Cerebrospinal Fluid Aβ42, Phosphorylated Tau\textsubscript{181}, and Resting-State Functional Connectivity

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IMPORTANCE Resting-state functional connectivity magnetic resonance imaging has great potential for characterizing pathophysiological changes during the preclinical phase of Alzheimer disease.

OBJECTIVE To assess the relationship between default mode network integrity and cerebrospinal fluid biomarkers of Alzheimer disease pathology in cognitively normal older individuals.

DESIGN, SETTING, AND PARTICIPANTS Cross-sectional cohort study at The Charles F. and Joanne Knight Alzheimer’s Disease Research Center at Washington University in St Louis, St Louis, Missouri, among 207 older adults with normal cognition (Clinical Dementia Rating, 0).

MAIN OUTCOMES AND MEASURES Resting-state functional connectivity magnetic resonance imaging measures of default mode network integrity.

RESULTS Decreased cerebrospinal fluid Aβ42 and increased cerebrospinal fluid phosphorylated tau\textsubscript{181} were independently associated with reduced default mode network integrity, with the most prominent decreases in functional connectivity observed between the posterior cingulate and medial temporal regions. Observed reductions in functional connectivity were unattributable to age or structural atrophy in the posterior cingulate and medial temporal areas. Similar resting-state functional connectivity magnetic resonance imaging findings in relation to cerebrospinal fluid biomarkers were obtained using region-of-interest analyses and voxelwise correlation mapping.

CONCLUSIONS AND RELEVANCE Both Aβ and tau pathology affect default mode network integrity before clinical onset of Alzheimer disease.

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Accumulation of Aβ and tau proteins, the pathologic hallmarks of Alzheimer disease (AD), starts years before clinical onset.\textsuperscript{1-4} Pathophysiological abnormalities in the preclinical phase of AD may be detected using cerebrospinal fluid (CSF) or neuroimaging biomarkers. Cerebrospinal fluid biomarkers have been recognized as key elements of research criteria for the preclinical phases of AD.\textsuperscript{5,6} Resting-state functional connectivity magnetic resonance imaging (rs-fcMR) imaging,\textsuperscript{7} a noninvasive measure of brain integrity, has considerable potential in investigations of preclinical AD.\textsuperscript{5,8,9}

Cerebrospinal fluid Aβ42 and amyloid imaging tracers, such as Pittsburgh compound B, measure amyloid burden in the brain.\textsuperscript{10,11} Both CSF tau and phosphorylated tau (ptau) are hypothesized to reflect neurodegeneration or tau pathology.\textsuperscript{12,13} Patients with symptomatic AD typically have a characteristic biomarker profile consisting of reduced CSF Aβ42, increased Pittsburgh compound B binding in the brain, and elevated CSF tau and ptau.\textsuperscript{11,14} Cognitively normal individuals can also exhibit biomarker evidence of AD pathology, with Aβ abnormalities more prevalent than alterations in tau or ptau.\textsuperscript{15-17}

Resting-state functional connectivity magnetic resonance imaging abnormalities have been consistently observed in the default mode network (DMN) in patients with symptomatic AD.\textsuperscript{18} Aβ preferentially deposits in cortical association areas that prominently include nodes of the DMN.\textsuperscript{19} Tau accumulation initially occurs in the limbic system,\textsuperscript{20} a subcomponent of the DMN.\textsuperscript{21} More recent rs-fcMR imaging investigations have detected DMN changes...
in asymptomatic individuals with increased amyloid deposition using Pittsburgh compound B. However, the association between functional connectivity in the DMN and CSF biomarker abnormalities requires further study.

We investigated the relationship between CSF biomarkers (eg, Aβ42 and ptau181) and rs-fcMR imaging in a large sample of cognitively normal individuals (N = 207). We hypothesized that decreased CSF Aβ42 and increased ptau181 would be associated with reduced DMN functional connectivity.

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, St Louis, Missouri. Detailed information about recruitment has been previously published. Inclusion criteria were (1) completion of MR imaging and CSF collection within 12 months of clinical assessment and (2) normal cognition, determined by a Clinical Dementia Rating (CDR) of 0, at the assessments closest to the time of MR imaging and CSF collection. Individuals were excluded if they had a medical or psychiatric illness that could affect longitudinal follow-up data 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 semistructured interviews with each participant and an informant and determined the presence or absence of dementia based on the principle of intradividual cognitive decline relative to prior functional level. Only individuals with a CDR of 0 (ie, cognitively normal) were included in the primary analysis. In total, 207 cognitively normal participants had both MR imaging and CSF collection within 12 months of clinical assessment. Demographic information and CSF biomarker profiles are given in Table 1.

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

CSF Collection and Analysis
Cerebrospinal fluid (20-30 mL) was collected at 8 AM after overnight fasting as previously described. Samples were analyzed for Aβ42, tau, and ptau181 by plate-based enzyme-linked immunosorbent assay (INNOSTEST; Innogenetics).

Because CSF tau was highly correlated with CSF ptau181 in the present cohort (Spearman ρ = 0.836, P < .001), we report only CSF ptau181 in relation to functional connectivity. In addition, neither CSF tau nor ptau181 was correlated with CSF Aβ42 (P ≥ .47 for both).

Table 1. Demographics and Cerebrospinal Fluid (CSF) Biomarkers in Cognitively Normal Participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>70.8 (6.3)</td>
</tr>
<tr>
<td>Age range, y</td>
<td>60-88</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>37.2</td>
</tr>
<tr>
<td>Education, mean (SD), y</td>
<td>15.7 (2.8)</td>
</tr>
<tr>
<td>Mini-Mental State Examination score, mean (SD)*</td>
<td>28.8 (1.3)</td>
</tr>
<tr>
<td>Clinical Dementia Rating sum of boxes, mean (SD)b</td>
<td>0.03 (0.13)</td>
</tr>
<tr>
<td>APOE genotype, % with ≥1 ε allele</td>
<td>31.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CSF biomarkers</th>
<th>Aβ42, mean (SD), pg/mL</th>
<th>624 (254)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF biomarkers</td>
<td>Aβ42 negative vs positive</td>
<td>136:71</td>
</tr>
<tr>
<td>CSF biomarkers</td>
<td>Phosphorylated tau181, mean (SD), pg/mL</td>
<td>61 (31)</td>
</tr>
<tr>
<td>CSF biomarkers</td>
<td>Phosphorylated tau181 negative vs positive</td>
<td>161:46</td>
</tr>
</tbody>
</table>

Abbreviation: APOE, apolipoprotein E.

* The range of scores is 30 (best) to 0 (worst).
b The sum of boxes (the sum of individual domain scores) ranges from 0 to 18, with lower scores indicating better performance.

Image Acquisition and Preprocessing of rs-fcMR Imaging Data
Participants were imaged using a scanning system (Trio 3T; Siemens Medical Systems). Two high-resolution structural images were obtained with T1-weighted magnetization-prepared rapid gradient-echo sequence (echo time, 16 milliseconds; repetition time, 2400 milliseconds; inversion time, 1000 milliseconds; flip angle, 8°; 256 × 256-pixel acquisition matrix; and 1 × 1 × 1-mm voxels). High-resolution T2-weighted fast spin-echo images were acquired (echo time, 455 milliseconds; repetition time, 3200 milliseconds; 256 × 256-pixel acquisition matrix; and 1 × 1 × 1-mm voxels). Two runs of rs-fcMR images (164 volumes each) were acquired using a gradient-echo sequence (echo time, 27 milliseconds; repetition time, 2.2 seconds; 64 × 64-pixel acquisition matrix; and flip angle, 90°). Thirty-six axial sections 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 crosshair, remain still, and not fall asleep during imaging. Details on rs-fcMR imaging preprocessing are provided in the eMethods of the Supplement.

Quality Assurance of rs-fcMR Imaging Data
The quality assurance procedures for rs-fcMR imaging data have been previously described. Briefly, functional MR imaging data quality was assessed by computing voxelwise root mean squared temporal variance (SD) averaged over the whole brain. Individuals with a mean preprocessed functional MR imaging signal SD exceeding 2.5% (before nuisance regression) or root-mean-squared head motion exceeding 1.25 mm were excluded. In addition, frames (volumes) with high variance were identified and removed. The number of frames excluded because of high variance did not correlate with any CSF variable (eMethods in the Supplement).
Definition of Regions of Interest

Generation of regions of interest (ROIs) within the DMN has been previously described. Briefly, rs-fcMR imaging data were analyzed from a separate cohort of 8 participants with mild AD dementia (CDR, 1) and 8 cognitively normal participants (CDR, 0). A 6-mm-radius sphere centered on the posterior cingulate cortex (PCC) (Montreal Neurological Institute coordinates, −2, −54, 16) was used as a seed. Correlation maps using this PCC seed were obtained for each participant and were averaged separately for the mild AD and cognitively normal groups. A group difference map was produced by subtracting the averaged map of the mild AD group from the cognitively normal group. 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, the left and right medial temporal lobe (MTL), and the medial prefrontal cortex (eTable 1 in the Supplement). The 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 rank partial correlation was used to assess the relationships between CSF biomarkers and DMN integrity. The ROI-based functional connectivity measures of the DMN (ie, PCC–retrosplenial cortex, PCC–left inferior parietal lobe, PCC–right inferior parietal lobe, PCC–medial prefrontal cortex, PCC–left MTL, and PCC–right MTL) were analyzed separately for each CSF biomarker, controlling for potential confounding effects. Because prior evidence has suggested that APOE genotype (the presence or absence of an ε4 allele) might affect Aβ42 in the CSF and DMN functional connectivity, we first assessed whether APOE ε4 status modulated the relationship between CSF Aβ42 and DMN functional connectivity. We compared the correlations obtained from APOE ε4 noncarriers with those from APOE ε4 carriers and reported these correlations if a significant difference was found. Otherwise, the relationship between CSF Aβ42 and functional connectivity was assessed within the entire cohort after adjusting for age, PCC and MTL volumes separately, and CSF ptau181. Details of the comparisons of correlation coefficients between APOE ε4 noncarriers and carriers are provided in the eMethods in the Supplement. Moreover, the associations between CSF ptau181 and functional connectivity were examined after adjusting for age, PCC and MTL volumes separately, and CSF Aβ42. Computation was implemented using R (version 2.15.1; R Foundation for Statistical Computing), with a statistical threshold for significance of P < .05, uncorrected for multiple comparisons.

Voxelwise Whole-Brain Investigation of Relationships Between CSF Biomarkers and DMN Integrity

Participants were classified as CSF Aβ42 negative (>500 pg/mL) or positive (≤500 pg/mL) and as CSF ptau181 negative (<80 pg/mL) or positive (≥80 pg/mL). 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 gray matter mask showing significant group contrast effects. These second-level analyses were conducted using 2-sample t tests implemented in Statistical Parametric Mapping (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/); the resulting t maps were thresholded at a cluster-level significance of P < .05.

Effects of PCC and MTL Volumes on rs-fcMR Imaging Changes

To assess the likelihood that observed functional connectivity changes were attributed to PCC and MTL atrophy, high-resolution structural images were processed using FreeSurfer (version 5.10; http://surfer.nmr.mgh.harvard.edu) to obtain regional volumes from the isthmus cingulate gyrus (ie, PCC) and the hippocampus, entorhinal cortex, and parahippocampal gyrus (combined to form MTL volume) (eMethods in the Supplement). Each PCC and MTL volume was correlated separately for CSF Aβ42 and ptau181 using a Spearman rank correlation. Specifically, CSF Aβ42–volumetric relationships were assessed after adjustment for age and CSF ptau181, and CSF ptau181–volumetric relationships were evaluated after adjustment for age and CSF Aβ42.

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 methods. Spearman rank partial correlations were computed between the functional connectivity measures and each CSF biomarker, with adjustment for potential confounding factors (Table 2). Correlations (Spearman ρ) between CSF Aβ42 and functional connectivity were not significantly different between APOE ε4 noncarriers and carriers (P ≥ .15 for all) (eTable 2 in the Supplement). Therefore, we pooled APOE ε4 carriers and noncarriers in subsequent analyses. Decreased CSF Aβ42 was associated with reduced functional connectivity between the PCC and left MTL (Spearman ρ = 0.155, P = .03) and the PCC and right MTL (Spearman ρ = 0.231, P < .001), after adjusting for age, PCC and MTL volumes separately, and CSF ptau181. Increased CSF ptau181 were associated with reduced functional connectivity between the PCC and left MTL (Spearman ρ = −0.182, P = .008) and with a trend-level decrease for the PCC and right MTL (Spearman ρ = −0.122, P = .08) and the PCC and medial prefrontal cortex (Spearman ρ = −0.115, P = .10), after adjusting for age, PCC and MTL volumes separately, and CSF Aβ42. All other associations between functional connectivity and CSF Aβ42 or CSF ptau181 were not significant (P ≥ .17 for all).
Topographies of Altered Functional Connectivity as a Function of CSF Biomarker

In the voxelwise analyses, each CSF biomarker was treated as a dichotomous variable. Group contrast correlation maps are shown in the Figure. The CSF Aβ42-positive individuals (≤500 pg/mL) compared with cerebrospinal fluid Aβ42-negative individuals (>500 pg/mL) (A) and in cerebrospinal fluid phosphorylated tau181-positive individuals (≥80 pg/mL) compared with cerebrospinal fluid phosphorylated tau181-negative individuals (<80 pg/mL) (B). Maps were displayed at a voxel-level *t* exceeding 2.5 and cluster size exceeding 35 voxels. Circles represent regions reaching a significance level of *P* < .05 (solid) and *P* < .10 but *P* > .05 (dashed), corrected at the cluster level. Detailed information about anatomical location and statistics of observed functional connectivity differences is given in Table 3 in the Supplement.

**Figure. Voxelwise Analyses Assessing Functional Connectivity of the Posterior Cingulate Cortex (PCC) in Cognitively Normal Individuals With Abnormal Levels of Cerebrospinal Fluid Aβ42 or Phosphorylated Tau181**

### Table 2. Associations Between Cerebrospinal Fluid (CSF) Aβ42 or Phosphorylated Tau181 and Default Mode Network Integrity*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Aβ42</th>
<th></th>
<th>Phosphorylated Tau181</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spearman ρ</td>
<td><em>P</em> Value</td>
<td>Spearman ρ</td>
<td><em>P</em> Value</td>
</tr>
<tr>
<td>PCC-retrosplenial cortex</td>
<td>-0.009</td>
<td>.89</td>
<td>-0.084</td>
<td>.23</td>
</tr>
<tr>
<td>PCC-left inferior parietal lobe</td>
<td>-0.028</td>
<td>.69</td>
<td>-0.100</td>
<td>.16</td>
</tr>
<tr>
<td>PCC-right inferior parietal lobe</td>
<td>0.087</td>
<td>.22</td>
<td>-0.096</td>
<td>.18</td>
</tr>
<tr>
<td>PCC-medial prefrontal cortex</td>
<td>0.096</td>
<td>.17</td>
<td>-0.115</td>
<td>.10</td>
</tr>
<tr>
<td>PCC-left medial temporal lobe</td>
<td>0.155</td>
<td>.03</td>
<td>-0.182</td>
<td>.008</td>
</tr>
<tr>
<td>PCC-right medial temporal lobe</td>
<td>0.231</td>
<td>&lt;.001</td>
<td>-0.122</td>
<td>.08</td>
</tr>
</tbody>
</table>

Abbreviation: PCC, posterior cingulate cortex.

* The CSF biomarkers were treated as continuous variables. Default mode network integrity was measured by interregional functional connectivity between independently defined default mode network regions. The associations between CSF Aβ42 or phosphorylated tau181, and default mode network integrity were assessed using Spearman rank partial correlation (ρ), with confounding effects being controlled for. Boldface print indicates the relationship is significant (*P* < .05), and data in italics indicate the relationship is at trend-level significance (*P* < .10 but *P* > .05).

**A** Adjusted for age, PCC and medial temporal lobe volumes separately, and CSF phosphorylated tau181.

**B** Adjusted for age, PCC and medial temporal lobe volumes separately, and CSF Aβ42.
Relationship Between CSF Biomarkers and PCC and MTL Volumetrics

We computed PCC and MTL volumes using Freesurfer-defined ROIs. The CSF Aβ42 was correlated with MTL volume (Spearman \( p = 0.172, P = .01 \)) but not with PCC volume (Spearman \( p = -0.044, P = .53 \)) after adjustment for age and CSF ptau. The CSF ptau was not correlated with MTL volume or with PCC volume (\( P \geq .10 \) for all).

Discussion

We assessed the relationship between neuroimaging indexes of brain functional network integrity and well-validated CSF biomarkers of AD pathology in cognitively normal older individuals. We demonstrated that decreased CSF Aβ42 and increased CSF ptau were associated with reduced magnitude of correlations in the DMN and within areas normally anticorrelated with the DMN. The most prominent decreases in functional connectivity were seen between the PCC and MTL regions. The effects of CSF Aβ42, CSF ptau, 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. Reduced functional connectivity among these regions is well documented in cognitively normal older adults with high amyloid burden. However, the MTL is not an early site of plaque formation. Therefore, the link between reduced PCC-MTL functional connectivity and lower CSF Aβ42 may be related to other mechanisms. One possibility is that functional connectivity changes are more related to soluble than fibrillar forms of Aβ. Animal studies have demonstrated that oligomeric Aβ directly impairs synaptic function or causes synaptic loss, particularly within the MTL. We observed that reduced CSF Aβ42 was associated with volume loss in the MTL but not the PCC, which is consistent with the oligomeric toxicity hypothesis. Further work examining CSF Aβ42 in relation to soluble forms of Aβ is 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. 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.

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 transentorhinal cortex and spread in a topographically stereotypical manner, possibly via anatomical connections. In our data, increased CSF ptau was associated with reduced functional connectivity within the anatomical pathways through which neurofibrillary tangles spread. 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, and these tangles are associated with little or no neuronal loss. In contrast, patients with very mild AD dementia (CDR, 0.5) have substantial neuronal loss (30%-50%) in the entorhinal cortex. It is possible that elevated CSF ptau may be associated with neuronal and synaptic loss in the MTL that is yet insufficient to produce overt clinical symptoms and is undetectable by the present volumetric measure but is detectable at the group level using rs-fcMR imaging.

The convergent effects of decreased CSF Aβ42 and increased CSF ptau on the DMN provide insights into the early pathogenesis of AD. The PCC and MTL are 2 critical nodes of a larger network supporting episodic memory. Stronger PCC-MTL functional connectivity is associated with better performance on memory tasks. Structural atrophy of the PCC-MTL pathway is consistent with commonly recognized memory impairments in AD. Therefore, 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 voxelwise whole-brain exploration, we observed that abnormal levels of CSF Aβ42 and ptau 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 presymptomatic individuals. Replication of our findings is needed in additional independent samples. Although we found no evidence that rs-fcMR imaging 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-fcMR imaging 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 mild symptomatic AD exhibit changes in multiple resting-state networks. Further work is warranted to examine the effects of CSF biomarker abnormalities in resting-state networks other than the DMN.
CSF Aβ42, Tau, and Resting-State Connectivity

Study supervision: Snyder, Benzing, Holtzman, Ances.

Conflict of Interest Disclosures: Dr Fagan consults for Roche and Lilly USA. Dr Benzing consults for Biomedical Systems, Inc and ICON Medical Imaging and receives research support from Avid Radiopharmaceuticals. Dr Holtzman reports consulting for Pfizer, Bristol-Myers Squibb, and Innogenetics and is on the scientific advisory boards of En ViVo, Satori, and C2N Diagnostics. Dr Morris has participated or is participating in clinical trials of antidementia drugs sponsored by Janssen Immunotherapy and by Pfizer; he has served as a consultant for Eisai, Esteve, Jansen Alzheimer Immunotherapy Program, Glaxo-Smith-Kline, Novartis, and Pfizer and receives research support from Eli Lilly and Avid Radiopharmaceuticals. No other disclosures were reported.

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REFERENCES