**Online First**

**Effect of APOE ε4 Status on Intrinsic Network Connectivity in Cognitively Normal Elderly Subjects**

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**Objective:** To examine default mode and salience network functional connectivity as a function of APOE ε4 status in a group of cognitively normal age-, sex-, and education-matched older adults.

**Design:** Case-control study.

**Subjects:** Fifty-six cognitively normal APOE ε4 carriers and 56 age-, sex- and education-matched cognitively normal APOE ε4 noncarriers.

**Main Outcome Measure:** Alterations in in-phase default mode and salience network connectivity in APOE ε4 carriers compared with APOE ε4 noncarriers ranging from 63 to 91 years of age.

**Results:** A posterior cingulate seed revealed decreased in-phase connectivity in regions of the posterior default mode network that included the left inferior parietal lobe, left middle temporal gyrus, and bilateral anterior temporal lobes in the ε4 carriers relative to APOE ε4 noncarriers. An anterior cingulate seed showed greater in-phase connectivity in the salience network including the cingulate gyrus, medial prefrontal cortex, bilateral insular cortex, striatum, and thalamus in APOE ε4 carriers vs noncarriers. There were no groupwise differences in brain anatomy.

**Conclusions:** The observation of functional alterations in default mode and salience network connectivity in the absence of structural changes between APOE ε4 carriers and noncarriers suggests that alterations in connectivity may have the potential to serve as an early biomarker.


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**The Study of Intrinsic Connectivity Networks is Rapidly Emerging as a Tool for Understanding Both Normal Brain Function and Pathologic States Such as Neurodegenerative Disorders.** The default mode network (DMN) has been extensively studied and is one of the resting state networks that undergoes changes as a result of normal aging and is also affected by Alzheimer disease (AD). Brain regions that constitute the DMN include the posterior cingulate, lateral parietal, and medial frontal cortices and the hippocampus. Many of these same regions also undergo preferential amyloid plaque formation in AD.

The APOE ε4 allele is a well-established genetic susceptibility factor for late-onset AD. Individuals who carry the APOE ε4 allele are at a 3- to 4-fold increased risk of developing late-onset or sporadic AD. There is also a strong relationship between APOE ε4 carrier status and β-amyloid deposition. Recently, studies have shown a relationship between amyloid burden, measured with Pittsburgh compound B, and disruption of functional connectivity of the DMN.

Functional connectivity studies reveal changes in the DMN in APOE ε4 carriers decades prior to the typical age at onset of clinical symptoms of AD. For example, a study of young APOE ε4 carriers (individuals aged 20-35 years) examined the DMN at rest and found increased coactivation in retrosplenial, medial prefrontal, and medial temporal lobe regions in the APOE ε4 carriers relative to noncarriers. Middle-aged and older APOE ε4 carriers also show changes in resting-state DMN connectivity. Fleisher et al found increased nodal connectivity in APOE ε4 carriers in several brain regions including the medial and dorsolateral prefrontal cortex and temporal lobe structures, while there was decreased connectivity in the precuneus and medial orbital frontal...
A more recent study compared DMN resting-state connectivity in Pittsburgh compound B–negative APOE ε4 carriers with Pittsburgh compound B–negative APOE ε4 noncarriers and found decreased connectivity from the precuneus to temporal regions and the dorsal anterior cingulate as well as increased connectivity from the precuneus to the dorsal occipital cortex and anterior frontal regions.14

Little is known about the effect of APOE ε4 status on the relationship between intrinsic connectivity networks late in life. The salience network (SN) is anticorrelated with the DMN.15 Recent evidence shows disease specificity in the interplay between the DMN and SN, in particular that connectivity of the SN is intensified in AD.16,17 The objective of this study was to examine the DMN and SN during task-free functional magnetic resonance imaging (fMRI) in an age-, sex-, and education-matched sample of cognitively normal elderly carriers vs noncarriers of the APOE ε4 allele.

### METHODS

#### PARTICIPANTS

Subjects were cognitively normal individuals enrolled in the Mayo Alzheimer’s Disease Research Center (n=9), which is not population based, or the Mayo Clinic Study of Aging (n=103), which is a prospective, population-based study of randomly selected residents of Olmsted County, Minnesota, between the ages of 70 and 89 years at the time of entry who had undergone task-free fMRI and genotyping for the APOE ε4 allele. A group of 56 cognitively normal APOE ε4 carriers were identified and matched 1 to 1 with a group of cognitively normal APOE ε4 noncarriers by age, sex, and education. This study was approved by the Mayo Clinic institutional review board and followed Health Insurance Portability and Accountability Act guidelines. Informed consent was obtained from every subject. Additional details of subject recruitment and design are described in a previous article.18

The criteria for being cognitively normal were (1) being independently functioning community dwellers, (2) not having an active neurologic condition, (3) not having cognitive concerns, (4) and normal findings on neurological and neurocognitive examination. The classification of cognitively normal was based on input from 3 sources: the neurologist’s clinical opinion, based solely on the neurologist’s interview and examination of the participant, neuropsychological test results, as interpreted by the neuropsychologist, and the nurse’s opinion, based exclusively on information about the participant obtained from an informant and reflected in the Clinical Dementia Rating Summary Score.19 On completion of these evaluations, the 3 evaluators discussed each patient and assigned a final consensus diagnosis. Exclusion criteria were (1) medical contraindications to MRI scanning and (2) structural abnormalities (eg, intracranial neoplasms, infarctions).

#### IMAGE ACQUISITION PROTOCOLS

The task-free fMRI signal time series was acquired using a gradient echo–planar sequence (time to repetition, 3000 milliseconds; echo time, 3 milliseconds; field of view, 256 × 256 in-plane matrix; phase field of view, 0.94; and slice thickness, 1.2 mm). All MPRAGE images underwent preprocessing correction for gradient nonlinearity and intensity nonuniformity.20

### TASK-FREE fMRI PREPROCESSING AND ANALYSIS

Preprocessing and data analysis were performed using a combination of the statistical parametric mapping software (SPM5; Wellcome Department of Cognitive Neurology, University College London, London, England), the resting-state fMRI data analysis toolkit (http://www.restfmri.net/forum/index.php)21 group independent component analysis (ICA) of the fMRI toolbox (GIFT) software,22 and in-house–developed software implemented in MATLAB (Mathworks Inc, Natick, Massachusetts).

Preprocessing steps included discarding the first 3 volumes to obtain steady-state magnetization, realignment, slice-time correction, normalization to International Consortium for Brain Mapping gradient echo–planar template, smoothing with a 4-mm full-width-at-half-maximum Gaussian kernel, linearly detrending to correct for signal drift, and 0.01- to 0.08-Hz bandpass filtering to reduce nonneuronal contributions to blood oxygen-dependent fluctuations. In addition, regression correction for spurious variables included rigid body transformation motion effects, global mean signal, white matter, and cerebrospinal fluid.15,23 Removal of global signal by regression improves specificity of connectivity analysis24 and is an attractive alternative to using physiologic cardiac and respiratory inputs as regressors25 to reduce spurious direct correlations when magnetic resonance–compatible physiological measuring systems are not available. This is necessary because gray matter has significantly greater capillary density than white matter,26 and this variability is not accounted for by cerebrospinal fluid and white matter regression alone.24 These preprocessed images were used for both ICA and seed-based connectivity analyses.

### INDEPENDENT COMPONENT ANALYSIS

We used the task-free fMRI preprocessed data described earlier for ICA. The intrinsic connectivity networks were first identified using the group ICA method of GIFT,22 with a low-dimensional estimation of 20 independent components.23 The group ICA analysis on the APOE ε4 noncarrier group was run 100 times using the ICASSO function to ensure stability of the 20 estimated components. The DMN and SN were identified by visual inspection of the group-independent components. The individual subject independent components were derived using the spatial and temporal dual-regression method. The individual subject’s components were then entered into a 1-sample t test analyzed at a threshold of a P < .05, corrected for multiple comparisons using the false discovery rate method. These maps were used to define seed locations described later.

### SEED-BASED Voxelwise Connectivity Analysis

Although ICA can show group changes, we chose seed-based analysis because it allows one to directly evaluate the disease effect on the connectivity of a seed to the rest of the brain. The posterior cingulate is one of the major hubs of the DMN, and the anterior cingulate is one of the major hubs of the SN.28 Therefore, we selected seed locations based on the coordinates with the highest z scores in the posterior cingulate cortex (2,−45,34) in the DMN and in the anterior cingulate cortex (−3,18,42) in the SN identified with ICA in the APOE ε4 noncarrier group.
The average blood oxygen level–dependent signal time course in each seed was correlated with every voxel in the brain for each subject using the Pearson’s correlation coefficient. Prior to group comparisons, the correlation coefficients were converted to z scores using the Fischer r-to-z transformation. Regions that have positive z scores between 2 fluctuating time courses indicate in-phase connections, and the regions with negative z scores indicate out-of-phase connections. These z score images were entered into the statistical analysis.

One-sample t tests were used to display voxelwise connectivity maps. Two-sample 2-sided t tests were performed to compare voxelwise connectivity between APOE ε4 noncarriers and carriers. To assess only in-phase connections, the group comparisons were masked by the out-of-phase connectivity maps identified in the 1-sample t test of APOE ε4 noncarriers. The interpretation of out-of-phase connectivity is an active area of research. Conclusions based on out-of-phase results should be evaluated with the understanding that the interpretation of out-of-phase connectivity may change as the field matures.

All analyses were corrected for multiple comparisons using familywise error at the cluster level [http://www.sph.umich.edu/~nichols/JG2/CorrClusTh.m].

OUT-OF-PHASE CONNECTIVITY IN APOE ε4 NONCARRIERS

The voxel-based morphometry analysis did not detect a significant difference between the groups (false discovery rate P < .05), suggesting that atrophy did not make a marked contribution to the present findings.

We investigated the effect of APOE ε4 status on intrinsic connectivity in an age-, sex-, and education-matched sample of cognitively normal older adults. The major findings were that APOE ε4 carriers show (1) diminished connectivity of the posterior DMN and (2) a relative increase in connectivity in the SN.

The DMN and SN are widely distributed anticorrelated neuroanatomical networks. 15 The DMN is consistently shown to have relatively more activity when individuals are at rest, ie, not performing cognitive tasks. 34 The SN is involved in cognitive control functions such as attention, working memory, and response selection 35 carriers in the left temporo-parietal junction, left middle temporal gyrus, and bilateral anterior temporal lobes (Figure 1).

### RESULTS

#### DEMOGRAPHICS

Our sample consisted of 56 cognitively normal elderly APOE ε4 noncarriers and 56 APOE ε4 carriers matched for age, sex, and education. A Wilcoxon 2-sided rank sum test showed that the APOE ε4 carriers scored lower than the noncarriers on the Short Test of Mental Status though their group mean remained in the normal range. 33 All APOE ε4 carriers and noncarriers had a Clinical Dementia Rating of 0 (Table). 21 (38) 21 (38)

#### POSTERIOR CINGULATE CORTEX SEED CONNECTIVITY

In APOE ε4 noncarriers, the posterior cingulate cortex seed (−3, 18, 42) showed connectivity within the anterior cingulate gyrus and medial prefrontal regions that were posterior to the medial prefrontal areas of connectivity with the posterior cingulate seed. There was also connectivity with the lateral prefrontal cortex, insular cortex, striatum, thalamus, and inferior parietal lobes.

Between-group comparisons showed increased connectivity in APOE ε4 carriers relative to APOE ε4 noncarriers in the cingulate gyrus, medial prefrontal cortex, bilateral insular cortex, striatum, and thalamus (Figure 2).

#### VOXEL-BASED MORPHOMETRY

The voxel-based morphometry analysis did not detect a significant difference between the groups (false discovery rate P < .05), suggesting that atrophy did not make a marked contribution to the present findings.

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#### ANTERIOR CINGULATE CORTEX SEED CONNECTIVITY

In APOE ε4 noncarriers, the anterior cingulate seed (−3, 18, 42) showed connectivity within the anterior cingulate gyrus and medial prefrontal regions that were posterior to the medial prefrontal areas of connectivity with the posterior cingulate seed. There was also connectivity with the lateral prefrontal cortex, insular cortex, striatum, thalamus, and inferior parietal lobes.

Between-group comparisons showed increased connectivity in APOE ε4 carriers relative to APOE ε4 noncarriers in the cingulate gyrus, medial prefrontal cortex, bilateral insular cortex, striatum, and thalamus (Figure 2).

### Table. Sample Demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CN No APOE ε4 (n=56)</th>
<th>CN APOE ε4 (n=56)</th>
</tr>
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<tbody>
<tr>
<td>Women, No (%)</td>
<td>21 (38)</td>
<td>21 (38)</td>
</tr>
<tr>
<td>APOE ε4 genotype, No. (%)</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>1 (2)</td>
<td>0</td>
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<tr>
<td></td>
<td>2/3</td>
<td>2/3</td>
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<tr>
<td></td>
<td>5 (9)</td>
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<td></td>
<td>3/3</td>
<td>3/3</td>
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<td></td>
<td>30 (51)</td>
<td>0</td>
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<td>2/4</td>
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<td>1 (2)</td>
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<td></td>
<td>3/4</td>
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<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>51 (91)</td>
<td>51 (91)</td>
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<td>4/4</td>
<td>4/4</td>
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<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>4 (7)</td>
<td>4 (7)</td>
</tr>
<tr>
<td>Median age, y (range)</td>
<td>79 (64-91)</td>
<td>78 (63-90)</td>
</tr>
<tr>
<td>Median education, y (range)</td>
<td>14 (8-20)</td>
<td>13 (8-20)</td>
</tr>
<tr>
<td>Median short test score (range)^b</td>
<td>36 (30-38)</td>
<td>35 (29-38)</td>
</tr>
<tr>
<td>Median CDR sum of boxes (range)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Abbreviations: CN, cognitively normal; CDR, Clinical Dementia Rating scale.
^a Demographics with the cognitively healthy patients matched 1 to 1.
^b P=.008.
as well as uncertainty, pain, and other homeostatic challenges.\textsuperscript{29,36,37} When individuals engage in cognitively demanding tasks, the DMN deactivates as activity in the SN becomes more pronounced.\textsuperscript{38,39} The right fronto-insular cortex, which is a network hub of the SN, plays a critical role in switching between task-negative and task-positive networks.\textsuperscript{39}

The DMN and SN are in a dynamic balance during resting states. Recent literature comparing AD with other dementias emphasizes the unique relationship between the DMN and SN and how they differ.\textsuperscript{16,17} The DMN and SN have disease specificity; hence, we interrogated both networks. Our results suggest that there is a disruption in the balance between the DMN and SN that is observable even in cognitively normal, asymptomatic APOE\textsubscript{ε}4 carriers. One possible interpretation, although speculative, is that a reduction in the inhibitory control of the posterior DMN may result in an aberrant relative increase in the SN and that this may represent a loss of the ability to appropriately regulate functional networks in clinically asymptomatic individuals.

Sheline et al\textsuperscript{14} recently described functional connectivity changes of the DMN in APOE\textsubscript{ε}4 carriers vs noncarriers. All of their subjects were Pittsburgh compound B negative, which allowed for isolation of the effect of APOE status. They placed a single seed in the precuneus and, similar to our results with a posterior cingulate cortex seed, found a decrease in connectivity in regions of the posterior DMN including the left hippocampus, left parahippocampus, and middle temporal cortex in the APOE\textsubscript{ε}4 carriers relative to noncarriers. Unlike their study, however, we also specifically interrogated the SN with a second seed placed in the anterior cingulate gyrus. Another difference is that, on average, our APOE\textsubscript{ε}4 carriers are 20 years older than their sample.

Several fMRI task activation studies show enhanced activation of brain regions in older APOE\textsubscript{ε}4 carriers vs noncarriers during cognitive tasks.\textsuperscript{40-42} One of these studies used a cohort similar in age to ours and found that the APOE\textsubscript{ε}4 carriers showed greater activation during a verbal paired-associate learning task than the noncarriers in multiple regions in the right hemisphere despite an equivalent level of memory function and comparable brain volume.\textsuperscript{41} Han and Bondi\textsuperscript{43} revised the apolipoprotein E compensatory mechanism hypothesis and proposed that APOE\textsubscript{ε}4 carriers compensate for cognitive declines later in life by invoking additional brain regions to perform cognitive tasks, with a predilection for increases in right hemisphere activity. They further

### Figure 1

Results from within- and between-group comparisons of the posterior cingulate cortex seed. CN indicates cognitively normal; PCC, posterior cingular cortex.

<table>
<thead>
<tr>
<th>PCC seed 2, –45, 34</th>
<th>In-phase connectivity in CN APOE\textsubscript{ε}4 noncarriers</th>
<th>In-phase connectivity reductions in APOE\textsubscript{ε}4 carriers vs noncarriers</th>
</tr>
</thead>
</table>

### Figure 2

Results from within- and between-group comparisons of the anterior cingulate cortex seed. ACC indicates anterior cingular cortex.

<table>
<thead>
<tr>
<th>ACC seed –3, 18, 42</th>
<th>In-phase connectivity in CN APOE\textsubscript{ε}4 noncarriers</th>
<th>In-phase connectivity increases in APOE\textsubscript{ε}4 carriers vs noncarriers</th>
</tr>
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</table>
suggest that frontal-executive cognitive processes might mediate these compensatory mechanisms. Our results indicate a relative increase in the SN but suggest that, rather than compensation, this increase in resting-state connectivity occurs in the context of decreased posterior default mode network connectivity and thus may represent disruption of the balance between the 2 networks.15

The primary biologic effect of APOE ε4 appears to be an increase in brain β-amyloid burden earlier in life. Although our subjects were clinically normal, resting-state MRI shows that there are subclinical consequences that are evident even in cognitively normal elderly individuals.7,9 Our results reflect changes in resting state connectivity that may be a direct result of higher amyloid burden in APOE ε4 carriers who are more likely to harbor clinically silent amyloid plaques and who are more likely to develop AD in the future.39

Based on the observation that regions of the DMN that are metabolically active in young adults also show a striking correlation with the pattern of amyloid deposition in older adults with AD, Buckner et al12 suggested that the areas of enhanced metabolism may provide regional conditions that are conducive to amyloid deposition. Filippini and colleagues12 show that connectivity within the DMN is increased in young adult APOE ε4 carriers (mean age, 28 years) relative to noncarriers, thus potentially setting the stage for earlier deposition of amyloid in regions of the DMN in carriers of the APOE ε4 allele. We propose that, after an initial phase of increased resting metabolism in young adulthood, APOE ε4 carriers show a more rapid decline in DMN connectivity than APOE ε4 noncarriers as they age. Future studies of the resting state in APOE ε4 carriers and noncarriers throughout middle adulthood will ultimately provide a better understanding of the trajectory of the DMN during the life span and the age at which these declines begin to occur.

Our study has several strengths. We have a fairly large sample size of thoroughly evaluated and well-characterized individuals. Our groups are matched for age, sex, and education, effectively ruling out these variables as potential explanations for the group differences observed in resting-state connectivity. Second, we performed both ICA and seed-based analyses, strengthening our results. Third, results from our voxel-based morphometry analysis suggest that our findings are not the result of relatively greater gray matter loss in the APOE ε4 carriers.

Our cohort consists of older individuals. The changes we describe may not generalize to changes in connectivity in younger cohorts. Although nearly all of our patients are derived from a population-based sample, they represent a subset of individuals who are willing to undergo neuroimaging studies. This could potentially limit the generalizability of our findings. Finally, although studies clearly document an association between APOE ε4 status and amyloid, we do not have imaging evidence of amyloidosis to confirm a direct relationship.

There is a dynamic balance between the DMN and SN, and this balance is interrupted in cognitively normal APOE ε4 carriers relative to noncarriers. Specifically, there are reductions in posterior DMN connectivity but a concomitant increase in SN connectivity at rest. Our results add to our understanding of functional brain changes in individuals at risk of developing AD and suggest that prodromal alterations in connectivity may have the potential to serve as a biomarker.

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