Altered Nucleus Basalis Connectivity Predicts Treatment Response in Mild Cognitive Impairment

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Conflicts of interest are listed at the end of this article.

See also the editorial by Chiang in this issue.

Purpose: To determine whether functional connectivity (FC) mapping of nucleus basalis of Meynert (NBM) cholinergic network (hereafter, NBM FC) could provide a biomarker of central cholinergic deficits with predictive potential for response to cholinesterase inhibitor (ChEI) treatment.

Materials and Methods: The Alzheimer's Disease Neuroimaging Initiative (ADNI) was approved by the institutional review boards of all participating sites. All participants and their representatives gave written informed consent prior to data collection. NBM FC was examined in 33 healthy control participants, 102 patients with mild cognitive impairment (MCI), and 33 patients with AD by using resting-state functional MRI data from the ADNI database. NBM FC was compared between groups before and after 6 months of ChEI treatment in MCI. Associations between baseline NBM FC and baseline cognitive performance as well as cognitive outcomes after treatment were investigated.

Results: Compared with the healthy control group, NBM FC was decreased in patients with untreated MCI and increased in patients with AD treated with ChEI (corrected $P < .05$). Global cognition (Alzheimer's Disease Assessment Scale-Cognitive subscale score) was associated with NBM FC ($r = -0.349$; $P < .001$). NBM FC was higher 6 months after ChEI compared with before ChEI in treated MCI (corrected $P < .05$), but did not change at 6 months in patients with untreated MCI (corrected $P < .05$). Baseline NBM FC in MCI strongly predicted cognitive outcomes 6 months after ChEI ($R^2 = 0.458$; $P = .001$).

Conclusion: Functional dissociation of the nucleus basalis of Meynert from a cortical network may explain the cognitive deficits in dementia and allow for the selection of individuals who are more likely to respond to cholinesterase inhibitors at early disease stages.

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Online supplemental material is available for this article.

Cholinergic deficits are a hallmark of Alzheimer disease (AD). The link between cholinergic deficits and cognitive impairment is well established (1) and cholinesterase inhibitors (ChEIs) are the mainstay symptomatic pharmacotherapy, with moderate effectiveness in established AD (2). However, only 18%–48% patients undergoing treatment show clinically significant improvement, and about 7% of patients develop adverse effects severe enough to stop the treatment (3). Additionally, no clinical effect has been established in patients with mild cognitive impairment (MCI) (4).

Individual diagnosis of central cholinergic deficits could be beneficial to address the lack of treatment response in MCI and the variable treatment response in established AD. Conceivably, increasing the cholinergic tone in patients without a cholinergic deficit is unlikely to lead to cognitive improvement, and conversely may make patients particularly prone to adverse effects (5). Several markers of cholinergic deficits were previously proposed (6–9). However, none of these markers gained widespread use because

they are too expensive or invasive, lack robustness, or are not widely available. It would be advantageous to identify a simple and fast MRI test to reliably map cholinergic deficits as part of the routine MRI work-up (10). Additionally, it has been suggested that high-dose ChEI may be more efficacious for AD (11), increasing the need for a robust cholinergic biomarker to assist selection of candidates for potentially more aggressive ChEI treatment.

The nucleus basalis of Meynert (NBM) provides the primary source of cholinergic inputs to the cerebral cortex and its widespread projections form the NBM cholinergic network (12). Resting-state functional connectivity (FC) mapping of NBM cholinergic network (hereafter, NBM FC) has been demonstrated in healthy people (13), suggesting that it is possible to use NBM FC to delineate cholinergic deficits in AD and MCI. NBM FC has a number of key advantages for potential clinical use because of its noninvasive nature and expected high specificity to map cholinergic pathways, given that over 90% of NBM neurons are cholinergic (12). Taken together, NBM FC may

Abbreviations

AD = Alzheimer disease, ADAS-Cog = Alzheimer's Disease Assessment Scale-Cognitive subscale, ADNI = Alzheimer's Disease Neuroimaging Initiative, ChEI = cholinesterase inhibitor, CSF = cerebrospinal fluid, FC = functional connectivity, MCI = mild cognitive impairment, NBM = nucleus basalis of Meynert

Summary

Nucleus basalis of Meynert functional connectivity metrics obtained from resting-state functional MRI have promising prognostic and predictive biomarker properties for central cholinergic dysfunction.

Implications for Patient Care

- The findings suggest that nucleus basalis of Meynert functional connectivity metrics obtained from resting-state functional MRI have promising biomarker properties for measuring central cholinergic dysfunction and predicting treatment response to cholinesterase inhibition.

- If confirmed in future studies, then nucleus basalis of Meynert functional connectivity may assist treatment stratification and facilitate ongoing drug development for optimized restoration of cholinergic functions in people living with or at risk for dementia.

probe the central cholinergic dysfunction and underlying neuronal degeneration so that we may be able to identify those people most likely to benefit from treatment with ChEI.

We hypothesized that reductions of NBM FC are a feature of MCI and AD; underlie cognitive impairment across the cognitively normal, MCI, and dementia spectrum not treated with ChEI; can be partially reversed with ChEI; and predict treatment response to ChEI in MCI and AD. We then undertook a series of posthoc tests to address the role of concurrent use of anticholinergic drugs, presence of cerebrospinal fluid (CSF) biomarkers of amyloid pathology, apolipoprotein E *(APOE)* ε4 genotype, and disease progression.

Materials and Methods

Participants

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (available at *https://adni.loni.usc.edu*). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, PET, other biologic markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Written informed consent was obtained from all individuals.

Data were selected based on the availability of resting-state functional MRI data and Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog) at both screening and 6 months after initiation of ChEI. As described in the ADNI visit schedule, the maximum interval between baseline and screening visits was prescribed as 28 days. A total of 175 participants (34 healthy control participants, 59 patients with early MCI, 48 patients with late MCI, and 34 patients with AD) were included in this study (details of diagnostic criteria in Appendix E1 [online]). Demographic and clinical information was used to screen for existing pathologies, dementia diagnosis, and medication history

(14). All participants completed a series of cognitive assessments and we chose ADAS-Cog to reflect cognitive function. ADAS-Cog comprises a comprehensive assessment of cognitive functions including memory and executive function that are linked with cholinergic function (15) and is commonly used as a primary outcome neuropsychological measure for AD clinical trials.

Information of dosing schedules for ChEI, memantine, and medications with potential anticholinergic effect was manually extracted (details in Appendix E1 [online]). At the screening visit, five patients with early MCI (8.5%), three patients with late MCI (6.3%), and all patients with AD were already taking ChEI. Five healthy control participants (14.7%), nine patients with early MCI (15.2%), four patients with late MCI (8.3%), and five patients with AD (14.7%) were taking anticholinergic drugs. One patient with late MCI (2.1%) and two patients with AD (5.9%) were taking memantine at the screening visit. Eight patients with early MCI (13.6%) and 13 patients with late MCI (30.2%) were prescribed ChEI after the screening visit.

To explore interactions between NBM FC and genotype or amyloid pathology as potential confounding factors (16,17), we manually extracted the status of *APOE* ε4 genotype and presence of CSF amyloid-β 42 from *http://www.ADNI.org*. Participants were designated as *APOE* ε4 carriers if they had one or two copies of allele 4, and as noncarriers if they had no allele 4 in their genotype. CSF amyloid-β 42, rather than amyloid PET, was chosen to reflect abnormal amyloid accumulation, given that it has been recently shown that CSF amyloid-β 42 becomes abnormal in the earliest stages of AD before amyloid PET starts (18). The cutoffs for abnormal CSF amyloid-β 42 used in this study were as follows: normal CSF amyloid-β 42 (participants with negative CSF Aβ 42 status) greater than 201.6 ng/L, and abnormal amyloid-β 42 (participants with positive CSF Aβ 42 status) less than 182.4 ng/L (18). Thirteen participants did not have *APOE* data.

Resting-State Functional MRI Acquisition and Quality Assessment Protocol

All participants were imaged with a 3.0-T Philips MR unit at multiple sites with the same ADNI 3.0-T imaging protocol. Participants were instructed to keep their eyes open during imaging. The data had been acquired by using a standard echo-planar imaging functional MRI protocol (repetition time msec/echo time msec, 3000/30; flip angle, 80°; number of sections, 48; section thickness, 3.3 mm; 140 volumes; spatial resolution, $3 \times 3 \times 3$ mm³; matrix, 64×64).

To account for possible artifacts resulting from micromotion, a rigorous protocol of data quality assessment was applied, modified from a multicenter functional MRI protocol (19), resulting in a final data set of 168 participants (33 healthy control participants, 59 patients with early MCI, 43 patients with late MCI, and 33 patients with AD). Good-quality functional MRI images 6 months after ChEI initiation were available in 12 patients with late MCI and in six patients with early MCI. Table 1 summarizes demographic, clinical, *APOE* ε4 status, Aβ-42 status, and cognitive information of the included participants.

Table 1: Demographics and Clinical and Cognitive Information

A: Baseline

Parameter	HC (n = 33)	Early MCI (n = 59)	Late MCI (n = 43)	AD (n = 33)	P Value
Age (y)	77.1 ± 7.3	72.4 ± 6.9	73.8 ± 8.6	72.8 ± 7.2	.03
Female sex*	18 (54.5)	37 (62.7)	16 (37.2)	16 (48.5)	.08
ADAS-Cog	6.4 ± 3.3	8.2 ± 3.5	11.4 ± 5.4	23.3 ± 7.6	<.001†
No. of participants undergoing ChEI treatment at baseline*	0 (0)	5 (8.5)	3 (7.0)	33 (100)	…
No. of participants taking anticholinergic medication at baseline*	5 (15.1)	9 (15.2)	4 (9.3)	5 (15.2)	.82
Apolipoprotein ε4 carriers*	9 (29.0)	27 (50.9)	16 (40.0)	24 (77.4)	.001†
Positive CSF amyloid-β 42*	6 (24.0)	18 (36.7)	12 (35.3)	15 (53.6)	.16

B: 6 Months after ChEI Treatment

Parameter	HC	EMCI (n = 8)‡	LMCI (n = 13)‡	AD	P Value
Age (y)	…	73.4 ± 5.3	72.9 ± 8.6	…	.90
Female sex*	…	4 (50)	5 (38.5)	…	.60
ADAS-Cog	…	10.3 ± 6.4	13.7 ± 5.4	…	.21
ADAS-Cog changes from baseline	…	0.9 ± 2.7	2.4 ± 4.9	…	.44

Note.—Unless otherwise specified, data are means ± standard deviations. AD = Alzheimer disease, ADAS-Cog = Alzheimer's Disease Assessment Scale-Cognitive subscale, ChEI = cholinesterase inhibitor, CSF = cerebrospinal fluid, HC = healthy control participants, MCI = mild cognitive impairment.

* Data are numbers, with percentages in parentheses.

† Significant level at $P < .05$.

‡ In total, eight patients with early MCI and 13 patients with late MCI had ADAS-Cog score 6 months after undergoing ChEI treatment. Among these patients, six patients with early MCI and 12 patients with late MCI patients had good-quality resting-state functional MR images at 6 months after undergoing ChEI treatment.

Seed-based FC Analysis

Seed-based FC analysis, which identifies the pattern of brain areas displaying correlated time series with respect to a predefined region, was carried out with software (FMRI Expert Analysis Tool [FEAT], version 6.0) in FSL (version 5.0.10; *https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT*) (20) by manually creating a mask (16 voxels, 581 mm³) in the NBM on functional MRI data sets for each participant. The approach of seed-based analysis and the method of testing the robustness of manual seed region selection are provided in Appendix E2 (online). Standard statistical Z transformation was applied to the correlation coefficient of the time series between NBM and the rest of the brain in FSL (15); thus, a normal distributed score was computed to index NBM FC.

Statistical Analysis

One-way analysis of variance and χ^2 test in SPSS (version 21; SPSS, Chicago, Ill) were used to compare demographics and cognitive performance between healthy control participants, patients with early MCI, patients with late MCI, and patients with AD. Statistical significance was set at $P < .05$.

All main statistical tests and secondary posthoc tests were voxel based and corrected for multiple tests as implemented in FSL. Inference on first-level analysis was based on Z statistical images of each individual thresholded at Z greater than 2.3 and a corrected cluster significance threshold of $P < .05$. Z statistical images of voxel-based high-level analyses were estimated based on Z greater than 1.96, and a familywise error–corrected cluster significance threshold of $P < .05$. Age and mean relative displacement, which is the net amount of motion between consecutive functional volumes (21), were treated as further between-participant covariates of no interest in all higher-level analyses and was carried out by using FMRIB's Local Analysis of Mixed Effects (FLAME) (20) in FSL. Additional outlier deweighting in FEAT was used to automatically detect and account for outlier data points for each voxel.

Posthoc Shapiro-Wilk tests in SPSS were undertaken on Z scores extracted from results of the main tests for the four hypotheses (described next) to test for normality.

To test hypothesis 1—that NBM FC is lower in patients with MCI and patients with AD compared with healthy control participants with normal cognitive function—we undertook a voxel-based F test to compare the NBM FC maps between groups (healthy control participants, patients with MCI, and patients with AD) by using multiple test correction within FEAT in FSL. Posthoc pairwise t tests (healthy control participants vs patients with MCI and healthy control participants vs patients with AD) were then used to determine the direction of the effect if the F test was significant. Significant level of the t tests was at corrected $P < .025$. Posthoc tests were performed in cohorts not taking anticholinergic drugs, and to explore the effects of *APOE* and Aβ status.

To investigate hypothesis 2—that NBM FC correlates with cognitive performance but in the absence of an a priori hypothesis of the specific regions affected within the NBM network—we undertook voxel-based correlation analysis of NBM FC maps with ADAS-Cog in the study population not taking ChEI. Posthoc tests included correlation analysis in the subgroup of participants not taking anticholinergic drugs to prevent possible medication-induced confounding effects.
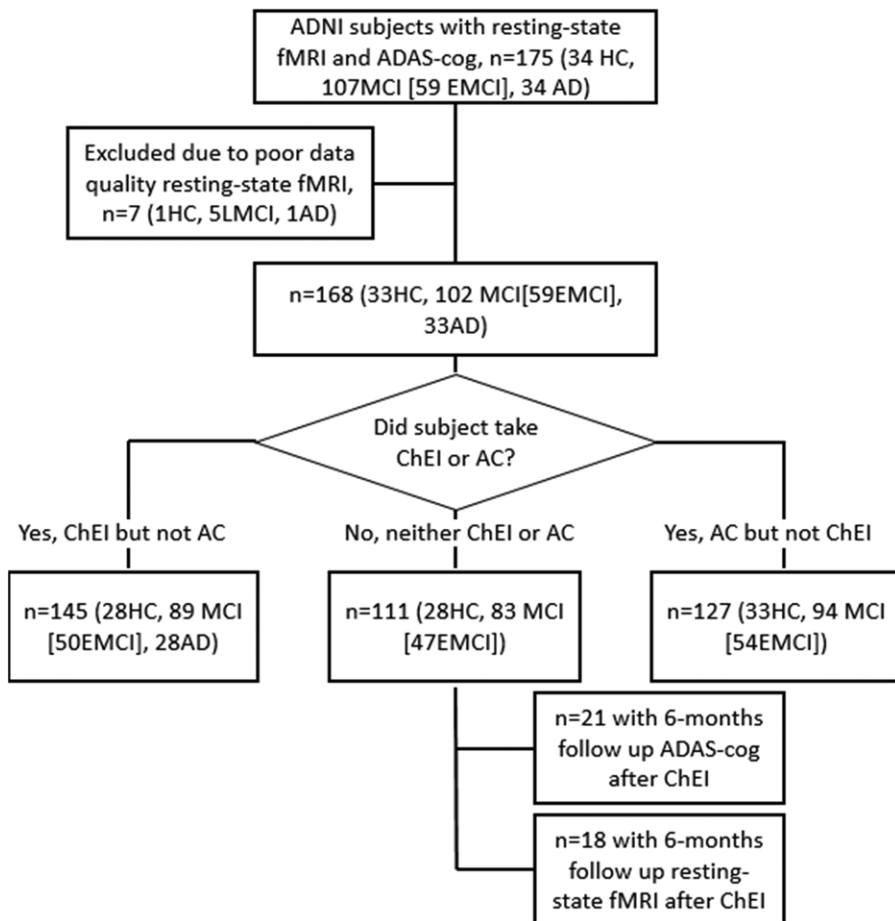
Figure 1: Flowchart shows participant selection. AC = anticholinergic treatment, AD = Alzheimer disease, ADAS-Cog = Alzheimer's Disease Assessment Scale-Cognitive subscale, ADNI = Alzheimer's Disease Neuroimaging Initiative, ChEI = cholinesterase inhibitor, EMCI = early mild cognitive impairment, HC = healthy control participants, LMCI = late mild cognitive impairment, MCI = mild cognitive impairment.

Results

A total of 168 participants (mean age ± standard deviation, 73.8 years ± 7.7; 87 women [51.8%] and 81 men [49.2]) were included. Figure 1 shows the flowchart of the participant selection. Healthy control participants were significantly older than were patients with MCI and patients with AD (*P* = .03) (Table 1). The manual method to define NBM FC maps was considered robust (Appendix E2 [online]).

NBM FC Pattern in Healthy Aging and Abnormalities in MCI and AD

In healthy control participants, the NBM was functionally connected with the anterior cingulate cortex, bilateral hippocampi, caudate, medial frontal gyri, insular cortex, superior temporal gyri, and occipital lobes (Fig 2, *A*). Patients with MCI (Fig 2, *B*) and patients with AD (Fig 2, *C*) showed qualitatively similar NBM FC patterns but additional NBM FC was seen in AD with the bilateral occipital lobes, bilateral medial temporal gyri, and posterior cingulate cortex. *F* test showed a significant between-group difference of NBM FC between healthy control participants, patients with MCI, and patients with AD (Fig E1 [online]). By using further pairwise *t* tests, we found that patients with MCI at baseline had decreased NBM FC in the anterior cingulate cortex, bilateral hippocampus, bilateral caudate, bilateral insular cortex, bilateral superior and medial temporal gyri, and bilateral occipital lobes, compared with healthy control participants (Fig 2, *D*; Table 2). Also, group comparison confirmed significantly increased NBM FC with the right hippocampus, left middle and inferior frontal gyri, and bilateral superior temporal gyrus in patients with AD compared with healthy control participants (Fig 2, *F*; Table 2) when age and mean relative displacement were controlled.

Effects of Age, Anticholinergic Drugs, Disease Progression, Genotype, and Amyloid Pathology on NBM FC

We ran several posthoc analyses to explore possible moderator factors. In healthy control participants and patients with MCI without ChEI or anticholinergic drugs, there was no significant correlation between age and *Z* score of clusters showing increased NBM FC in AD compared with healthy control participants (*P* = .74).

To study hypothesis 3—that ChEI would increase NBM FC without an a priori hypothesis of where these effects would be—we again used voxel-based change analysis of NBM FC maps comparing before ChEI treatment and 6 months after ChEI initiation in 18 patients with MCI (voxel-based paired *t* test FEAT in FSL corrected for multiple comparisons). In view of the naturalistic study design, we sought to control for possible disease progression effects that might confound our change analysis. Hence, we assessed possible serial disease progression effects of NBM FC maps over an interval of 6 months in a matched (for age, sex, and disease severity) group of 18 patients with MCI (six with early MCI and 12 with late MCI) who did not undergo ChEI treatment in the interval by using voxel-based paired *t* test in FEAT as posthoc test.

Last, we studied the hypothesis that low NBM FC would predict the cognitive response to ChEI. We used voxel-wise correlation analysis of baseline NBM FC maps with changes of ADAS-Cog 6 months after initiating ChEI. Again, because of the naturalistic study design, we assessed whether NBM FC at baseline might also predict cognitive change in an untreated cohort (posthoc test).
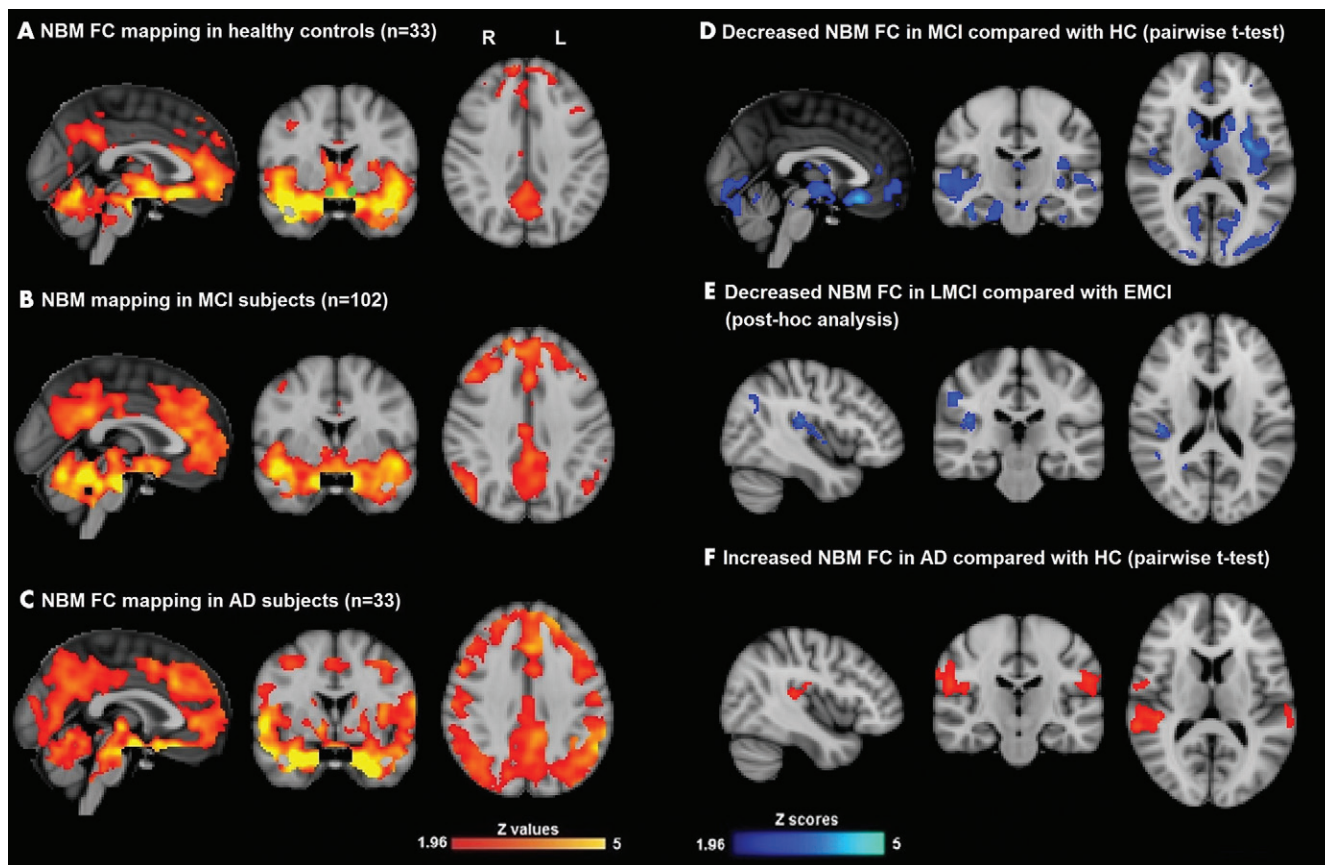
Figure 2: Images show functional connectivity (FC) mapping of nucleus basalis of Meynert (NBM) cholinergic network (hereafter, NBM FC) (whole cohort) in, *A*, healthy aging (healthy control participants [HC], *n* = 33; corrected *P* < .05) and also shows seed mask (green), *B*, in patients with mild cognitive impairment (MCI) (*n* = 102; corrected *P* < .05), and, *C*, in patients with Alzheimer disease (AD) (*n* = 33; corrected *P* < .05). Significance was set at *P* < .05. *D*, Difference NBM FC map between MCI and HC (blue–light blue indicates reductions in MCI; pairwise *t* test, corrected *P* < .025). *E*, Difference NBM FC between late MCI (LMCI) and early MCI (EMCI) (blue–light blue indicates reductions in LMCI; posthoc pairwise *t* test, *P* < .05). *F*, Difference NBM FC between AD and HC (red-yellow indicates increased FC in AD; pairwise *t* test, corrected *P* < .025). All results were masked by gray matter masks obtained from Montreal Neurological Institute 152 standard-space T1-weighted average structural template image. All *t* test analyses were controlled for age and mean relative displacement.

We repeated the between-group comparison after exclusion of participants who were taking anticholinergic drugs, which revealed similar results as in the whole cohort (Fig E2 [online]).

To further explore the effects of disease progression without confounding effects from ChEI or anticholinergic drugs, we also compared patients with untreated early MCI and untreated late MCI. The results revealed a decreased NBM FC with the right parietal lobe, right postcentral gyrus, right lateral occipital cortex, right insula, and precuneus in late MCI compared with early MCI (age and mean relative displacement–controlled, familywise error–corrected *P* < .05) (Fig 2, *E*; Table 2).

To explore the effect of genotype and amyloid pathology on NBM FC without confounding effects from ChEI or anticholinergic drugs, binary logistic regression analyses between the status of *APOE ε4*, amyloid-β 42 status, and *Z* scores of clusters showing difference of NBM FC (healthy control participants vs patients with AD) were conducted. No significant correlation was found between the *Z* score of clusters showing increased NBM FC in AD and the status of *APOE ε4* (*P* = .26) or amyloid-β 42 status (*P* = .27).

NBM FC and Cognitive Impairment

A significant but modest negative correlation (*r* = −0.349; *P* < .001) (Fig 3, Table 2) was found between baseline ADAS-Cog and NBM FC with the posterior cingulate cortex, anterior cingulate cortex, bilateral caudate, bilateral putamen, bilateral thalamus, left insular cortex, left hippocampus, left middle frontal gyrus, and right middle temporal gyrus in the group of participants not taking ChEI at baseline (*n* = 127 [33 healthy control participants and 54 patients with MCI]). This correlation was largely unchanged when 16 participants who were taking anticholinergic drugs were excluded (*n* = 111; *r* = −0.351; *P* < .001) (Fig E3 [online]).

To control for a possible general cognitive effect exerted by loss of global FC, and specifically impaired FC in the posterior cingulate cortex, we investigated whether posterior cingulate cortex or primary visual cortex FC was associated with ADAS-Cog. No significant correlation between cognitive performance and posterior cingulate cortex or primary visual cortex FC was identified, supporting a weak but specific association between NBM FC and cognition.

Table 2: Brain Regions Where NBM FC Are Associated with Cognition and Cholinesterase Inhibitory Effects

Parameter	Coordinates (mm)			Z Value[*]
	X	Y	Z	
Decreased NBM FC in patients with MCI compared with HC				
Left caudate	−16	22	2	2.49
Right caudate	14	24	2	2.62
Left insular cortex	−38	−12	4	2.56
Right insular cortex	40	4	6	3.38
Right superior temporal gyrus	60	−6	4	2.42
Left middle temporal gyrus	−56	−38	−2	2.90
Right middle temporal gyrus	54	−34	−2	3.35
Increased NBM FC in patients with AD compared with HC				
Right hippocampus	32	−20	−10	2.42
Left middle frontal gyrus	−42	56	0	2.89
Left inferior frontal gyrus	−50	24	4	3.03
Left middle occipital gyrus	−34	−90	4	2.45
Right superior temporal gyrus	54	−34	8	2.49
Right inferior parietal lobule	64	−40	24	3.12
Left inferior parietal lobule	−48	−38	24	2.89
Decreased NBM FC in late MCI compared with early MCI				
Right parietal lobe	42	−24	18	3.38
Right postcentral gyrus	54	−24	36	2.95
Right lateral occipital cortex	46	−68	38	2.11
Right insula	44	−28	14	3.06
Precuneus	22	−60	22	2.41
Negative correlation between baseline NBM FC and ADAS-Cog				
Posterior cingulate cortex	−6	−46	30	3.47
Anterior cingulate cortex	6	18	−14	3.62
Left caudate	−12	10	10	2.37
Right caudate	16	10	10	2.98
Left putamen	−26	−4	−4	2.89
Right putamen	20	10	−2	2.66
Left thalamus	−6	−14	2	2.60
Right thalamus	6	−12	2	2.76
Left insular cortex	−38	20	2	2.42
Left hippocampus	−28	−22	−14	2.36
Left middle frontal gyrus	−46	14	32	3.70
Right middle temporal gyrus	50	−66	16	3.27
Increased NBM FC at 6 mo after ChEI treatment compared with NBM FC at baseline				
Right putamen	20	10	10	2.89
Left superior frontal gyrus	−16	44	42	2.94
Right middle frontal gyrus	30	4	44	2.32
Anterior cingulate cortex	−6	8	46	2.38
Positive correlation between baseline NBM FC and ADAS-Cog changes				
Left lateral occipital cortex	−28	−74	44	2.63
Left medial frontal gyrus	−2	34	32	2.54
Negative correlation between baseline NBM FC and ADAS-Cog changes				
Anterior cingulate cortex	−4	26	16	3.37

Note.—AD = Alzheimer disease, ADAS-Cog = Alzheimer's Disease Assessment Scale-Cognitive subscale, ChEI = cholinesterase inhibitor, HC = healthy control participants, FC = functional connectivity, MCI = mild cognitive impairment, NBM = nucleus basalis of Meynert.

[*] All statistical images were thresholded at a familywise error–corrected threshold of $P < .05$.
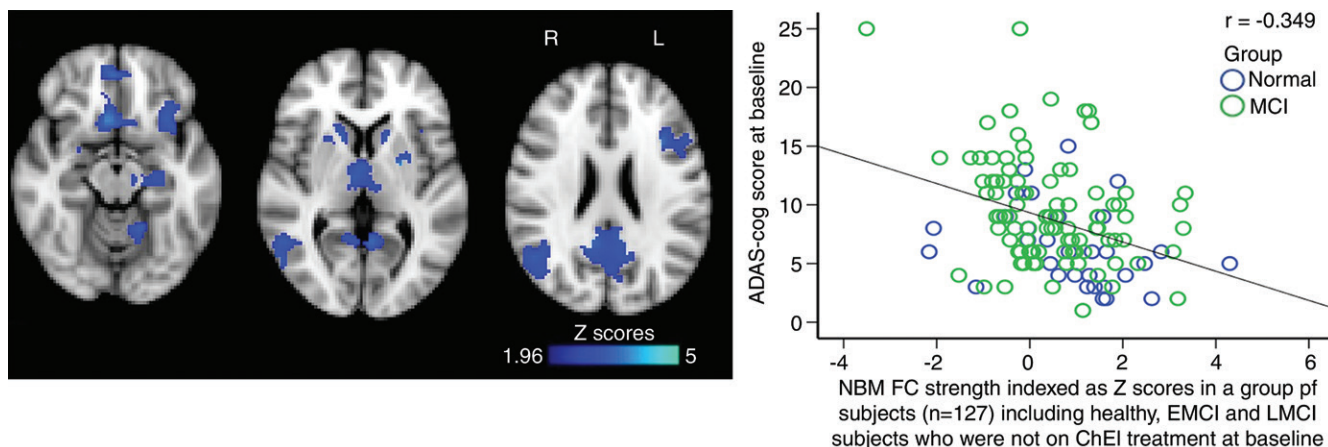
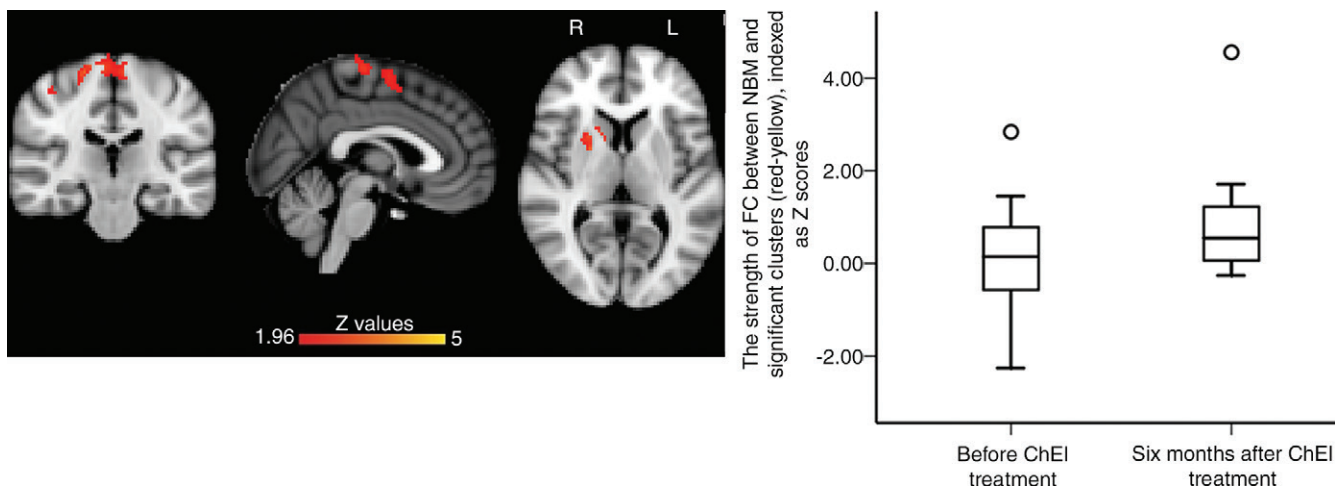**Figure 3:** Images show functional connectivity (FC) mapping of nucleus basalis of Meynert (NBM) cholinergic network (hereafter, NBM FC) and cognition. Negative correlation map between baseline Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog) and NBM FC located in significant clusters (blue-light blue) in group of participants including healthy control participants (HC), patients with early mild cognitive impairment (EMCI), and patients with late mild cognitive impairment (LMCI) who were not undergoing cholinesterase inhibitor (ChEI) treatment (*n* = 127). All results were masked by gray matter masks obtained from Montreal Neurological Institute 152 standard-space T1-weighted average structural template image. Scatterplot shows negative correlation between baseline ADAS-Cog and FC between NBM and significant clusters. All analyses were controlled for age and mean relative displacement. Significance was set at corrected *P* < .05. MCI = mild cognitive impairment.

Figure 4: Images show serial changes in functional connectivity (FC) mapping of nucleus basalis of Meynert (NBM) cholinergic network (hereafter, NBM FC) between baseline and 6 months after undergoing cholinesterase inhibitor (ChEI) treatment in 18 patients with mild cognitive impairment (MCI). NBM FC significantly increased from baseline to 6 months after ChEI in right anterior striatum and prefrontal cortex (red-yellow). Box plot shows distribution of strength of FC between NBM and significant clusters (red-yellow) before and 6 months after ChEI, indexed as Z score. All results were masked by gray matter masks obtained from Montreal Neurological Institute 152 standard-space T1-weighted average structural template image. Significance level was at familywise error–corrected *P* < .05 (corrected for multiple comparisons) and corrected for age and mean displacement.

NBM FC Changes after Initiation of ChEI

A total of 18 patients with MCI (mean age, 72.2 years ± 7.3; seven women [38.9%] and 11 men [61%]; six with early MCI and twelve with late MCI) underwent functional MRI before and 6 months after starting ChEI. Compared with pretreatment, the 6-month follow-up images showed significantly increased FC between NBM and the right putamen, right caudate, left superior frontal gyrus, right middle frontal gyrus, and anterior cingulate cortex (corrected *P* = .001) (Fig 4, Table 2). Because of the naturalistic ADNI cohort design, the observed serial changes may be related to disease progression rather than to ChEI treatment. To control for such potentially confounding effects, we compared the base-line NBM FC in 18 patients with untreated MCI (mean age, 72.8 years ± 5.1; seven women [38.9%] and 11 men [61%]; six with early MCI and 12 with late MCI) with their 6-month follow-up NBM FC, which did not reveal significant changes.

NBM FC at Baseline to Predict Cognitive Outcome after ChEI

We then assessed the potential of baseline NBM FC to predict the cognitive outcome after 6 months of ChEI. NBM FC in both the lateral cholinergic network (left lateral occipital cortex) and the medial cholinergic network (left medial frontal gyrus) was strongly and positively correlated with changes in
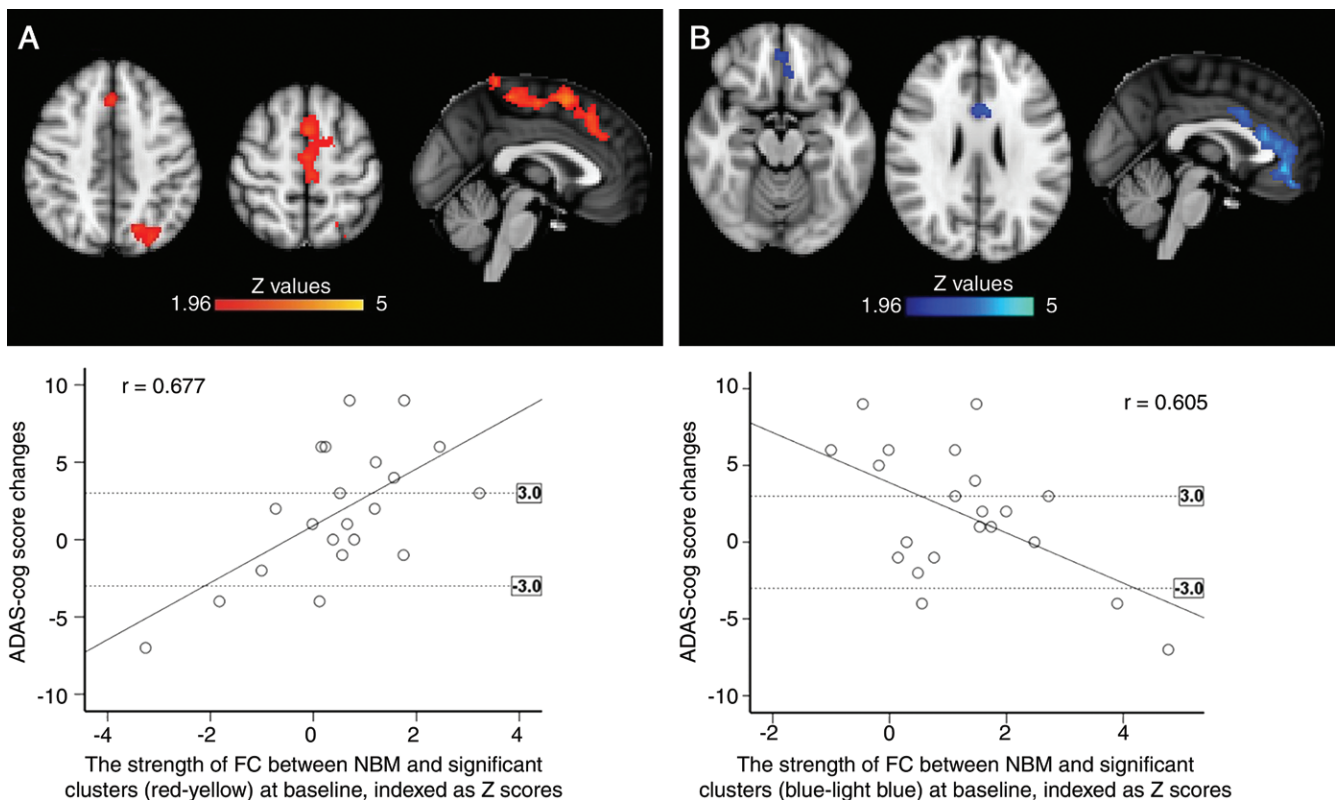
Figure 5: Images show correlation between baseline functional connectivity (FC) mapping of nucleus basalis of Meynert (NBM) cholinergic network (hereafter, NBM FC) and changes of cognitive performance assessed by Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog) over 6 months of treatment with cholinesterase inhibitor. *A,* Positive correlation between ADAS-Cog change (posttreatment minus pretreatment; positive difference represents worsening cognition) and baseline NBM FC (red) in patients with mild cognitive impairment (MCI). Scatterplot illustrates positive correlation for significant clusters. *B,* Negative correlation between changes of ADAS-Cog and NBM FC (blue) at baseline in patients with MCI. Scatterplot shows negative correlation in significant clusters. Dotted lines in scatterplots indicate three-point change (improve/decline) on ADAS-Cog, which is considered as clinically relevant. All analyses were controlled for age and mean relative displacement. All results were masked by gray matter masks obtained from Montreal Neurological Institute 152 standard-space T1-weighted average structural template image. Significance level was at corrected $P < .05$.

ADAS-Cog scores over 6 months of ChEI (R^2, 0.458; $P = .001$) (Fig 5, *A*; Table 2) in line with the hypothesis that lower FC may predict increased benefits from ChEI. Unexpectedly, we also found a similarly strong anticorrelation between NBM FC in the medial cholinergic network (anterior cingulate cortex) and ADAS-Cog changes 6 months after starting ChEI ($R^2 = 0.366$; $P = .006$) (Fig 5, *B*; Table 2). To further control for possible confounding predictor effects associated with disease progression but unrelated to ChEI, we studied the association between NBM FC at baseline and ADAS-Cog changes 6 months after baseline in 21 patients with MCI who did not take ChEI in the interval, which did not reveal significant associations. Additionally, to investigate whether *APOE ε*4 or amyloid-β 42 status had an effect on the predictive power of NBM FC on treatment response, the association between the *Z* score of clustering showing positive correlation with ADAS-Cog changes (Fig 5, *A*) and *APOE ε*4 and amyloid-β 42 status was explored, but no significant results were revealed (*APOE ε*4, $P = .94$; amyloid-β 42 status, $P = .25$).

Discussion

Our study investigated the potential of basal forebrain FC as a biomarker of cholinergic dysfunction in MCI and early AD

by using the publicly available ADNI data sets. Seeding FC maps in the NBM allowed us to identify established cortical and subcortical networks with known cholinergic innervation by the NBM. Four main findings demonstrated diagnostic, prognostic, and predictive biomarker properties. First, NBM FC was decreased in patients with untreated MCI, but was increased in patients with early AD who were taking ChEI compared with the control group. Second, global cognitive performance in healthy participants and patients with untreated MCI was correlated with NBM FC predominantly in the default mode network. Third, the NBM FC deficit was partially responsive to indirect cholinomimetic treatment in patients with MCI, showing significant increases 6 months after ChEI initiation, but not in an untreated parallel group. Fourth, baseline NBM FC strongly predicted cognitive outcomes in patients with MCI 6 months after starting ChEI, but not in an untreated parallel group.

The pattern of cortical and subcortical NBM FC, including hubs of the default mode and the salience networks and the bilateral caudate nuclei, is largely consistent with the anatomic identification of the medial and lateral cholinergic pathways (22) and a previous report in healthy younger people (13). Specifically, the medial cholinergic pathway matches the FC between

NBM and medial prefrontal cortices, anterior cingulate cortex, and subcortical regions. The capsular division of the lateral cholinergic pathway is consistent with the FC between NBM and bilateral hippocampi, as is the perisylvian division with the FC between NBM and bilateral insula. Different from Li et al (13), we did not find areas that were anticorrelated with NBM, which can be explained by the fact that we avoided to regress out global signal changes, thereby minimizing spurious negative cross-correlations between brain regions (23).

Compared with healthy control participants, patients with MCI showed decreased NBM FC within both the medial and lateral cholinergic system. This finding is well in line with the early neurodegeneration of the NBM and several PET studies showing a widespread reduction of acetylcholine activities in MCI (25,26). The results are also in broad accordance with two recent NBM FC studies in MCI (27) and MCI in Parkinson disease (28) showing reduced NBM FC associated with cognitive deficits. However, we found a more extended pattern of NBM FC deficit in MCI compared with Li et al (27), which may be related to our postprocessing method and the medication effects. Further decrease of NBM FC in cases of late MCI versus early MCI supports the progressive nature of cholinergic dysfunction.

In a large group of healthy control participants and patients with MCI who were not taking ChEI, we showed a significant but modest correlation between cognitive performance and NBM FC within the medial and lateral cholinergic system. Findings were not affected by excluding participants who were taking anticholinergic drugs. The regions within the NBM FC network showing associations with cognitive performance partly overlap with the default mode network such as the posterior cingulate cortex and the frontal, temporal, and parietal cortices. The pattern identified and the direction of association provide further support for the cholinergic hypothesis of dementia (2) in line with previous studies showing reduced acetylcholine activities in multiple brain regions by using PET imaging techniques (29,30).

Interestingly, we did not find a further decrease of NBM FC in AD, but we observed an increased NBM FC in patients with AD compared with healthy control participants. This finding—at first surprising—may be related to a treatment effect, as the ADNI repository only holds data from patients with AD already receiving treatment with ChEI. In line with pharmacologic imaging studies showing increased activation after cholinergic challenge (31,32), ChEI is expected to increase the cholinergic tone and the NBM FC. However, this interpretation remains speculative without comparison with NBM FC maps of patients with untreated AD.

To further characterize a possible treatment effect, we studied serial NBM FC changes between baseline and 6-month posttreatment data in a subgroup of patients with MCI. This showed significantly increased NBM FC within the right putamen, right caudate, left superior frontal gyrus, and right middle frontal gyrus compared with pretreatment, suggesting at least partial restoration of cholinergic modulation of neural activities. This result concords well with the expected treatment effects of ChEI to restore synaptic activities within the cholinergic pathways and enhanced brain activity as shown in pharmacologic functional MRI studies (33). Taken together, our results of increased FC between NBM and left superior frontal gyrus and right middle frontal gyrus at rest after ChEI treatment may explain increased task responsiveness shown previously (33). However, in our study, we cannot directly allocate the observed NBM FC change to a pharmacologic effect of ChEI because of the naturalistic design. Nevertheless, in a matched cohort of patients with untreated MCI, we did not find a serial effect of NBM FC over 6 months supporting our interpretation that the observed NBM FC changes after ChEI reflect cholinomimetic effects rather than the disease process.

It would be clinically useful to identify patients who will benefit most from ChEI to improve the treatment effectiveness in established AD, and to enable stratification of patients with MCI for future treatment trials. Although clinical effectiveness of ChEI has not been proven in patients with MCI, it is conceivable that only those patients with MCI with reduced NBM FC may benefit more from ChEI. We observed a pattern of reduced NBM FC at baseline that was associated with better cognitive outcomes at 6 months after taking ChEI, mainly located in the left lateral occipital cortex (lateral cholinergic pathway) and the left middle frontal gyrus (medial cholinergic pathway), supporting the notion that imaging metrics have the potential to predict the cognitive response to ChEI in MCI and AD (34,35). By contrast, baseline NBM FC was not associated with cognitive outcome in those patients with MCI who did not take ChEI in the 6-month interval, making a confounding predictive effect of cognitive decline from disease progression less likely. The predictive potential is large as the overall effect was strong, with several clusters explaining up to 48% of the variance in cognitive outcome. Interestingly, we also observed a pattern of negative correlation between baseline NBM FC, mainly located in the anterior cingulate cortex, and cognitive change 6 months after taking ChEI. The direction of this association may be explained by the known high degree of regional heterogeneity of cholinergic receptors and possible disease stage effects (36).

Clinical translational potential of NBM FC is particularly high because task-free resting-state functional MRI can be easily incorporated into the current routine diagnostic work-up of patients with memory complaints. If the prediction of treatment response can be confirmed in prospective studies, then NBM FC could broaden the clinical remit of MRI for work-up of memory impairment beyond the current diagnostic support to assist treatment stratification.

Our study was limited by the design of the ADNI study. All the participants with AD had already started to take ChEI when they were recruited. Thus, we could not investigate the effects of ChEI on NBM FC in patients with AD. Because of the observational nature of the ADNI study, our findings on ChEI effects on NBM FC and the prediction of cognitive outcomes by NBM FC could not be placebo controlled. However, serial data were available in matched patients with untreated MCI, allowing us to make a serial effect from disease progression unlikely. Because of the small cohort of

patients undergoing ChEI, for the comparison of NBM FC before and after 6 months of ChEI treatment, we chose a less rigorous multiple test correction at the voxel level alone. Larger, prospective studies are needed to confirm the reported treatment changes of NBM FC and the predictive value of NBM FC at baseline. Another potential limitation was the robustness of the seed-based analysis because of the small size and ill-defined borders of the NBM at functional MRI, making manual delineation challenging. We addressed this by showing the robustness of the manual drawing method against small shifts of the seeds by using an in-house Matlab (version R2016a; Mathworks, Natick, Mass) code. This step could be improved by semiautomatic seed selection.

In summary, our study shows that NBM FC metrics obtained from a brief task-free functional MRI at 3.0 T has promising prognostic and predictive biomarker properties for central cholinergic dysfunction. If confirmed in prospective studies, then NBM FC could assist treatment stratification and facilitate ongoing drug development for optimized restoration of cholinergic functions in people living with or at risk for dementia.

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