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The effect of *APOE* ε4 allele on cholinesterase inhibitors in patients with Alzheimer's disease: Evaluation of the feasibility of resting state functional connectivity magnetic resonance imaging

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Abstract

This work is to determine whether apolipoprotein E (*APOE*) genotype modulates the effect of cholinesterase inhibitor (ChEI) treatment on resting state functional connectivity magnetic resonance imaging (rs-fcMRI) in patients with Alzheimer's disease (AD). We retrospectively studied very mild and mild AD participants who were treated (N=25) or untreated (N=19) with ChEIs with respect to rs-fcMRI measure of 5 resting state networks (RSNs): default mode, dorsal attention (DAN), control (CON), salience (SAL), and sensory-motor. For each network, a composite score was computed as the mean of Pearson's correlations between pairwise time courses extracted from areas comprising this network. The composite scores were analyzed as a function of ChEI treatment and *APOE* ε4 allele. Across all participants, significant interactions between ChEI treatment and *APOE* ε4 allele were observed for all 5 RSNs. Within *APOE* ε4 carriers, significantly greater composite scores were observed in the DAN, CON and SAL for treated compared to untreated participants. Within *APOE* ε4 non-carriers, treated and untreated participants did not have significantly different composite scores for all RSNs. These data suggest

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that *APOE* genotype affects the response to ChEI using rs-fcMRI. Rs-fcMRI may be useful for assessing the therapeutic effect of medications in AD clinical trials.

Keywords

Alzheimer's disease (AD); functional magnetic resonance imaging (fMRI); Cholinesterase inhibitor; Apolipoprotein E (*APOE*); resting state functional connectivity

INTRODUCTION

At autopsy there is a marked loss of cholinergic neurons in the nucleus basalis of Meynert in Alzheimer's disease (AD) patients [1]. Loss of these neurons is the basis for the cholinergic deficit in the cerebral cortex [2,3], and may contribute to clinical symptoms seen in AD [4]. Cholinesterase inhibitors (ChEIs) attempt to restore this central cholinergic shortage and may help treat cognition and behavioral changes [5].

Mixed results have been observed concerning the modulation of cognitive response to ChEIs by apolipoprotein E (*APOE*) genotype in individuals with mild to moderate AD or those with mild cognitive impairment. Early studies showed that *APOE* ε4 carriers had a worse clinical response to an older ChEI (tacrine) compared to *APOE* ε4 non-carriers [6,7]. Recent studies have suggested that newer ChEIs (donepezil, rivastigmine, and galantamine) may lead to greater cognitive improvements in *APOE* ε4 carriers than non-carriers [8–11]. However, some studies have observed no differences between *APOE* ε4 carriers and noncarriers in response to treatment with ChEIs [12–14].

Resting state functional connectivity magnetic resonance imaging (rs-fcMRI) non-invasively measures the temporal correlation of spontaneous fluctuations of the blood oxygen level-dependent (BOLD) signal [15]. The correlated fluctuations can be observed across spatially distributed regions that recapitulate the topographies of BOLD response induced by performance for various cognitive tasks [16]. These rs-fcMRI-observed topographic patterns have been referred to as resting state networks (RSNs). Rs-fcMRI has great promise in assessing the pathophysiology of AD (see reviews by Greicius [17], Broyd et al.[18]). Our group has recently demonstrated that symptomatic AD participants exhibited rs-fcMRI abnormalities across multiple RSNs that progressively worsen with advancing disease stage [19]. However, a limited number of rs-fcMRI studies have investigated the effect of ChEI treatment, with most primarily focused on RSNs involving the hippocampus and cingulate cortex [20,21].

The primary objective of the present work was to retrospectively investigate the effect of ChEI treatment on the integrity of multiple RSNs in patients with very mild and mild AD. In particular, we sought to determine whether *APOE* genotype would modulate the effect of ChEI treatment on these RSNs.

METHODS

Participants

Participants were community-dwelling volunteers enrolled in studies of aging and memory at the Charles F. and Joanne Knight Alzheimer's Disease Research Center at Washington University in Saint Louis. Detailed information regarding recruitment has previously been published [22]. Inclusion criteria for this study were: 1) a diagnosis of very mild or mild AD dementia, and 2) either not receiving medication for AD or on a stable dose of ChEIs (donepezil, rivastigmine, or galantamine) for at least 15 days, and 3) *APOE* genotyping.

Individuals were excluded from this study if they had neurological, psychiatric or systemic illness that might impact cognition. This study was approved by the Human Research Protection Office at Washington University in St. Louis and the Institutional Review Board at St. Louis College of Pharmacy. All participants provided written informed consent prior to participating in this study.

Clinical assessment

An experienced clinician conducted separate semi-structured interviews with the participant and a collateral source (CS). The clinician then determined whether dementia was present or absent based on the principle of intra-individual cognitive decline relative to previously attained function. The clinician's judgment was operationalized using the Clinical Dementia Rating (CDR)[23], in which CDR 0, 0.5, 1, 2, and 3 corresponded to no dementia (i.e., cognitively normal), very mild, mild, moderate, and severe dementia, respectively. Only CDR 0.5 and CDR 1 participants were included in this study. In addition, CDR-sum of boxes [24] and Mini-Mental State Examination (MMSE) [25] were obtained.

Genotyping

DNA was extracted from peripheral blood samples. Genotyping for *APOE* was performed using standard procedures previously described [26].

Image acquisition and pre-processing of rs-fcMRI data

MRI data were collected using a Siemens Trio 3.0 Tesla scanner with a twelve-channel head coil. High-resolution structural images were acquired with T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) sequence (echo time [TE] = 16 msec, repetition time [TR] = 2,400 msec, inversion time [TI] = 1,000 msec, flip angle = 8°, 256 × 256 acquisition matrix, 1 × 1 × 1 mm voxels). A two-dimensional spin density/T2-weighted fast spin echo (T2W-FSE) scan was performed (TE = 455 msec, TR = 3,200 msec, 256 × 256 acquisition matrix, 1 × 1 × 1 mm voxels). Two rs-fcMRI scans (164 volumes each) were obtained using a gradient spin-echo sequence (TE = 27 msec, TR = 2.2 sec, 64 × 64 acquisition matrix, flip angle = 90°). Whole-brain coverage was achieved using thirty-six axial slices parallel to the anterior–posterior commissure line with approximately 4.0 mm cubic voxels in each volume. During rs-fcMRI scanning, participants were required to fixate on a visual cross-hair and not fall asleep. All rs-fcMRI data were preprocessed using previously described methods[27]. Additional details concerning rs-fcMRI data preprocessing and quality assurance are provided in Supplemental Material.

Definition of rs-fcMRI regions of interest

The procedure for generating regions of interest (ROIs) has been previously described[19,28]. Briefly, rs-fcMRI data acquired from a separate group of 17 healthy young adults were used to generate nodes that comprise 7 RSNs that included the default mode (DMN), dorsal attention (DAN), control (CON), salience (SAL), and auditory, visual, and somatomotor networks. rs-fcMRI data from this young adult group were analyzed using a group-wise spatial independent component analysis (sICA) based upon a published fastICA algorithm [29] implemented in Matlab. This dataset was also analyzed using seed-based correlation mapping, in which initial seed regions were created according to previously published coordinates[30]. Loci of matching peaks from the ICA and seed based correlation maps were selected as the centers of thirty-six spherical (6 mm radius) ROIs (representing seven RSNs) for the present analysis. Locations of these ROIs were displayed on correlation maps that were created using a representative seed from each RSN using all AD participants (Figure 1 and Supplemental Table S1). The three RSNs corresponding to the auditory, visual, and somatomotor cortices were combined into a single sensory-motor network (SMN).

Subject-level ROI-based measure of functional connectivity

The mean time course, extracted from the preprocessed rs-fcMRI data, was obtained from each ROI. Pearson's correlation coefficients were computed between pairwise ROI time courses across all ROIs within a given RSN. The correlation coefficients were converted to *z* values using Fisher's transformation. For each RSN, correlation coefficients [*z*(r)] across all ROI pairs included in this network were averaged to form a composite score. The composite score has been shown to correlate with disease severity of AD [19]. This approach to statistical inference achieves data reduction and reduces the impact of sampling error across node pairs. Additional analysis concerning the influence of negative correlation on composite score is presented in Supplemental Figure S1.

Statistical analysis

For each RSN, subject-level composite scores were analyzed as a function of ChEI (treated vs. untreated) and *APOE* genotype (presence vs. absence of at least one ε4 allele) by an analysis of variance (ANOVA). The main effects of ChEI and APOE genotype and their interaction were tested for each RSN. If a significant interaction was observed, the effect of ChEI was tested separately in *APOE* ε4 carriers and non-carriers, and the effect of *APOE* genotype was assessed separately in treated and untreated participants. The five RSNs were analyzed separately (using 5 ANOVA models) with a statistical threshold for significance of $p < 0.05$, uncorrected for multiple comparisons (SPSS 19.0 Chicago, IL).

RESULTS

Demographic information for the entire cohort is provided in Table 1. The demographic variables were not significantly different between the very mild and mild AD participants treated and untreated with ChEIs (all $p \geq 0.25$). Within the ChEIs-treated participants (N=25), 21 received donepezil, 3 were prescribed galantamine and 1 was taking rivastigmine. For ChEIs-treated patients, the duration between initiation of treatment and acquisition of MRI ranged from 4 to 78 months with a median of 18 months.

Effects of ChEI and APOE genotype on RSN composite scores for the entire cohort

For each RSN, an ANOVA model assessed the effects of ChEI, APOE genotype and their possible interaction on RSN composite scores of this RSN. The interaction between ChEI and *APOE* genotype was significant for each of the 5 RSNs (DMN: F = 4.903, $p = 0.033$; DAN: F = 5.022, $p = 0.031$; CON: F = 8.924, $p = 0.005$; SAL: F = 6.638, $p = 0.014$ and SMN: F = 4.523, $p = 0.040$). Across the 5 RSNs, neither the effects of ChEI nor *APOE* genotype were significant for any of the RSN composite scores (ChEI effect: all $p \geq 0.477$; *APOE* ε4 effect: all $p \geq 0.144$).

Effects of ChEI on RSN composite scores in APOE ε4 carriers

Demographic variables were not significantly different between ChEI-treated (N=16) and untreated (N=11) *APOE* ε4 carriers (all $p \geq 0.34$) (Supplemental Table S2A). In general, across all 5 RSNs, the treated *APOE* ε4 carriers exhibited greater RSN composite scores than those *APOE* ε4 carriers who remained untreated. However, statistically significant increases were only observed in the DAN, CON and SAL (all $p < 0.05$) (Figure 1).

Effects of ChEI on RSN composite scores in APOE ε4 noncarriers

Demographic variables were also similar between the treated (N=9) and untreated (N=8) *APOE* ε4 non-carriers (all $p \geq 0.20$) (Supplemental Table S2B). On average, treated *APOE* ε4 non-carriers had lower RSN composite scores than untreated APOE ε4 non-carrier individuals. However, group differences were not statistically significant (all $p \geq 0.114$) (Figure 1).

We examined the effect of *APOE* genotype on RSN composite scores in treated and untreated AD participants separately. Significant differences were found for several RSNs between *APOE* ε4 non-carriers and carriers in either treated or untreated groups (Supplemental Materials).

DISCUSSION

The present work demonstrates that among individuals with very mild and mild AD, *APOE* ε4 carriers and non-carriers were affected differentially by ChEI treatment with respect to rs-fcMRI measures of RSN integrity. Specifically, within *APOE* ε4 carriers, functional connectivity was increased in all 5 RSNs in treated compared to untreated individuals, with statistically significant increases seen in the DAN, CON and SAL. In contrast, within *APOE* ε4 non-carriers, functional connectivity was decreased across the 5 RSNs in treated compared to untreated individuals, but none of these effects were statistically significant.

We observed that ChEI treatment is associated with significant changes in functional connectivity that occur only in *APOE* ε4 carriers. In the largest randomized control trial evaluating the effect of a ChEI (donepezil) in persons with mild cognitive impairment, treatment significantly delayed the progression to AD over 24 months, but only within *APOE* ε4 carriers [8]. A sub-study derived from this trial has subsequently shown that patients treated with this ChEI had a trend toward lower rates of hippocampal atrophy compared to individuals receiving placebo, but this effect was only seen in *APOE* ε4 carriers [31]. Our

findings are consistent with these previous studies [8–11,31] suggesting that the presence of *APOE* ε4 allele may modulate the response to ChEIs. However, other studies [12–14] have reported that *APOE* ε4 allele does not affect the response to ChEI treatment. These conflicting results are difficult to reconcile and may reflect differences in sample size, outcome measures, follow-up periods, and ChEI pharmacodynamics. Although further studies are needed to elucidate the biological underpinnings for drug-genotype interactions, the present results suggest that genotyping for *APOE* may be beneficial for determining therapeutic strategies for very mild and mild AD individuals.

The topographic distribution of the RSNs significantly affected by ChEI treatment is consistent with prior reports. Previous studies [32,33] have compared the BOLD response to various cognitive paradigms before and after ChEI (donepezil) treatment. These studies have reported that treatment is associated with increased activation in the lateral prefrontal areas [32,33]. In a positron emission tomography study comparing acetylcholinesterase activity before and after ChEI (donepezil) therapy, the greatest inhibition of acetylcholinesterase activity was observed in the anterior cingulate cortex [34]. Since the lateral prefrontal cortex and the anterior cingulate comprise principle nodes of the CON and SAL respectively, the available data suggest that the enhancement of prefrontal activity may be involved by the mechanism of action for donepezil treatment.

The effect of ChEIs (in particular donepezil) on rs-fcMRI has been most recently studied using voxelwise whole brain analyses [20,21]. Goveas and colleagues observed that administration of donepezil increased functional correlations between the hippocampus and multiple cortical or subcortical regions [20]. Another study reports that donepezil treatment is associated with an enhanced functional connectivity between cingulate cortex and other brain areas [21]. We demonstrated that ChEI treatment was associated with significant increases in functional correlations within the DAN, CON and SAL. While further work incorporating distinct analytic strategies is needed, the available data may collectively suggest that rs-fcMRI is useful for detecting the therapeutic effect in AD clinical trials.

The present work has several limitations. The present study used global signal regression as a preprocessing technique[35]. This technique provides a simple way to remove noise correlations associated with variations in heart rate and breathing[36], however, it may mathematically induces negative correlations in seed-based analysis[37,38]. We defined the ROIs only from regions that showed positive correlations with a priori seeds. We computed a RSN composite score from only ROIs belonging to a particular network. These procedures maximally mitigate the confounding impacts of global signal regression on the present results. Further studies are needed to confirm our findings using alternative methods correcting for variations in heart rate and breathing[39,40]. Our study was limited by both cross-sectional design and small sample size. We were unable to collect data before and after treatment to determine whether the clinical response was consistent with rs-fcMRI observations (i.e., treatment-associated symptomatic improvement occurs in *APOE* ε4 carriers but not in *APOE* ε4 non-carriers). In addition, the present statistical results fail to survive stringent Bonferroni correction due to our rather limited sample size. A prospective, placebo-controlled trail with a larger sample size is needed to confirm observed effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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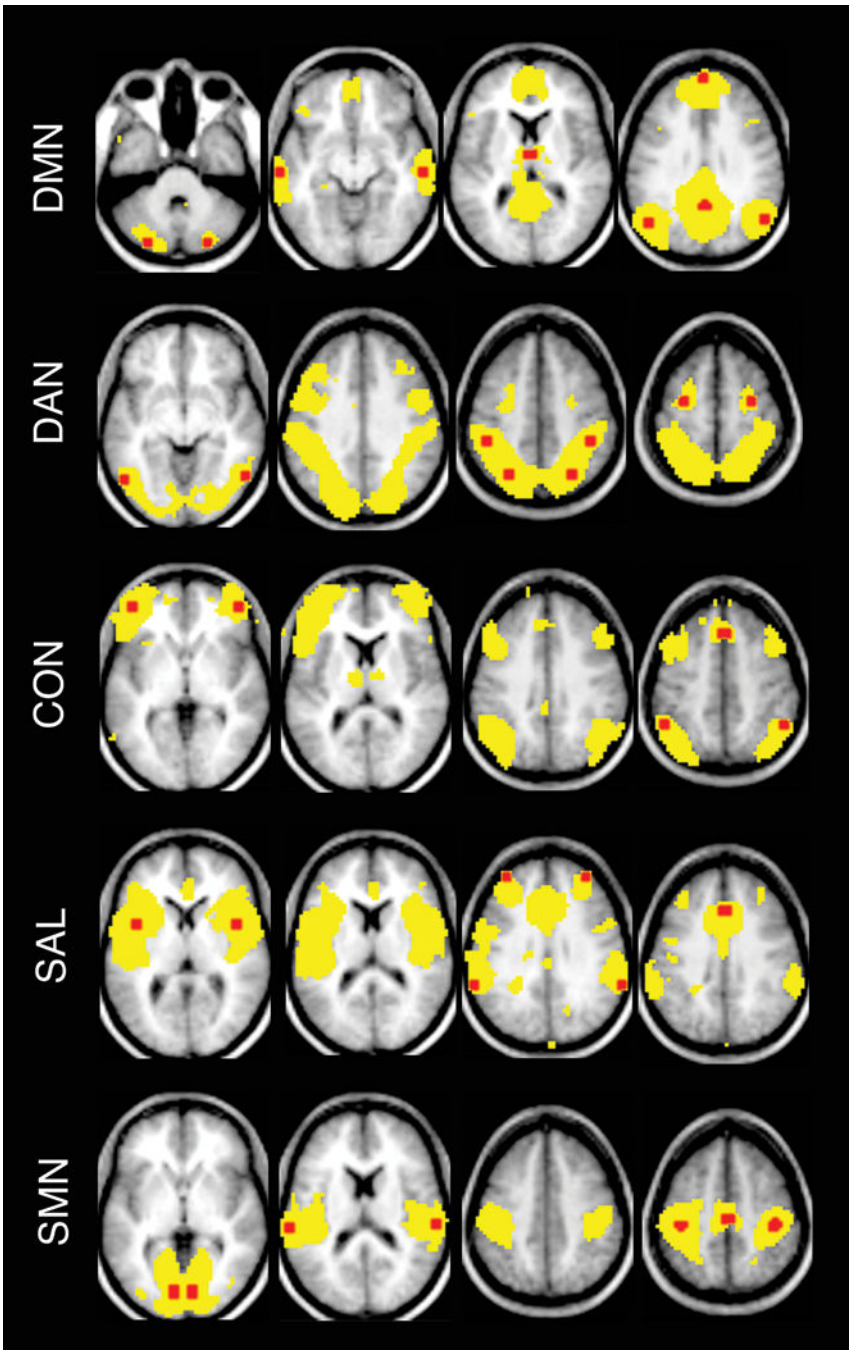

Figure 1.

Topographies of resting state networks (RSNs) and locations of seed regions.

Using a representative seed for each RSN, correlation maps were created for all AD participants. Group average maps thresholded at z(r)>0.1 are shown using yellow. All a priori seed regions (6-mm spheres) (red) from a RSN are overlaid on the group average map. Atlas coordinates for each seed region are provided in Supplemental Table S1. In particular, the posterior cingulate cortex was used as seed region for default mode network (DMN); the left posterior intraparietal sulcus for dorsal attention network (DAN); the left anterior

prefrontal cortex for control network (CON), the left insular cortex for salience network (SAL), and the left primary visual cortex, the left primary auditory cortex and the left motor cortex for the sensory-motor network (SMN).

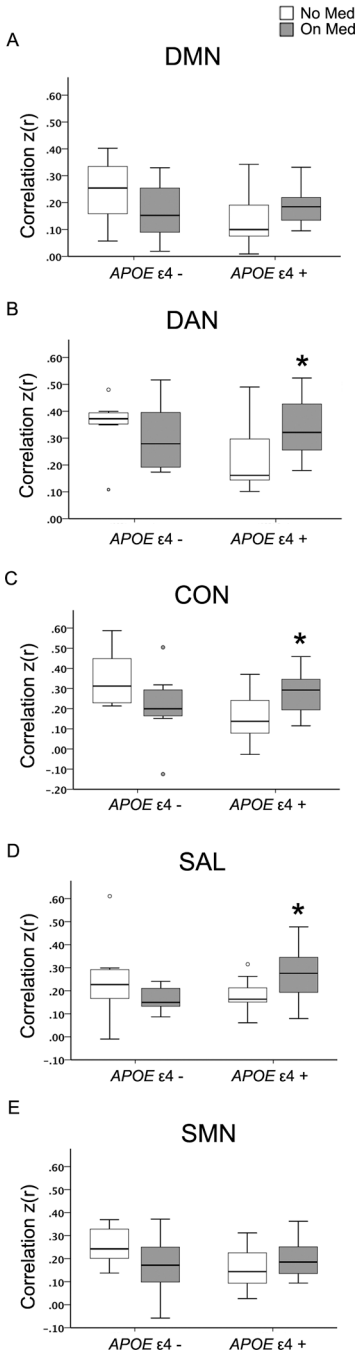

Figure 2.

The effect of cholinesterase inhibitor (ChEI) treatment on composite score resting state functional connectivity in apolipoprotein E (*APOE*) ε4 allele carriers and noncarriers with very mild and mild Alzheimer's disease.

Boxes plot with network composite scores with whiskers extending to 1.5 interquartile range.

*: $p < 0.05$. The circles represent outliers.

On Med refers to participants receiving ChEI treatment, No Med refers to participants not received treatment for AD. See Figure 1 for additional abbreviations.

Table 1

Participants demographics

Participants	Untreated	Treated	p value
N = 44	N = 19	N = 25	
Mean Age (SD), year	75.8 (6.9)	76.2 (5.1)	0.81
Age range, year	64-88	65-84	
Sex, %Male	42.1	48.0	0.77
Mean Education (SD), year	14.7 (2.9)	14.7 (2.8)	1.00
Mean MMSE (SD)	25.5 (3.7)	26.5 (3.1)	0.33
CDR (No. of 0.5/1)	16/3	21/4	1.00
Mean CDR sum of boxes (SD)	2.3 (1.6)	2.8 (1.5)	0.25
APOE genotype, % ε4+	57.9	64.0	0.76

SD: standard deviation, MMSE: mini-mental state examination, for which the range of scores is from 30 (“best”) to 0 (“worst”), CDR: Clinical Dementia Rating, for which CDR 0.5 and CDR 1 indicate very mild and mild AD respectively, the CDR sum of boxes (the sum of individual CDR domain scores) range from 0 to 18, with lower scores indicating better performance. *APOE*: Apolipoprotein E