Relationship between Basal Forebrain Resting-State Functional Connectivity and Brain Amyloid-β Deposition in Cognitively Intact Older Adults with Subjective Memory Complaints

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This retrospective study was approved by the local ethics committee and written informed consent was obtained from all participants. Resting-state functional connectivity (RSFC) of anterior and posterior basal forebrain seeds was investigated, as well as PET-measured global amyloid-β load by using standardized uptake ratio value (SUVR) in 267 older cognitively intact individuals with subjective memory complaints (age range, 70–85 years; overall mean age, 75.8 years; 167 women [mean age, 75.9 years] and 100 men [mean age, 75.8 years]). The participants were from the Investigation of Alzheimer’s Predictors in Subjective Memory Complainers (INSIGHT-preAD) cohort (date range, 2013–present). The relationship between SUVR and the basal forebrain RSFC was assessed, followed by the effects of apolipoprotein E (APOE) genotype and sex on the basal forebrain RSFC.

Results: Higher SUVR values correlated with lower posterior basal forebrain RSFC in the hippocampus and the thalamus (Pearson $r = -0.23$; $P < .001$ corrected for familywise error [FWE]), Both sex and APOE genotype impacted the associations between basal forebrain RSFC and the global amyloid deposition ($r > 3.59$; $P < .05$ corrected for FWE).

Conclusion: Data indicate a distinct in vivo association between posterior basal forebrain dynamics and global fibrillary amyloid-β pathology in cognitively intact older adults with subjective memory complaints; both apolipoprotein E and sex moderate such association.

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Purpose: To evaluate the association between the global fibrillary amyloid-β pathology and the basal forebrain connectivity at rest in cognitively intact older adults at risk for Alzheimer disease.

Materials and Methods: This retrospective study was approved by the local ethics committee and written informed consent was obtained from all participants. Resting-state functional connectivity (RSFC) of anterior and posterior basal forebrain seeds was investigated, as well as PET-measured global amyloid-β load by using standardized uptake ratio value (SUVR) in 267 older cognitively intact individuals with subjective memory complaints (age range, 70–85 years; overall mean age, 75.8 years; 167 women [mean age, 75.9 years] and 100 men [mean age, 75.8 years]). The participants were from the Investigation of Alzheimer’s Predictors in Subjective Memory Complainers (INSIGHT-preAD) cohort (date range, 2013–present). The relationship between SUVR and the basal forebrain RSFC was assessed, followed by the effects of apolipoprotein E (APOE) genotype and sex on the basal forebrain RSFC.

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Connectivity measures are emerging as potential intermediate biomarkers for Alzheimer disease (AD), although a considerable heterogeneity exists in terms of the networks affected (1). Overall, the most severe dysfunctions were found in key nodes of the default mode network, including the posterior cingulate cortex, the precuneus, the inferior parietal cortex, and the hippocampus (1). Interestingly, neuroimaging genetics studies showed lower connectivity in the posterior default mode network regions and higher fronto default mode network connectivity in individuals carrying risk genes of sporadic AD, such as allelic variations of the apolipoprotein E (APOE) genotype (2). Importantly, two recent studies further indicated the presence of an interaction effect between APOE ε4 and sex on the default mode network, showing that female APOE ε4 carriers exhibited reduced functional connectivity compared
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**Basal Forebrain Resting-State Functional Connectivity and Brain β-Amyloid Deposition**

Here, we studied the association of global amyloid load with male carriers (3,4). These results are in line with recent findings suggesting that sex might constitute an important additional factor influencing phenotypic variability across the spectrum of AD.

In the last few decades, resting-state functional MRI studies have provided consistent evidence that alterations in the neuronal networks reflect the underlying pathologic alterations (1), such as senile plaques, containing extracellular deposits of aggregated amyloid-β peptides and the intracellular accumulation of neurofibrillary tangles (5). Notably, network dysfunctions are not fully spatially coterminous with regional amyloid or tau pathology. Such observation suggests that network disruption itself may represent a remote consequence of regional pathology or pathophysiology, triggering and driving pathologic hallmarks including the amyloid cascade (6).

Several recent lines of evidence suggest a tight interrelation between the amyloid cascade and cerebral cholinergic functions (7–10). The basal forebrain, which serves as the primary cholinergic source of neurons projecting to the cerebral cortex, seems to be particularly affected by pathophysiological alterations, as shown in postmortem brains with AD (11) and in cognitively intact individuals (12–14). Recent studies combining amyloid PET with structural MRI as indirect measure of synaptic and neuronal atrophy reported a robust in vivo association between cortical amyloid-β burden and basal forebrain neuronal loss, both in AD dementia (15,16) and in individuals with preclinical AD (17). However, the impact of in vivo amyloid-β load on functional basal forebrain mechanisms remains to be identified.

Here, we studied the association of global amyloid load as determined by using PET with basal forebrain functional connectivity from resting-state functional MRI data derived from cognitively intact older individuals with subjective memory complaints, recruited from the Investigation of Alzheimer’s Predictors in Subjective Memory Complainers (INSIGHT-preAD) cohort (18). Older people with subjective memory complaints are twice as likely to develop dementia as individuals without subjective memory complaints (19).

Clarifying the role of in vivo fibrillary amyloid-β in relation to cholinergic functional connectivity is crucial to understand the pathophysiologic link between amyloid accumulation and cholinergic dysfunction in the earliest stages of AD. Thus, our main aim was to determine whether in vivo fibrillary amyloid-β is associated with cholinergic functional connectivity in cognitively older adults at risk for AD. Additionally, we aimed to address the influence of both sex and *APOE* ε4 allele.

**Materials and Methods**

*The INSIGHT-preAD Study* This retrospective study was approved by the local institutional review board and has been conducted in accordance with the Helsinki Declaration of 1975. Written informed consent was provided by all participants. Our study data were obtained from the INSIGHT-preAD research program (18). The same cohort has been used in previously published studies (18,20–23), but none of them evaluated the functional connectivity at rest of these individuals. The INSIGHT-preAD study is an ongoing single-center observational cohort study being performed at the Institute of Memory and Alzheimer’s Disease, Pitié-Salpêtrière University Hospital, Paris, France (date range, 2013–present; B.D. is the principal investigator and H.H. is the scientific director). The study was promoted by INSERM in collaboration with Brain & Spine Institute (Institut du Cerveau et de la Moelle Épinière [ICM]), IHU-A-ICM, and Pfizer and has received support from the Investissement d’Avenir (Agence Nationale de la Recherche 10-AIHU-06). The study was promoted in collaboration with the Bordeaux Hospital University Center (Centre Hospitalier Universitaire [CHU], coordination Center for Clinical Investigation EC7), the promoter of Memento cohort, funded by Fondation Plan Alzheimer. The study was further supported by Avid/Lilly. The research leading to these results was supported by the Colam Initiatives and the Fondation pour la Recherche sur Alzheimer, Paris, France. This publication is within the framework of the program MIDAS led by the Sorbonne University Foundation and sponsored by MSDAVENIR. None of the funders of the study participated in the analysis, interpretation, or writing of the report.

*The INSIGHT-preAD study includes 318 cognitively intact older adults aged 70 to 85 years, with subjective memory complaints defined as follows: First, participants answered “yes” to both questions: “Are you complaining about your memory?” and “Is it a regular complaint that has lasted now more than 6 months?” Second, participants presented intact cognitive functions based on the Mini-Mental State Examination (score ≥27), Clinical Dementia Rating scale (score of 0), and Free and Cued Selective Reminding Test (total score ≥41). Amyloid status; *APOE* genotype; demographic, cognitive, functional, nutritional, biologic, and genomic information; and imaging, electrophysiologic, and other assessments were determined at baseline.
Clinical and Neuropsychologic Assessments
A comprehensive neuropsychologic battery was administered to all participants of the INSIGHT-preAD cohort to assess all relevant cognitive domains (B.D. and H.H.). More information about the neuropsychologic battery can be found in Appendix E1 (online). In our study, we considered only participants' performance at the Mini-Mental State Examination to assess the general cognition.

APOE Genotype
Genomic DNA was prepared from frozen blood samples with the ArchivePure DNA purification system (5 Prime, Gaithersburg, Md) according to the manufacturer's instructions (M.C.P.). The APOE genotypes were determined by using Sanger method. Exon 4 from APOE gene holding the single-nucleotide polymorphism related to the APOE 3/4 alleles was amplified by using polymerase chain reaction with the following primers: APOE sense, 5'-TAAGCTTGCCAGGCTGTCCCAAGGA-3'; APOE antisense, 5'-ACAGAATCCGCCCGGCCTGGTGACAC-3'. For each sample, the reaction mixture (50 μL) contained 200 ng of genomic DNA, 10 μL polymerase chain reaction buffer (5X), 1 μL deoxyribonucleotide triphosphate (10 mmol/L), 1 μL of each forward and reverse primers (10 μmol/L), and 0.25 μL GoTaq DNA polymerase (Promega, Madison, Wis). The cycling program was carried out after a preheating step at 95°C for 2 minutes and 35 cycles of denaturation at 95°C for 1 minute, annealing at 68°C for 1 minute and extension at 72°C for 1 minute. The amplified fragments were then purified and sequenced by using the same primers.

Two APOE single-nucleotide polymorphisms rs429358 and rs7412 allowed identification of the e2, e3, and e4 alleles (24). In line with previous evidence suggesting the e4 allele is associated with increased risk of developing AD (25), participants were divided into two groups based on the APOE status. Individuals carrying at least one APOE e4 allele were classified as APOE e4 carriers (APOE e4–positive), and the others as APOE e4 noncarriers (APOE e4–negative).

PET Data Acquisition and Processing
PET scans were acquired in a single session with a PET/CT scanner (Gemini XLS; Philips Healthcare, Cleveland, Ohio) around 50 minutes after injection of approximately 370 MBq (333–407 MBq) of florbetapir (Amyvid; Avid Radiopharmaceuticals, Philadelphia, Pa) (M.O.H.). Images (three consecutive acquisitions of 5 minutes each; acquisition matrix, 128 × 128; voxel size, 2 × 2 × 2 mm³) were reconstructed by using iterative LOR-RAMLA algorithm (10 iterations), applying a smooth postreconstruction filter and integrating all corrections (attenuation, scatter, and random coincidence) (22). PET data were realigned, averaged, visually inspected for possible artifacts, and analyzed with a pipeline developed by the CATI team, a neuroimaging platform funded by French Plan Alzheimer (http://catti-neuroimaging.com). Given that structural MR images were coregistered to florbetapir PET images, mismatch between CT and emission scans was also evaluated by using statistical parametric mapping (SPM, version 8; Wellcome Depart-
ment of Imaging Neuroscience, London, England; available at https://www.fil.ion.ucl.ac.uk/spm). By using inverse deformation fields and matrix, the florbetapir standardized uptake value was calculated in a reference region (ie, whole cerebelum) and in six bilateral cortical regions (precuneus, anterior cingulum, posterior cingulum and parietal, temporal, and orbitofrontal cortices) derived from a combination of both the Avid (https://www.avidrp.com) and the CAEN (https://nimh.unicaen.fr/accueil) methods (26). The partial volume effect correction was applied to the PET images. Parametric PET images were created for each individual by dividing each voxel with the mean value extracted from the reference region. Standard uptake value ratios (SUVRs) were calculated by averaging the mean activity of all cortical regions of interest in the individual PET native space.

Resting-State Functional MRI Data Acquisition and Preprocessing
All images were acquired by using a 3.0-T Siemens Trio MRI system (Siemens Medical Systems, Erlangen, Germany) at the Center for Neuroimaging Research (Centre de NeuroImagerie de Recherche, CNIR) at the Brain & Spine Institute (ICM, CNRS/Inserm/Sorbonne Université), Pitié-Salpêtrière University Hospital, Paris, France (P.A.C). During the resting-state functional MRI examination, participants were instructed to keep their eyes closed and stay as still as possible. The resting-state functional MR images were collected by using an echo-planar imaging sequence (repetition time, 2460 msec; echo time, 30 msec; section thickness, 3 mm; matrix, 64 × 64; voxel size, 3 × 3 × 3 mm³; number of volumes, 250; number of sections, 45; run, 1) sensitive to blood oxygenation level–dependent contrast (T2* weighting). Only one resting-state functional MRI run was acquired for each participant.

The resting-state functional MRI data were preprocessed by using Data Processing Assistant for Resting-State MRI (DPARSFA) (27) implemented in DPABI (http://rfmri.org/dpabi), based on SPM version 8. Each participant's first 10 volumes were excluded to avoid potential noise related to the equilibrium of the magnet and participant's adaptation to the imager. The remaining 240 volumes were preprocessed in a series of steps including section-timing correction, realignment, and segmentation by using SPM priors for cerebrospinal fluid and white matter. We regressed out the global mean and the confounding effects of cerebrospinal fluid and white matter to reduce the influence of physiologic noise. The Friston 24-parameter model, which includes six head motion parameters, six head motion parameters one time point before, and the 12 corresponding squared items, was used to regress out head motion effects (28). We excluded from the analyses participants who had a maximum displacement in one or more of the orthogonal directions greater than 2.5 mm or rotation greater than 2.5 degrees (Fig 1). A temporal band-pass filtering (passband, 0.01–0.1 Hz) was applied to reduce the effect of low-frequency drift and high-frequency physiologic noise.

The motion-corrected functional volumes were subsequently spatially normalized to the T1 unified segmentation template in Montreal Neurologic Institute (MNI) coordinates derived from SPM version 8 software and resampled to 3 × 3 × 3-mm³ voxels.
Definition of the Functional Basal Forebrain as Region of Interest

The seed regions consisted of anterior and posterior functional subdivisions of the basal forebrain, as recently identified in a resting-state functional connectivity (RSFC)-based clustering parcellation of the basal forebrain in an independent sample of healthy adults (29) (M.J.G. and S.J.T.). The details of this independent methodologic study are described in the original article (29); we summarize here all the necessary information to understand the functional definition of the used basal forebrain seed regions. All voxels in a stereotactically defined basal forebrain region of interest (30) were clustered into functionally homogeneous partitions by applying a k-means clustering algorithm to the voxelwise brain-wide functional connectivity profiles as derived from resting-state functional MRI data (31). This approach identified a principal parcellation of the basal forebrain into two functional subdivisions with clearly differing functional connectivity profiles (Fig 2): an anterior basal forebrain (ABF) region (green in Fig 2), mainly corresponding to the rostral nuclei of the medial septum and diagonal band, as well as anterior-medial parts of the nucleus basalis of Meynert, and a posterior basal forebrain (PBF) region (red in Fig 2), covering anterior-lateral, intermediate, and posterior parts of the nucleus basalis of Meynert.

The DPARSFA toolbox was used to create individual subject seed-to-voxel connectivity maps including several steps. First, the mean time series of each seed region was extracted and correlated (Pearson correlation) with that of each whole brain voxel. Then, the Fisher r-to-z transformation was applied to standardize the resulting correlation maps. Finally, individuals’ standardized maps were entered in a one-sample t test (SPM version 8) to identify brain regions within the gray matter that showed correlations with each seed region (P < .05 corrected for familywise error [FWE] at voxel level; P < .05 corrected for FWE at the cluster level). Age and Mini-Mental State Examination score were included as covariates.

Statistical Analyses

Statistical analyses were performed by P.A.C. and M.H. with contributions from M.J.G. and S.J.T by using DPABI (SPSS, version 21; Chicago, Ill) and R (version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria; available at https://www.r-project.org). Age and Mini-Mental State Examination score were used as nuisance covariates. Statistical analyses included the following steps: creating a binary mask for either ABF or PBF, evaluating common and differential patterns between ABF and PBF binary maps, evaluating the correlation between the basal forebrain RSFC and the amyloid load (ie, SUVR values), and evaluating the influence of sex and APOE ε4 allele on the basal forebrain RSFC–SUVR association.

Basal forebrain RSFC binary masks.—Individual basal forebrain RSFC maps were initially extracted to determine which brain voxels showed activity fluctuations that are positively correlated with those of the ABF and PBF seed regions. The subject-level statistical maps were then used to generate an ABF and PBF group-level map. These group-level maps were finally used as binary masks—three-dimensional images at the same resolution and in the same space as the preprocessed data sets and used to filter voxels of interest—for the subsequent correlation analyses. Because apparent negative correlations between networks on the group level can be driven by global signal regression (32), they were not considered in the analyses.

Conjunction and difference analyses.—Differential and conjunction analyses were performed to evaluate different and common pattern of functional connections between the two parts of the basal forebrain (ABF and PBF; P < .001 corrected for FWE).

Association between basal forebrain RSFC and global brain amyloid load.—Correlations among voxelwise brain RSFC measures (z scores) and global SUVR scores were computed in DPABI. In the model, the individual seed-to-voxel connectivity map is the dependent variable and SUVR score is the independent variable. The DPABI toolbox implements the regression model for all voxels in the seed-to-voxel connectivity map and then converts the voxelwise t values (t) to Pearson r values (r) by using the formula:

\[ r = \frac{t}{\sqrt{df + t^2}} \]

where df is degrees of freedom. (See the DPABI website at http://rfmri.org/dpabi for further details.) The final output is a matrix/image of Pearson correlation coefficients (P < .05 corrected for FWE; minimum voxels, 10).
Conjunction analysis.—The conjunction analyses showed a common functional connectivity pattern between the ABF and PBF in the bilateral insulae, the hippocampi, and the basal ganglia as well as the poster cingulate cortex and the frontal area partially (5882 voxels, peak MNI coordinates: 9, 9, 29; *P*, .001 corrected for FWE; 739 voxels, peak MNI coordinates: 23, 54, 26; *P*, .001 corrected for FWE). The common regions are represented in green in Figure 4.

Differential analyses.—An exclusive conjunction analysis was applied to evaluate the specific RSFC pattern of ABF and PBF (Fig 4). We found that the ABF connections involved regions belonging to anterior-posterior midline and medial temporal default mode network nodes (Fig 3, A). Regions showing positive RSFC with the PBF were mainly located in the typical nodes of the ventral salience network, such as dorsal anterior cingulate cortex and insula (Fig 3, B).

Correlations between Positive RSFC of Basal Forebrain and Brain Amyloid Uptake
To evaluate the association between basal forebrain RSFC and brain global amyloid load, we performed a voxel-based cor-

Impact of APOE genotype and sex on the basal forebrain RSFC–SUVR association.—Two separate full factorial models added APOE genotype or sex, and their respective interaction terms with SUVR score, as predictor variables to assess possible moderating effects of these variables on the association between SUVR and basal forebrain functional connectivity (*P* < .05 corrected for false discovery rate at the cluster level).

Results
From the INSIGHT-preAD cohort, we considered only participants who underwent the resting-state functional MRI acquisition (*n* = 297; date range, 2013–2014). Thirty participants were excluded due to incidental imaging abnormalities and excessive head motion during imaging (see Fig 2 for details). Demographic characteristics (age range, 70–85 years; overall mean age, 75.8 years; 167 women [mean age, 75.9 years] and 100 men [mean age, 75.8 years]), cognitive performance, in vivo brain amyloid SUVRs, and APOE genotype of the final subset (267 participants) are shown in Table 1.

Basal Forebrain Functional Connectivity Patterns at Rest
The group-level *t* test was used to create positive basal forebrain RSFC masks for each basal forebrain region of interest (ABF and PBF) (Fig 3). Brain regions showing positive RSFC with the ABF were mainly located in the anterior-posterior midline and medial temporal default mode network nodes (Fig 3, A). Regions showing positive RSFC with the PBF were mainly located in the typical nodes of the ventral salience network, such as dorsal anterior cingulate cortex and insula (Fig 3, B).

Correlation between Positive RSFC of Basal Forebrain and Brain Amyloid Uptake
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Table 1: Demographic Characteristics, Global Cognitive Performance, and Amyloid SUVRs of the Final Subset of 267 Cognitively Intact Older Individuals, APOE e4 Non-carriers, and APOE e4 Carriers

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall</th>
<th>APOE e4 Non-carriers</th>
<th>APOE e4 Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>267</td>
<td>192/267 (72)</td>
<td>53/267 (20)</td>
</tr>
<tr>
<td>No. of women</td>
<td>167/267 (63)</td>
<td>136/192 (71)</td>
<td>31/53 (58)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>75.8 ± 3.5</td>
<td>75.7 ± 3.6</td>
<td>76.1 ± 3.6</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.7 ± 1.0</td>
<td>28.7 ± 1.0</td>
<td>28.7 ± 1.0</td>
</tr>
<tr>
<td>SUVR</td>
<td>0.78 ± 0.19</td>
<td>0.76 ± 0.23</td>
<td>0.83 ± 0.23</td>
</tr>
</tbody>
</table>

Note.—Unless otherwise indicated, data are numbers of patients, with percentages in parentheses. APOE = apolipoprotein, MMSE = Mini-Mental State Examination, SUVR = standard update value ratio.

* Data are means ± standard deviations.
relation analysis between global amyloid SUVR values and voxelwise RSFC of both ABF and PBF, separately. Age and Mini-Mental State Examination score were used as nuisance covariates. The search space for the voxelwise analyses were restricted to the significant group-level positive basal forebrain RSFC maps reported in the previous analysis ($P < .05$ corrected for FWE). For the PBF seed, we found one significant cluster located in the left hippocampus and thalamus where RSFC negatively correlated with SUVR (43 voxels, peak MNI coordinates: $[-21, -30, -6]$; $P < .001$ corrected for FWE; Pearson $r$ in the peak voxel, $-0.23$). No brain regions with significant positive correlations were found.

In the analysis correlating SUVR values with RSFC of the ABF seed, no voxels passed the significance threshold of $P < .05$ corrected for FWE.

Role of APOE Genotype and Sex on the Basal Forebrain RSFC–SUVR Association
We then tested the moderating effect of APOE and sex on the association of basal forebrain RSFC and global amyloid load. The analysis of the impact of APOE genotype on the PBF RSFC–SUVR association showed that $APOE\,\epsilon4$ noncarriers are less positively correlated than are $APOE\,\epsilon4$ carriers in the thalamus and posterior areas (left occipital middle gyrus [Brodmann area 19] extended to the left inferior parietal lobule [Brodmann area 39]) (Fig 5, A, Table 2). No effect was found on the ABF RSFC–SUVR association.

The interaction effect between sex and SUVR on the basal forebrain RSFC revealed that women are more positively correlated than are men in the superior frontal gyrus (Brodmann area 10) considering the ABF RSFC, and in both the caudate nucleus and the thalamus considering PBF RSFC (Fig 5, B).

Discussion
In our study, we reported in vivo evidence for the association between basal forebrain functional connectivity at rest (or RSFC) and global cortical amyloid load (or SUVR) in older adults with subjective memory complaints. Given the structural and functional variability of cholinergic subdivisions within the basal forebrain (33), we explored the RSFC separately for the anterior and the posterior basal forebrain seed regions (ABF and PBF, respectively) based on their largely differing resting-state...
absence of objective cognitive decline, the cholinergic functional connectivity is affected by global amyloid accumulation in cognitively intact older adults. In particular, we disclosed a significant cluster in the left hippocampus extending to the thalamus that showed lower functional connections with the PBF as SUVR levels increased. This in vivo association is in line with earlier findings reporting the basal forebrain cholinergic neurons to be particularly vulnerable to age- and AD-related neurodegeneration (eg, reference 32). Such functional alterations might reflect, and possibly anticipate, a robust link between amyloid pathology and cholinergic degeneration and fiber loss as previously observed in postmortem brain tissue of older individuals without dementia (14,38), as well as in patients with AD dementia (39,40). More regional-specific measures should be performed to allow conclusions on the relation to regional brain amyloid-β accumulation. Because cross-sectional designs do not imply causality, further longitudinal studies combining resting-state functional MRI, structural MRI, and measures of brain amyloidosis are needed to delineate the potential sequence of causative events, if any.

We also investigated the role of sex and APOE genotype on the associations between basal forebrain RSFC and the global amyloid deposition. We disclosed a significant effect

**Figure 5:** Functional MR images show modulating effect of apolipoprotein E (APOE) genotype and sex on basal forebrain resting-state functional connectivity (RSFC). A, Top panel illustrates significant clusters where APOE ε4 noncarriers present less positive correlations than do APOE ε4 carriers in anterior basal forebrain (ABF) RSFC. B, Bottom panel illustrates significant voxels where women presented a stronger association between standardized update value ratio and functional connectivity of ABF (in red) and posterior basal forebrain (in green) compared with men (P value < .05 corrected for familywise error).
of both factors: the APOE ε4 allele showed stronger correlation between the global brain amyloid deposit and the PBF functional dynamics in occipital regions and in the thalamus. We provided further evidence that the APOE ε4 allele is linked to abnormal amyloid-β aggregation (41–44) and leads to differences in the brain functional organization in cognitively intact individuals (2). This result supports the hypothesis of different binding characteristics of the APOE ε4 isoform with amyloid-β (45).

Previous findings of APOE effects on brain functional dynamics associated with AD neuropathology were also available in combination with the sex factor: cognitively intact APOE ε4 female carriers showed lower default mode network (4) and hippocampal connectivity (3), larger hypometabolism, and atrophy compared with male carriers (46). There is a vast literature on sex differences in human brains that encompasses multiple domains from healthy to diseased conditions (47); sex might therefore constitute a key factor influencing phenotypic variability in AD (48). Evidence of structural (49,50) and functional (51) differences between men and women have started to emerge in AD phenotypic variability (48,50) and functional (51) differences between men and women. The specific role of sex in basal forebrain functioning and functional reorganization.

In contrast, a previous study showed that the association between basal forebrain volume and cortical amyloid deposition in presymptomatic and preclinical stages of AD did not depend on age, sex, and APOE ε4 genotype (17). Such a difference might be because we investigated specific functional basal forebrain subregions, functional rather than structural indexes, and a specific population (namely, individuals with subjective memory complaints); also, sex was only included as covariate regressor. In general, we believe that both sex and APOE genotype should be integrated in future investigations to clarify those discrepancies comparing different networks and populations.

Our study had limitations that warrant discussion, including its retrospective nature and the use of global cortical amyloid-β deposition. Because of the resolution limits of PET, however, it may be difficult to define the membrane boundary of small regions. Although the sample size is large enough to support our conclusions, the inclusion of a control group may strengthen the results. The comparison of our population with individuals without subjective memory complaints may demonstrate that the association between amyloid burden and neurophysiologic alterations is specific for individuals at risk for AD.

Overall, our findings indicate that the basal forebrain cholinergic pathway might be impaired at an early stage during the neurodegenerative process. Therefore, basal forebrain functional alterations may be a promising candidate for an early preclinical biomarker of AD and a potentially useful functional outcome in time, to our knowledge, that a sex effect is demonstrated in neuroimaging measures of basal forebrain function in the human brain. We found that women showed more positive correlation between the global brain amyloid deposit and the basal forebrain functional connectivity in frontal and subcortical areas. The general greater activity in women during different cognitive engagement (54–56) and at rest (57,58) may spread the brain amyloid-β deposition (6). Alternatively, neuromodulator properties of sex hormones on neurite outgrowth, synaptogenesis, dendritic branching, myelination, and other important mechanisms of neural activity and plasticity may play a role in age-related changes of brain structure and function (59). Further investigations are necessary to identify the specific role of sex in basal forebrain functioning and functional reorganization.

Table 2: Significant Clusters of the Functional Connectivity at Rest for Moderating Effect of APOE and Sex on the Association of Basal Forebrain RSFC and Global Amyloid Load (SUVR)

<table>
<thead>
<tr>
<th>Contrast and Brain Region</th>
<th>Cluster</th>
<th>Coordinates (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE genotype</td>
<td>P Value*</td>
<td>P Value†</td>
</tr>
<tr>
<td>PBF</td>
<td>APOE ε3 &lt; APOE ε4</td>
<td>Thalamus</td>
</tr>
<tr>
<td>Left occipital middle gyrus‡</td>
<td>.041</td>
<td>.027</td>
</tr>
<tr>
<td>Sex</td>
<td>AFB</td>
<td>Female &gt; male</td>
</tr>
<tr>
<td>Superior frontal gyrus§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBF</td>
<td>Female &gt; male</td>
<td>Right nucleus caudate</td>
</tr>
<tr>
<td>Thalamus</td>
<td>.05</td>
<td>.049</td>
</tr>
</tbody>
</table>

Note.—Threshold of P < .05 corrected for false discovery rate and cluster size k > 10. Coordinates of peak voxels (x, y, z) are given in Montreal Neurologic Institute space. AFB = anterior basal forebrain, APOE = apolipoprotein, PBF = posterior basal forebrain, RSFC = resting-state functional connectivity, SUVR = standard update value ratio.

1 Indicates correction for familywise error.
2 Indicates correction for false discovery rate.
3 Indicates Brodmann area 19.
4 Indicates Brodmann area 10.
clinical therapy trials. Further longitudinal analyses, including volumetric measures of the basal forebrain and the comparison between individuals with AD dementia and cognitively intact older individuals, will provide insights on the temporal dynamics characterizing early AD pathophysiology.

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