Reduced Regional Cortical Thickness Rate of Change in Donepezil-Treated Subjects With Suspected Prodromal Alzheimer's Disease

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ABSTRACT

Objective: Cortical thinning, previously identified during prodromal stages of Alzheimer's disease (AD), is a "candidate" biomarker implemented in AD clinical therapy trials. We investigated the effect of donepezil treatment on cortical thickness in mild cognitively impaired subjects with the amnestic syndrome of the hippocampal type, a prodromal at-risk group for progression to AD dementia.

Methods: Data were from a longitudinal analysis of a community-based multicenter suspected prodromal AD cohort diagnosed by the Free and Cued Selective Reminding Test (81 donepezil vs 92 placebo) enrolled in a double-blind, randomized, placebo-controlled parallel group design using donepezil (10 mg/day). The study started in November 2006 and concluded in August 2010. All subjects underwent 2 brain structural magnetic resonance imaging (MRI) scans, at baseline and at the end of the trial. Structural MRI images had been processed using the automated pipeline for longitudinal segmentation and surface reconstruction implemented in FreeSurfer. The primary outcome measure of this post hoc study was the annualized percentage change (APC) of cortical thickness.

Results: The donepezil group exhibited reduced APC cortical thinning compared to placebo in the rostral anterior cingulate (right: $P = .048$; left: $P = .032$), the orbitofrontal (right: $P = .012$; left: $P < .048$), and the right inferior frontal ($P = .022$) cortices and in the right insula ($P = .010$). These results were not statistically significant after Bonferroni correction likely due to insufficient power for cortical thickness measurements in the study group powered for the predefined hippocampus outcome.

Conclusions: Our findings support the hypothesis that cortical thickness is a reliable candidate surrogate outcome in early predementia AD trials. In addition, donepezil treatment may have an impact on cortical structure/morphology in areas innervated by the medial and lateral cholinergic pathways.

Trial Registration: ClinicalTrials.gov identifier: NCT00403520

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The current approved treatments with cholinesterase inhibitors are generally considered to be moderately and only symptomatically beneficial for late-stage dementia in patients with Alzheimer's disease (AD).[1] Evidence of potential biological and disease-modifying properties of cholinesterase inhibitors is controversially and critically discussed. No agent tested in randomized clinical trials in mild cognitive impairment (MCI) has met its primary efficacy objectives, measured by clinical rating instruments.[2–10]

To date, only 1 study investigating the use of in vivo biomarkers of AD in a clinical trial of donepezil has been conducted.[11] On the other hand, further clinical studies on structural imaging markers in subjects with MCI have reported conflicting results.[12–14]

Although cortical thickness represents a promising structural imaging end point in clinical trials, as it offers a direct assessment of effect sizes expressed in a meaningful metric,[15–17] no study has yet investigated the donepezil effect on the cortical mantle.

This current study is a post hoc analysis based on the data collected in the context of the Hippocampus Study clinical trial (ClinicalTrials.gov identifier: NCT00403520).[14] The first aim of the trial was to investigate the effect of donepezil treatment using the hippocampal rate of change as primary outcome.[14] Here, we will consider the impact of donepezil treatment on the rate of change in cortical thickness in patients with suspected prodromal AD diagnosed using the Free and Cued Selective Reminding Test (FCSRT), a test allowing prognostication of prodromal AD within the group of individuals with MCI.[18,19]

Clinical Points

- Cortical thickness measurements may represent reliable surrogate markers of disease progression and underlying Alzheimer's disease at prodromal stages.
- Our data indicate that donepezil treatment may reduce cortical thickness expressed as annualized percentage change mainly in cortical areas associated with the cholinergic system, supporting the hypothesis that donepezil may impact brain structure/morphology.

METHODS

Study Population

Participants included in the present study were all patients who underwent a baseline and a follow-up magnetic resonance imaging (MRI) scan during the Hippocampus Study.[14] The primary efficacy outcome of the Hippocampus Study was the annualized percentage change (APC) of total hippocampal volume measured by an automated segmentation method. In this post hoc analysis, we explored the effect of donepezil on cortical thickness. Each patient received at least 1 dose of double-blind study medication and had baseline and follow-up clinical and MRI assessment.

The details of the patient characteristics have been previously described.[14] Briefly, a total of 332 patients were screened within the French national network of Memory Resources and Research Centres (MRRC) consisting of 28 regional university expert centers with neurologists, geriatricians, and neuropsychologists and biological and neuroimaging resources in each center.

Inclusion criteria were (1) more than 50 years of age, (2) a progressive hippocampal amnestic syndrome defined by Free Recall ≤ 17 or Total Recall < 40 on the FCSRT, and (3) no dementia, with a Clinical Dementia Rating stage of 0.5 and preserved cognition and functional performance. Subjects who met the eligibility criteria were enrolled in the randomization phase beginning with visit 1. From the total population of individuals randomized in the clinical trial (placebo = 103 and donepezil = 113), for the present study we considered only patients who performed MRI at baseline and at the end of the treatment (placebo = 92 and donepezil = 82).

Study Design

This was a multicenter double-blind, randomized, placebo-controlled trial with parallel group design and a treatment period of 12 months. The sample power of the study was calculated according to the primary aim of the Hippocampus Study clinical trial, and not for the aim of the present study. The study started in November 2006 and concluded in August 2010. At baseline visit, patients underwent a baseline MRI scan and a cognitive evaluation including the Alzheimer's Disease Assessment Scale-Cognitive subscale, MCI version (ADAS-Cog-MCI)[20]; Mini-Mental State Examination (MMSE); modified Isaacs test; California Verbal Learning Test (CVLT)[21]; Trail Making Tests (TMT) A and B[22]; and the Benton Visual Retention Test.[23] Following baseline evaluation, patients were randomly assigned to 1 of 2 groups, corresponding to double-blind treatment with either active treatment or placebo (active treatment: 1 capsule of 5 mg of donepezil daily for weeks 0 to 6, then 2 capsules of 5 mg of donepezil [ie, 10 mg] daily from week 6 to month 12; placebo: 1 placebo capsule daily for weeks 0 to 6, then 2 capsules daily from week 6 to month 12). The study protocol was approved by the institutional review board of each site, and informed consent was obtained from all subjects.

Acquisition of MRI Images

Brain MRI scans were acquired in each center at baseline and at the end of the treatment period. All MRI scans were performed using 1.5 Tesla or 3 Tesla MRI scanners qualified by the central MRI analysis core at the Cogimage team, Research Center of the Institut du Cerveau et de la Moelle Épinière (CRICM), Paris, France. The sequences used in the present article were 3-dimensional T1-weighted images. The 3-dimensional T1-weighted scan parameters for scans at 1.5T were repetition time (TR) = 10 ms, echo time (TE) = minimum, flip angle: 10, PREP time: 600, bandpass: 12.50 khz, axial orientation, 1.3-mm slice thickness, contiguous (124 slices), imaging matrix size: 256 × 256, and field of view: 240 × 240. The 3D T1-weighted scan parameters for scans at 3T were TR = 3.9 ms, TE = 2,100 ms, flip angle: 15, PREP time: 1,100 ms, sagittal orientation with 1-mm slice thickness, contiguous (144 slices), acquisition matrix size: 256 × 192, and field of view: 256 × 192. The expert neuroradiologists in charge of quality control verified artifacts of movements, ringing, wrap around, and metal artifacts; moreover, they attested that the sequences were acquired according to protocol parameters. After the quality control of images, one 3-dimensional T1-weighted scan was identified as corrupted, and it was excluded from all statistical and neuroimaging analyses.

Cortical Thickness Reconstruction and Surface Analysis

To extract reliable thickness estimates, images were automatically processed with the longitudinal stream[24] in FreeSurfer, version 5.1 (http://surfer.nmr.mgh.harvard.edu/). Details on the longitudinal pipeline used to analyze data are described at the following link: https://surfer.nmr.mgh.harvard.edu/fswiki/LongitudinalProcessing. Specifically, an unbiased within-subject template space and image is created using robust, inverse consistent registration.[25] Several processing steps, such as skull stripping, Talairach transforms, atlas registration, surface maps, and parcellations, are then initialized with common information from the within-subject template, significantly increasing reliability and statistical power.[26] On the basis of gyral and sulcal anatomy, the cortex was segmented using the Desikan-Killiany atlas.[27] For each of the cortical regions, mean cortical thickness was calculated as the distance (in millimeters) between the pial and gray/white matter surfaces. A quality control of data was performed; the pial and the white matter surfaces were checked in order to remove any non-brain tissue and

Table 1. Baseline Demographic and Clinical Characteristics of Patients Undergoing Baseline and Follow-Up MRI[a]

Characteristic	Placebo (n = 92)	Donepezil (n = 81)	*P* Value
Age, y	73.17 (6.63)	73.24 (6.67)	.966
Sex			
Male	44 (47.8)	39 (48.1)	.544
Female	48 (52.2)	42 (51.9)	
APOE ε4 carriers	18 (19.6)	18 (22.2)	.215
Missing, n	54	43	
Education			
No education	1 (0.01)	0 (0.0)	.330
Primary	7 (7.6)	8 (9.9)	
Certificate of primary education	43 (46.7)	30 (37.0)	
Secondary	14 (15.2)	21 (25.9)	
Higher education	27 (29.3)	22 (27.2)	
Follow-up MRI (mo)	9.65 (4.78)	10.07 (4.43)	.537
FCSRT (Free Recall)	11.32 (5.61)	12.21 (5.40)	.289
FCSRT (Total Recall)	29.55 (9.93)	31.37 (8.91)	.210
Hamilton Depression Rating Scale	3.22 (2.94)	2.83 (2.49)	.352
ADAS-Cog-MCI score	12.32 (4.40)	12.32 (4.61)	.997
MMSE score	25.86 (2.79)	25.94 (2.26)	.838
3 Tesla MRI	22 (24.0)	23 (28.4)	.534

[a]Means and standard deviations are reported for continuous variables, numbers and percentages for dichotomous values. *P* values denote significant differences by analysis of variance and χ^2 tests.
Abbreviations: ADAS-Cog-MCI = Alzheimer's Disease Assessment Scale-Cognitive subscale, MCI version; *APOE* = apolipoprotein E; FCSRT = Free and Cued Selective Reminding Test; MMSE = Mini-Mental State Examination; MRI = magnetic resonance imaging.

non-white matter tissue, respectively. The cortical thickness was smoothed with a 10-mm full width at half maximum Gaussian kernel to improve the signal-to-noise ratio and statistical power.

Statistical Analysis

Demographic, clinical, and neuropsychological features were compared between subject groups (placebo vs donepezil). The χ^2 test was performed on categorical variables, while the 1-way analysis of variance (ANOVA) was utilized for continuous variables. Since the assumption of normality was violated, baseline cortical thickness measures were transformed according to a 2-step approach for transforming continuous variables to normal. The first step involves transforming the original variable toward statistical uniformity (ie, satisfies the preponderance of diagnostic tests for uniformity) by calculating the fractional rank of each score; then, the fractional ranks obtained from the previous step were transformed to normal using the inverse normal distribution function.[28] Normalized baseline cortical thickness measures were compared between groups using the ANOVA.

Annualized percentage change (APC) of cortical thickness, in all the regions extracted from the FreeSurfer pipeline, was computed as follows:

$$\text{APC} = \frac{\text{Change From Baseline}}{\text{Value at Baseline}} \times \frac{365}{\text{MRI Delay}} \times 100$$

Then, the cortical thickness APCs were compared between placebo and donepezil using the Wilcoxon–Mann-Whitney test.

The cortical thickness APC comparisons were supplemented post hoc by the linear mixed-effects regression model with random intercepts, controlling for scanner platforms, to compare the difference in the cortical thickness change between placebo and donepezil patients in relation to treatment. Bonferroni correction for multiple comparisons was applied to the cortical thickness APC comparisons and to the mixed-effect regression model results. The statistical analyses were performed using SPSS, version 22.00 (IBM Corp).

Surface analyses were performed using MATLAB (http://fr.mathworks.com) and the Qdec toolbox of FreeSurfer (https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/QdecGroupAnalysis_freeview). One-year changes in bilateral cortical structures were investigated using the linear mixed-effects models. The results were projected onto the template. The surface analyses were re-thresholded using a 2-stage false discovery rate of .05 (FDR2 < .05).

Power Analysis

Since the original Hippocampus Study clinical trial was not designed to examine cortical thickness, sample size needed to detect a thickness change of a certain number of millimeters between placebo and treatment patients was estimated. Sample size requirements were estimated using the following formula:

$2 \times K \times \left(\frac{\sigma}{\mu 1 - \mu 2}\right)^2$, where K = constant, which is a function of α (.05) and β = .2 (80%); σ is the standard deviation of cortical thickness APC in the untreated group, μ values are the mean values of cortical thickness APC in the treatment and placebo groups. The following modification was included: the sample size thus derived was increased by 10% to allow for losses at follow-up.

RESULTS

From the total sample of 316 individuals (103 placebo and 113 donepezil) randomly assigned in the trial, 174 individuals (92 placebo and 82 donepezil) underwent both baseline and follow-up MRI scans. One subject in the donepezil group was then excluded from analysis due to a corrupted scan.

No significant differences were found for baseline sociodemographic as well as cognitive features between groups (Table 1). The apolipoprotein E (*APOE*) genotype was present in 18 patients (19.6%) in the placebo group and 18 (22.2%) in the donepezil group showing no substantial differences (*P* = .215) between the 2 groups.

The baseline cortical thickness, for all brain regions considered, was not significantly different between the 2 groups (Table 2).

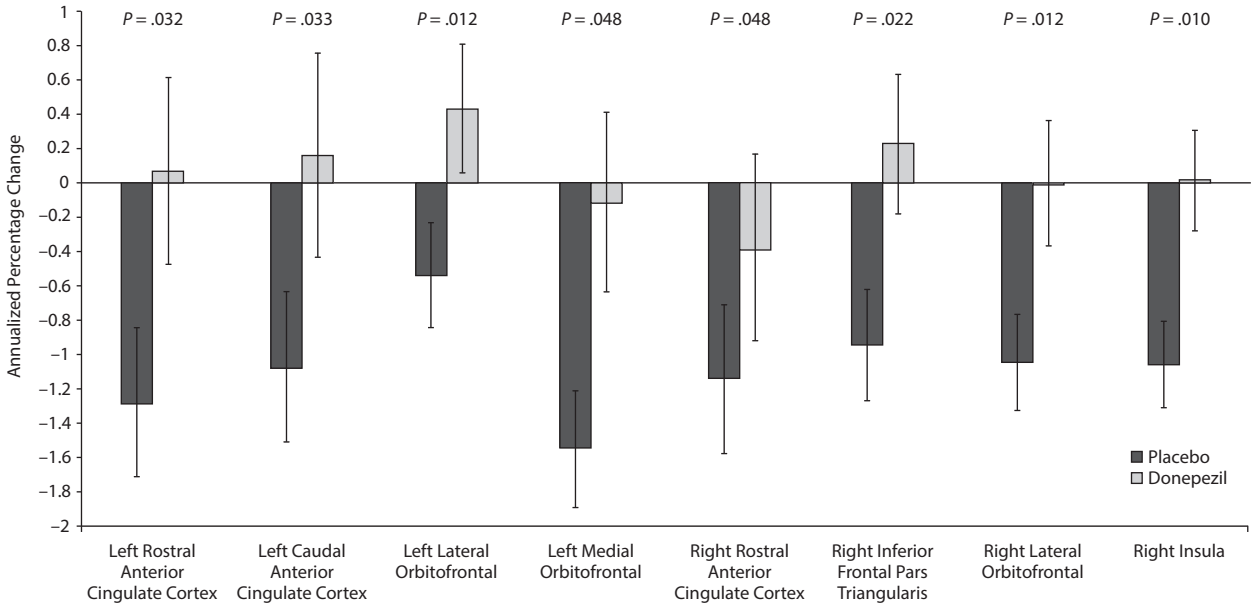
As detailed in Figure 1, a significant difference in the regional cortical thickness APC was observed between groups. In particular, the placebo group compared with the donepezil group showed a higher APC in the right and left rostral anterior cingulate cortex (−1.14% vs −0.38% [*P* = .048]

Table 2. Baseline Right and Left Cortical Thickness (mm) of 173 Patients[a]

	Left Hemisphere			Right Hemisphere		
Area	Placebo (n = 92)	Donepezil (n = 81)	*P* Value	Placebo (n = 92)	Donepezil (n = 81)	*P* Value
Cingulate cortex						
Rostral anterior	2.74 ± 0.02	2.80 ± 0.02	.113	2.72 ± 0.02	2.75 ± 0.03	.698
Caudal anterior	2.57 ± 0.03	2.60 ± 0.04	.820	2.57 ± 0.03	2.68 ± 0.03	.064
Posterior	2.31 ± 0.01	2.34 ± 0.02	.452	2.31 ± 0.02	2.34 ± 0.02	.345
Isthmus	2.15 ± 0.02	2.17 ± 0.01	.337	2.12 ± 0.01	2.14 ± 0.02	.538
Frontal lobe						
Pre-central	2.32 ± 0.01	2.35 ± 0.01	.355	2.34 ± 0.01	2.35 ± 0.01	.664
Superior frontal	2.49 ± 0.01	2.50 ± 0.01	.996	2.51 ± 0.01	2.51 ± 0.01	.560
Middle frontal caudal	2.33 ± 0.01	2.35 ± 0.01	.350	2.37 ± 0.01	2.38 ± 0.01	.985
Middle frontal rostral	2.20 ± 0.01	2.22 ± 0.01	.472	2.21 ± 0.01	2.23 ± 0.01	.823
Inferior frontal pars orbitalis	2.51 ± 0.02	2.54 ± 0.02	.255	2.49 ± 0.02	2.45 ± 0.02	.239
Inferior frontal pars triangularis	2.27 ± 0.01	2.27 ± 0.01	.941	2.26 ± 0.01	2.24 ± 0.01	.392
Inferior frontal pars opercularis	2.38 ± 0.01	2.39 ± 0.01	.557	2.40 ± 0.01	2.40 ± 0.01	.887
Frontal pole	2.61 ± 0.03	2.65 ± 0.03	.192	2.67 ± 0.03	2.66 ± 0.03	.916
Orbitofrontal lateral	2.45 ± 0.01	2.44 ± 0.01	.892	2.44 ± 0.01	2.44 ± 0.01	.607
Orbitofrontal medial	2.36 ± 0.02	2.36 ± 0.01	.308	2.34 ± 0.01	2.36 ± 0.01	.143
Parietal						
Postcentral	1.95 ± 0.01	1.99 ± 0.02	.460	1.98 ± 0.01	2.00 ± 0.01	.472
Supra marginal	2.31 ± 0.01	2.32 ± 0.01	.885	2.33 ± 0.01	2.34 ± 0.01	.848
Superior parietal	2.01 ± 0.01	2.04 ± 0.01	.182	2.05 ± 0.01	2.06 ± 0.01	.814
Inferior parietal	2.22 ± 0.01	2.26 ± 0.01	.166	2.22 ± 0.01	2.25 ± 0.01	.164
Precuneus	2.12 ± 0.01	2.14 ± 0.01	.541	2.12 ± 0.01	2.13 ± 0.01	.798
Temporal						
Inferior temporal	2.56 ± 0.01	2.58 ± 0.02	.447	2.57 ± 0.01	2.57 ± 0.01	.986
Superior temporal	2.5 ± 0.1	2.5 ± 0.1	.980	2.5 ± 0.1	2.5 ± 0.1	.852
Transverse temporal	2.25 ± 0.02	2.22 ± 0.02	.770	2.26 ± 0.02	2.27 ± 0.02	.824
Middle temporal	2.59 ± 0.01	2.60 ± 0.01	.778	2.56 ± 0.01	2.55 ± 0.01	.504
Temporal pole	3.3 ± 0.3	3.2 ± 0.3	.376	3.32 ± 0.04	3.31 ± 0.03	.991
Entorhinal	2.94 ± 0.04	2.88 ± 0.04	.554	2.84 ± 0.05	2.87 ± 0.04	.669
Parahippocampal	2.38 ± 0.03	2.41 ± 0.03	.936	2.34 ± 0.02	2.28 ± 0.04	.498
Fusiform	2.43 ± 0.01	2.45 ± 0.01	.322	2.41 ± 0.01	2.43 ± 0.01	.270
Occipital						
Lateral occipital	2.03 ± 0.01	2.05 ± 0.01	.713	2.02 ± 0.01	2.03 ± 0.01	.887
Cuneus	1.75 ± 0.01	1.78 ± 0.01	.250	1.74 ± 0.01	1.75 ± 0.01	.725
Peri-calcarine	1.56 ± 0.01	1.56 ± 0.01	.940	1.51 ± 0.01	1.51 ± 0.01	.702
Lingual	1.85 ± 0.01	1.86 ± 0.01	.718	1.83 ± 0.01	1.82 ± 0.01	.889
Insula	2.81 ± 0.01	2.80 ± 0.01	.748	2.86 ± 0.01	2.82 ± 0.02	.111

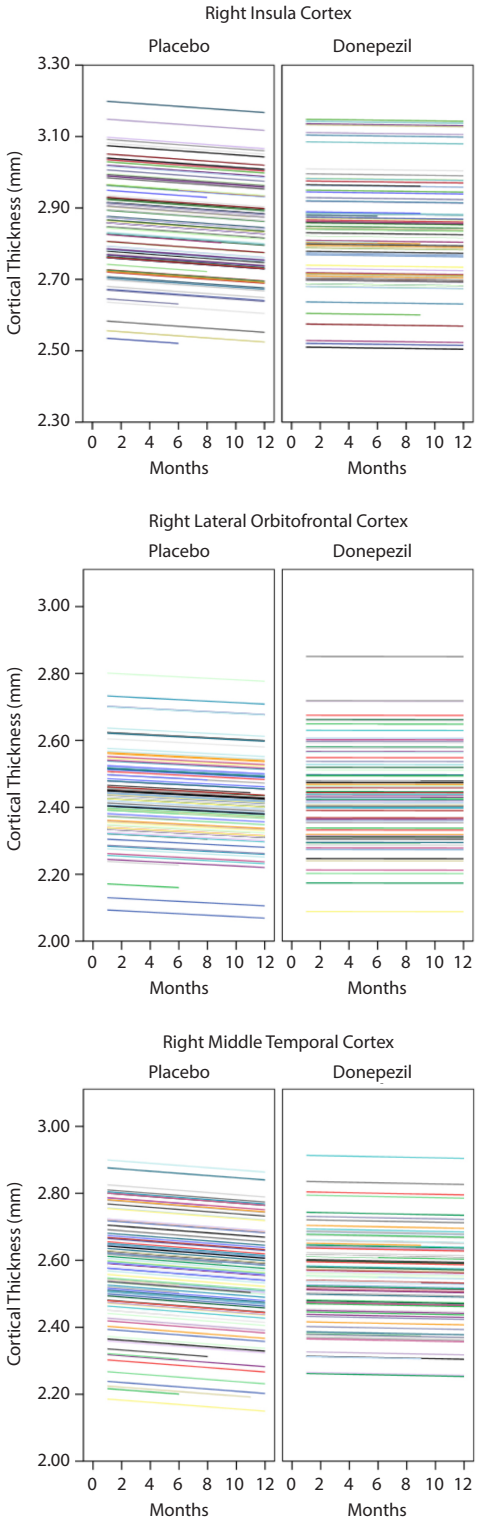
[a]Means and standard deviations are reported; *P* values denote significant differences by analysis of variance.

Figure 1. Significant Differences in the Left and Right Brain Cortical Thickness Annualized Percentage Changes[a]



[a]Patients treated with donepezil showed an increase or stable change of the frontal and insula cortical thicknesses compared to the placebo group, which revealed a cortical thinning in all the cortical regions. Numbers indicate means (standard errors) of annual percentage change; *P* values denote the significance by Wilcoxon–Mann-Whitney test.

Figure 2. Individual Trajectories of Cortical Thickness Change[a]



[a]Graphs display significant changes over time that resulted from the linear mixed-effects regression models with random intercepts in the donepezil and placebo groups based on cortical thickness measurements from baseline (month 0) to follow-up scan.

and −1.28% vs 0.07% [P=.032], respectively), left caudal anterior cingulate cortex (−1.07% vs 0.16% [P=.033]), right and left orbitofrontal cortex (−1.04% vs −0.001% [P=.012] and −0.54% vs 0.43% [P<.048], respectively), right inferior frontal cortex (−0.94% vs 0.23% [P=.022]), and right insula (−1.06% vs 0.013% [P=.010]).

Post hoc analysis by the mixed-effects model (Figure 2) revealed that, during the 12 months of the treatment period, cortical thickness was significantly decreased in the placebo group compared with the donepezil group in the right lateral orbitofrontal cortex (difference in slope 0.0023; P=.026), right middle temporal cortex (difference in slope 0.0027; P=.027), and right insula (difference in slope 0.0027; P=.015). Both the cortical thickness APC comparisons and the results on the mixed-effects model did not survive after Bonferroni correction.

Surface differences revealed a cortical thinning in the placebo group compared to the donepezil group in the left superior temporal, left orbitofrontal, right supramarginal, and right insula cortices (Figure 3). This result did not survive after correction for false discovery rate.

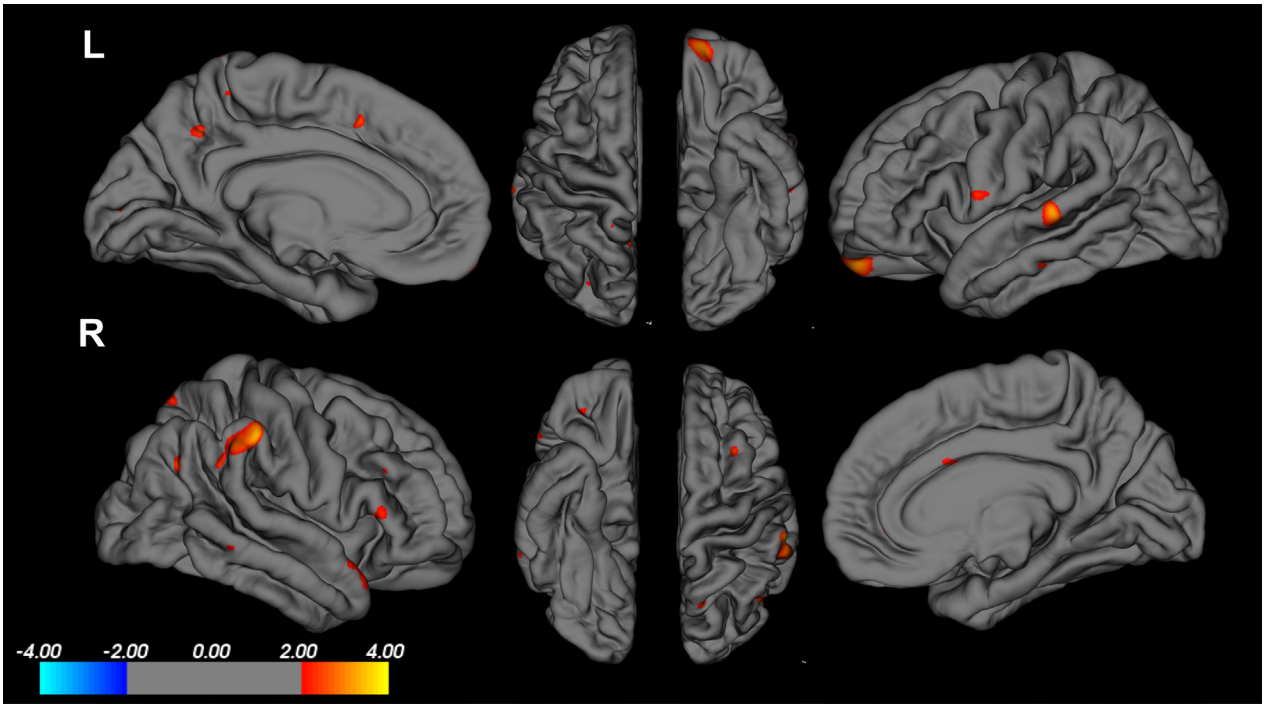
Power Analysis

The mean (SD) cortical thickness APC for the donepezil group was −0.51% (2.18%), while for the placebo group, −0.95% (2.10%). On the basis of these values, to have 80% power to detect a drug effect at 5% significance level (α=.05), 187 patients would be needed for each treatment arm. This assumes a 1-year placebo-controlled trial with a 10% patient dropout rate.

DISCUSSION

To our knowledge, this is the first study investigating the effect of donepezil treatment on regional brain cortical thickness in suspected prodromal AD patients with amnestic syndrome of hippocampal type. Our results highlight the presence of a trend of stability in the cortical thickness APC in the anterior cingulate cortex, left orbitofrontal cortex, inferior frontal cortex, and right insula in patients with suspected prodromal AD treated with donepezil. These findings have been further supported by data obtained from the longitudinal mixed model and the cortical surface comparison of cortical thickness APC between groups. Indeed, we have found a trend of reduced cortical surface in the placebo group compared with the donepezil group after 1 year of donepezil treatment in the following cortical regions: orbitofrontal, superior, caudal middle frontal, superior temporal, supramarginal, precuneus, inferior parietal, and insula. Some of these areas have been recognized to be of key importance since they are supposed to represent the cortical signature of patients with prodromal AD.[29,30] Although our results did not survive after multiple comparisons, they still remain promising since they have shown a statistical trend toward a significant impact of donepezil treatment in preserving cortical thickness in patients with suspected prodromal AD. One possible reason explaining why our

Figure 3. Surface Differences Between Placebo and Donepezil Groups Detected by the Linear Mixed-Effects Model[a]



Cortical Surface	Clusters Size (mm^2)	Coordinates			*P* Value
		X	Y	Z	
Left superior temporal	70.02	−64.6	−27.4	4.2	< .001
Left orbitofrontal	113.19	−11.4	51.7	−17.9	< .001
Left superior frontal	41.01	−10.5	14.0	41.2	.002
Left precuneus	61.98	−8.9	−52.0	40.3	.003
Right supramarginal	279.94	58.7	−27.8	38.8	< .001
Right insula	43.70	30.1	20.9	7.5	< .001
Right inferior parietal	61.87	46.1	−61.0	29.3	.002
Right caudal middle frontal	44.26	33.0	18.8	43.5	.003

[a]Results are false discovery rate uncorrected.

results did not survive to multiple comparison corrections might be that the sample power of the current study was not calculated for the present purpose but for the original aim of the Hippocampus Study clinical trial. Indeed, the necessary sample size to obtain a statistically significant effect in the cortical thickness APC between placebo and donepezil should be substantially higher (187 patients for each arm), as revealed by the sample power analysis.

Notably, in line with a previous study revealing an increased resting state metabolism in the left prefrontal cortex (and in the right hippocampus) of AD patients treated with donepezil,[11] we also found a significant reduced cortical thinning in the left orbitofrontal cortex and anterior cingulate cortex in this group.

Previous studies investigating the donepezil effect on different structural imaging markers, such as the hippocampus, the brain lateral ventricles, and the whole brain volumes, showed contrasting results. A randomized double-blind, placebo-controlled single-center study[31] of 67 patients with mild-to-moderate AD found a small decrease in left hippocampal volume after 24 weeks of donepezil treatment compared with the placebo-treated subjects. Results from the Alzheimer's Disease Cooperative Study (ADCS) of donepezil/vitamin E conducted in 131 patients with MCI showed a statistical trend toward a possible slowing of the hippocampal atrophy rate by donepezil.[12] A more recent study conducted by Schuff and colleagues[32] on subjects with MCI reported significant differences in favor of the donepezil group for the total ventricular region and the cortical region (whole brain volumes) but not for the hippocampal volume. Recently, our group[14] confirmed the data by Schuff and colleagues[32] for ventricular and whole brain volumes. In addition, we observed a significant reduction in the annual rate of hippocampal atrophy in suspected prodromal AD patients treated with donepezil.[14]

Regional cortical thickness reduction is used as a predictive indicator of AD-related neurodegeneration in subjects with MCI.[30,33] Our results revealed unilateral treatment differences in several brain regions that might be due to normal variation and specialization of function and structure. Brain asymmetry is believed to be evolutionally adaptive, reducing possible interference between hemispheres.[34]

Based on the hypothesis that donepezil may attenuate amyloid-induced neuronal toxicity,[35,36] it is conceivable that 12 months of donepezil treatment might have a

neuroprotective effect on the cortex. In vitro studies demonstrated that lesions of the cholinergic nucleus basalis of Meynert promote the ex vivo synthesis of Aβ precursor protein (AβPP) in the cerebral cortex.[37] Recently, an in vivo neuroimaging study described a correlation between basal forebrain atrophy and elevated cortical amyloid load in preclinical and predementia stages of AD,[38] thus reflecting the association found in several human autopsy studies between amyloid pathology and cholinergic atrophy in AD.[39,40,41] Cortical amyloid accumulation might induce cholinergic cell death involving alterations in the levels of intracellular calcium and/or production of toxic and inflammatory mediators such as nitric oxide, cytokines, and reactive oxygen intermediates.[42] On the other hand, a cholinergic stimulation positively alters the mechanisms of amyloid processing, thus protecting neurons from neurodegeneration induced by Aβ.[35]

In this study, we found a trend toward statistical significance for cortical thinning reduction in the cortical areas innervated by the medial and lateral cholinergic pathways[43] in patients with suspected prodromal AD receiving 1 year of donepezil treatment.

One of the main strengths of the present study is that the refined target population derived from a large-scale community-based multicenter cohort of subjects included on the basis of the FCSRT, a memory test reported to be highly correlated with hippocampal volume and cerebral spinal fluid level changes of the Alzheimer type.[18,19]

The current study presents potential limitations. First, the data used in the present research were not specifically powered for the aims of the present study, thus reducing the significance of the results. The protocol of the study did not include information on the settings of the subjects of the population or on race and ethnicity characteristics or data in terms of lifestyle. The latter, in particular, given its practical aspects—nutrition, hydration, and physical activity—has become significant in terms of AD prevention. We did not collect sufficient data on *APOE ε4* genotype to exclude the hypothesis that our results were not significantly impacted by the presence of *APOE ε4* genotype. Finally, the subjects have not been followed for a long enough period of time to determine the incidence of incipient AD in each group.

Overall, our findings support the hypothesis that cortical thickness might be used as surrogate outcome in predementia AD clinical trials. Moreover, our findings suggest that donepezil may have an impact on cortical morphology in patients with prodromal AD. A potentially disease-modifying effect of approved cholinesterase inhibitors represents a result of pivotal clinical interest. Further studies are needed to confirm this first investigation. Moreover, established measurements of the basal forebrain cholinergic nuclei volume, such as the nucleus basalis of Meynert and its subnuclei, as well as their in vivo white matter connections to subcortical and cortical areas, are of interest to detect the specific donepezil effect on the brain's cholinergic system.

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