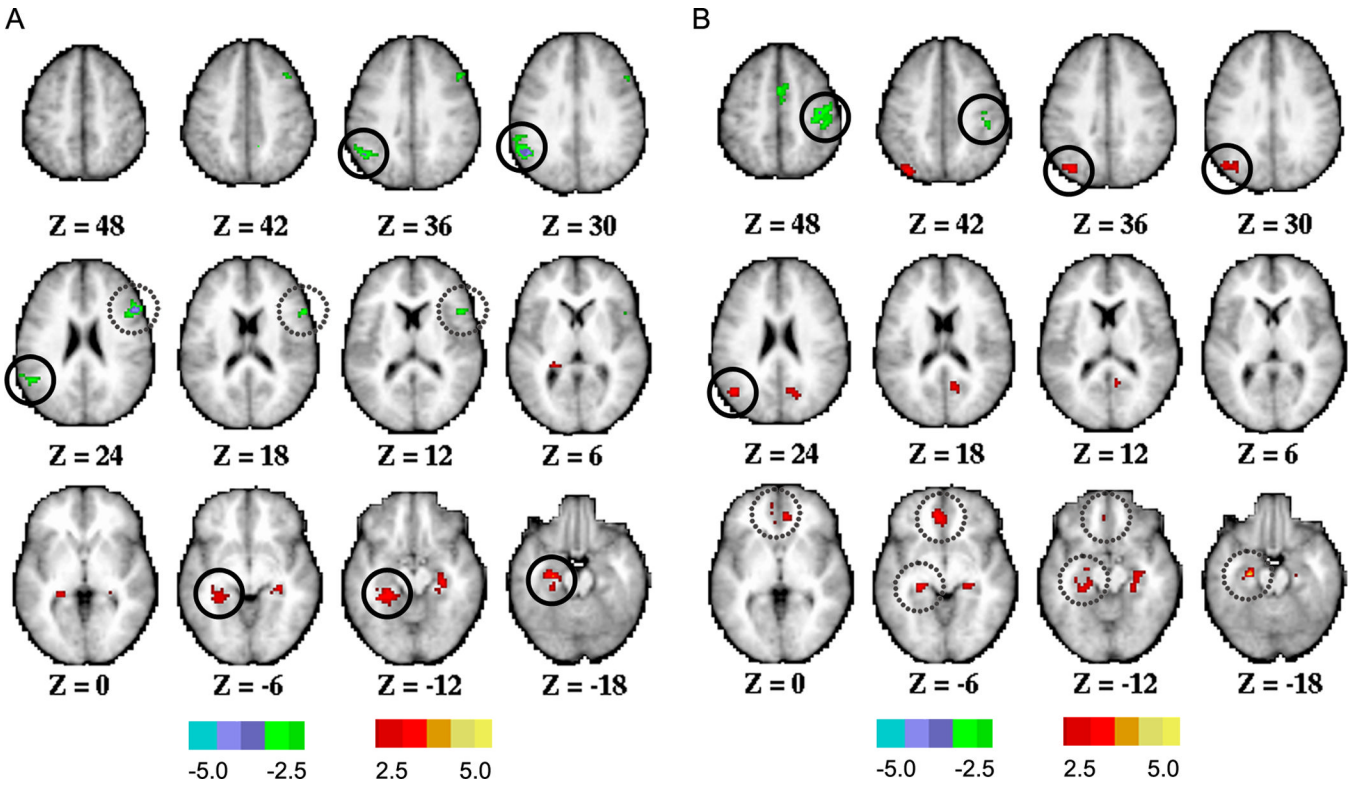

Figure 1. Voxel-wise analyses assessing functional connectivity of the posterior cingulate cortex (PCC) in cognitively normal individuals with abnormal levels of cerebrospinal fluid (CSF) $A\beta_{42}$ or $ptau_{181}$

Two-sample *t*-test assessed decreases (hot color) or increases (cold color) in functional connectivity of the PCC in CSF $A\beta_{42}$-positive individuals (≤500 pg/ml) compared to CSF $A\beta_{42}$-negative individuals (>500 pg/ml) (A), and in CSF $ptau_{181}$-positive individuals (≥80 pg/ml) compared to CSF $ptau_{181}$-negative individuals (<80 pg/ml) (B). Maps were displayed at a voxel-level $|t| > 2.5$ and cluster size > 35 voxels. Solid and dashed circles represent regions reaching a significance level of $p < 0.05$ and $0.05 < p < 0.1$ respectively, corrected at cluster-level. Detailed information about anatomic location and statistics of observed functional connectivity differences are listed in eTable 3.

Table 1

Demographics and cerebrospinal fluid biomarkers in cognitively normal participants

Mean age (SD), year	70.8(6.3)
Age range, year	60–88
Sex, % Male	37.2
Mean Education (SD), year	15.7 (2.8)
Mean MMSE score (SD)	28.8 (1.3)
CDR sum of boxes [mean (SD)]	0.03 (0.13)
APOE genotype, % at least one ε4 allele	31.4
Cerebrospinal fluid biomarkers	
Mean $A\beta_{42}$ (SD), pg/ml	624 (254)
$A\beta_{42}$-negative vs. positive	136:71
Mean $ptau_{181}$ (SD), pg/ml	61 (31)
$Ptau_{181}$-negative vs. positive	161:46

SD: standard deviation, MMSE: mini-mental state examination, for which the range of scores is from 30 (“best”) to 0 (“worst”), CDR sum of boxes: Clinical Dementia Rating sum of boxes (the sum of individual CDR domain scores) range from 0 to 18, with lower scores indicating better performance.

APOE: Apolipoprotein E, Aβ: amyloid-β, ptau: phosphorylated tau.

Table 2

Associations between cerebrospinal fluid levels of $A\beta_{42}$ or $ptau_{181}$ and default mode network integrity

	CSF $A\beta_{42}$ †		**CSF ptau §**	
	rho	***p***	**rho**	***p***
PCC-RSC	−0.009	0.894	−0.084	0.233
PCC-Left IPL	−0.028	0.689	−0.100	0.155
PCC-Right IPL	0.087	0.217	−0.096	0.175
PCC-MPFC	0.096	0.174	*−0.115*	*0.100*
PCC-Left MTL	**0.155**	**0.026**	**−0.182**	**0.008**
PCC-Right MTL	**0.231**	**<0.001**	*−0.122*	*0.081*

Cerebrospinal fluid (CSF) biomarkers were treated as continuous variables. Default mode network (DMN) integrity was measured by inter-regional functional connectivity between independently defined DMN regions. The associations between CSF levels of $A\beta_{42}$, tau, or $ptau_{181}$ and DMN integrity were assessed using Spearman partial correlation (rho) with confounding effects being controlled.

† rho values were adjusted for age, PCC and MTL volumes separately, and CSF $ptau_{181}$ levels.

§ rho values were adjusted for age, PCC and MTL volumes separately, and CSF $A\beta_{42}$ levels. Bold indicates the relationship is significant ($p < 0.05$), and italics indicate the relationship is at trend-level significance ($0.05 < p < 0.10$).

PCC: posterior cingulate cortex, RSC: retrosplenial cortex, IPL: inferior parietal lobule; MPFC: medial prefrontal cortex; MTL: medial temporal lobe.