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Functional alteration patterns of default mode network: comparisons of normal aging, amnesic mild cognitive impairment and Alzheimer's disease

Jungho Cha¹, Hang Joon Jo², Hee Jin Kim³, Sang Won Seo³, Han-Soo Kim¹, Uicheul Yoon⁴, Hyunjin Park⁵, Duk L. Na³, and Jong-Min Lee^{1,*}

¹Department of Biomedical Engineering, Hanyang University, Seoul, South Korea

²Scientific and Statistical Computing Core, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892, USA

³Department of Neurology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea

⁴Department of Biomedical Engineering, College of Health and Medical Science, Catholic University of Daegu, Gyeongsan-si, South Korea

⁵School of Electronic Electrical Engineering, Sungkyunkwan University, South Korea

Abstract

Most of default mode network (DMN) studies in patient with amnesic mild cognitive impairment (aMCI) and Alzheimer's disease (AD) were based only on the comparing two groups, namely patients and controls. Information derived from comparing the three groups, normal, aMCI, and AD group, simultaneously may lead us to better understand the progression of dementia. The purpose of this study was to evaluate functional connectivity of DMN in the continuum from normal through aMCI to AD. Differences in functional connectivity were compared between the three groups using independent component analysis. The relationship between the functional connectivity and disease progression was investigated using multiple regression analysis with Mini-Mental State Examination (MMSE) scores. The results revealed differences throughout the left posterior cingulate cortex (PCC), left middle temporal gyrus (MTG), right middle frontal gyrus (MFG), and bilateral parahippocampal gyrus (PHG). Both patients with aMCI and AD showed decreased connectivity in the left PCC and left PHG compared with healthy subjects. Furthermore, patient with AD also showed decreased connectivity in the left MTG and right PHG. Increased functional connectivity was observed in the right MFG of patients with AD compared with other groups. MMSE scores exhibited significant positive and negative correlations with functional connectivity in PCC, MTG and MFG regions. Taken together, an increased functional connectivity in the MFG for AD patients might compensate for the loss of function in the PCC, MTG via compensatory mechanisms in cortico-cortical connections.

Keywords

Resting-state; Functional magnetic resonance imaging; Alzheimer's Disease; Mild Cognitive Impairment; Default Mode Network

Introduction

Alzheimer's disease (AD) is the most common cause of dementia and is characterized by the formation of neuritic plaques and neurofibrillary tangles (Braak & Braak, 1996), whereas mild cognitive impairment (MCI) refers to a transitional state between the cognition of normal aging and dementia (Petersen *et al.*, 1999). Although MCI may evolve into dementia with various etiologies or even return to a normal cognitive state, still many studies have suggested that amnesic subtype of MCI (aMCI), characterized by episodic memory loss, is a robust indicator of an increased likelihood of development of MCI into AD (Gauthier *et al.*, 2006; Yaffe *et al.*, 2006).

Recently, low-frequency fluctuations approach during the resting-state (RS) has been widely used to analyze functional connectivity in functional magnetic resonance imaging (fMRI) (Biswal *et al.*, 1995). One major finding of RS fMRI studies was notion of default mode network (DMN), which is internally driven conceptual processing that occurs during the conscious RS (Binder *et al.*, 1999; Greicius *et al.*, 2003). The DMN has been shown to have high RS metabolism (Raichle *et al.*, 2001). Most previous studies mainly reported decreased connectivity in several DMN regions in patients with AD or aMCI compared with healthy individuals (Greicius *et al.*, 2004; Rombouts *et al.*, 2005; Sorg *et al.*, 2007). Conversely, some studies showed several regions that showed increased functional connectivity in patients with aMCI compared with healthy individuals (Bai *et al.*, 2009; Qi *et al.*, 2010). Nevertheless, the progression of DMN connectivity in patients with aMCI or AD remains uncertain. Most of these studies were based on the comparison of only two groups (Greicius *et al.*, 2004; Sorg *et al.*, 2007; Wang *et al.*, 2007; Bai *et al.*, 2009; Zhang *et al.*, 2009; Qi *et al.*, 2010). The alteration patterns of DMN connectivity in patients with AD, aMCI, and healthy individuals remain, therefore, poorly understood. Comparison of functional connectivity in DMN between three groups simultaneously may provide better information than studies that only compared between two groups in the analysis of the progression of dementia; it would provide alteration patterns of decreased and increased functional connectivity as the disease progresses from normal aging to aMCI and AD.

The purpose of our study was to investigate patterns of functional alteration in the RS DMN using independent component analysis (ICA) across the continuum from normal aging to aMCI and AD, and explore whether the differences in functional connectivity are associated with differences in cognitive ability. Thus, this study examined (1) altered functional connectivity of the DMN in patients with AD or aMCI compared with healthy individuals; and (2) whether the regions exhibiting altered functional connectivity varied with a well known score of disease progression, Mini-Mental State Examination (MMSE) score, in patients with AD or aMCI. The MMSE score can quantify general cognitive function and reflects the progression of dementia (Folstein *et al.*, 1983). The evaluation of the relationship between functional connectivity of DMN and cognitive abilities (i.e., MMSE scores) may provide important information to understand how functional alterations in patients with aMCI or AD progresses over time.

Materials and Methods

Participants

Sixty-two healthy subjects (male/female ratio, 17/45; age, 68.5 ± 8.0 years), 34 elderly patients with aMCI (18/16, 68.4 ± 7.9 years), and 37 elderly patients with AD (10/27, 72.8 ± 8.2 years) participated in this study. We obtained written informed consent, according to the Declaration of Helsinki, from all patients and controls and the study was approved by the Institutional Review Board of the Samsung Medical Center, Seoul, South Korea. Patients with aMCI met Petersen's criteria for MCI, with the following modifications (Petersen *et al.*,

1999): (1) subjective memory complaint by the patient or his/her caregiver; (2) normal general cognitive function above the 16th percentile on the MMSE; (3) normal activities of daily living (ADL), as judged by both an interview with a clinician and the standardized ADL scale described previously (Seo *et al.*, 2007); (4) objective memory function decline below the 16th percentile on neuropsychological tests; and (5) no dementia. All patients with AD fulfilled the criteria for probable AD proposed by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann *et al.*, 1984). The healthy control group consisted of 62 individuals who had no history of neurological or psychiatric illnesses and exhibited normal performance in the neuropsychological tests. All participants underwent clinical interviews and neurological examinations, as described previously (Seo *et al.*, 2007). The demographic and clinical data of the participants are presented in Table 1.

Data acquisition

All imaging was carried out at the Samsung Medical Center using a Philips Intera Achieva 3.0 Tesla scanner equipped with an 8-channel SENSE head coil (Philips Healthcare, the Netherlands). Whole-brain echo planar imaging (EPI) time-series scans (TR= 3s; TE = 35 ms; flip angle = 90°; $1.7 \times 1.7 \times 4 \text{ mm}^3$ voxel resolution) consisted of 100 volumes and lasted for about 5 min. During each scan, participants were instructed to rest with their eyes open and fixate a crosshair that was centrally projected in white against a black background. A high-resolution T1-weighted anatomical image was also acquired using a magnetization-prepared gradient echo (MPRAGE) sequence (TR = 9.9 ms; TE = 4.6 ms; flip angle = 8°; $0.5 \times 0.5 \times 0.5 \text{ mm}^3$ voxel resolution).

fMRI data preprocessing

Imaging data were assessed and processed using AFNI (Analysis of Functional NeuroImages, <http://afni.nimh.nih.gov/afni/>) and FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) (Cox, 1996; Jenkinson & Smith, 2001; Jenkinson *et al.*, 2002; Smith, 2002) software. EPI data were screened for head motion artifacts (Power *et al.*, 2012) using the threshold level 0.3 mm for the Euclidean L2 norm of motion displacement per TR interval, which passed the sudden motion qualification of AFNI software. Head-coil artifacts were also visually checked by observing the contribution of local white matter signals in a time series, and no strong artifact was detected across brain tissue types (Jo *et al.*, 2010). The first three volumes from each functional image were discarded to allow for the stabilization of the magnetic field. Subsequently, the slice timing and head motion of RS EPI time series were corrected and spatial smoothing was performed using a 6mm full-width-at-half-maximum (FWHM) Gaussian kernel and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with $\sigma=100$ s). Preprocessed EPI data were aligned to the T1-weighted anatomical image and normalized to the MNI152 template space for each individual (Mazziotta *et al.*, 2001).

Independent component analysis

To identify time series coherences across voxels or regions, probabilistic ICA was performed, as implemented in FSL's Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) software (www.fmrib.ox.ac.uk/fsl/melodic; Beckmann & Smith, 2004). MELODIC automatically estimates the number of components from each datum using the Laplace approximation to the Bayesian evidence of the model order (Minka, 2000; Beckmann & Smith, 2004). The whitened observations were decomposed into sets of vectors that described signal variation across the temporal (time courses) and spatial (maps) domains by optimizing for non-Gaussian spatial source distributions using a fixed-point iteration technique (Hyvarinen, 1999). The application of ICA to fMRI data allows the removal of high- and low-frequency artifacts (McKeown *et al.*,

1998; Quigley *et al.*, 2002). For each component, the z value reflected the degree to which the time series of each voxel was associated with one of the specific components. We used spatial differences across z values of each component to determine functional connectivity levels. Therefore, the alterations of “functional connectivity” refers to the spatial changes in z values of each component. All components were then transformed into stereotaxic space and resampled to a voxel dimension of $2 \times 2 \times 2 \text{ mm}^3$. The chosen component of the DMN was selected from the independent components of each individual using a combination of manual and template-matching procedures (Greicius *et al.*, 2004). The manual selection procedure has the advantage of being highly accurate, but it may be subject to human error and is a time-consuming process in which all components must be checked (Franco *et al.*, 2009). The template-matching procedure has the advantage of reducing time and human error compared with manual selection procedure. The combination of manual and automated techniques might be the best approach for selecting the DMN component (Franco *et al.*, 2009). For the template-matching procedure, a template of the DMN was developed based on the peak regions reported previously (Greicius *et al.*, 2004; Wu *et al.*, 2011). Each region in the template was a sphere with a radius of 5mm. The difference of the average z values of voxels between inside and outside the template was calculated for each component. Among the five components exhibiting the greatest differences, manual selection of one component was performed by two trained researchers (JHC and HSK). This selection was based on the functional connectivity of the posterior cingulate cortex (PCC), prefrontal cortex, and inferior parietal lobule (IPL), which are assumed to be the core regions associated with the DMN (Buckner *et al.*, 2008). Finally, the component selected by the raters was determined to be the DMN component.

Group comparisons

Intra- and intergroup analysis—To explore the within-group functional connectivity differences of the DMN patterns, a one-sample t test was performed on the individual normalized functional connectivity of DMN maps of each group. The significance threshold was set at uncorrected $P < 0.001$ ($|t| > 3.46$ (healthy subjects), > 3.61 (aMCI), and > 3.58 (AD)) with a cluster size $> 312 \text{ mm}^3$. An analysis of covariance (ANCOVA) was performed to determine the differences of functional connectivity between the three groups using age, sex, and education as covariates. Using the AFNI's AlphaSim program, Monte Carlo simulations were performed to control for Type I errors (parameters: individual voxel p -value=0.01, simulated 10,000 times iteratively, 6mm FWHM Gaussian filter width with a whole-brain mask). The AlphaSim program provides an estimate of the overall significance level achieved for various combinations of individual voxel probability thresholds and cluster size thresholds (Poline *et al.*, 1997). We obtained a corrected significance level of $P_{\alpha} < 0.05$ (uncorrected individual voxel height threshold of $P < 0.01$, $F > 4.776$ with a minimum cluster size of $1,000 \text{ mm}^3$). Subsequently, to examine the inter-group differences, *post hoc* two-sample t tests were performed between pairs of groups for voxel-wise statistics (corrected significant level of $P_{\alpha} < 0.05$).

Relationship between functional connectivity of DMN and MMSE scores—To investigate the relationship between the functional connectivity of DMN and disease progression in the patients, we performed multiple regression analysis between the functional connectivity and MMSE scores in each patient group. Age, sex, and education were entered as covariates in the regression analysis.

Results

Group comparisons

Intra- and inter-group analysis—One-sample *t* test was used to generate group maps of DMN connectivity, including the PCC/precuneus, middle frontal gyrus (MFG), IPL, and middle temporal gyrus (MTG). The majority of DMN clusters were consistent across all groups (Fig 1 and see Supporting Information Table S1 for the details of clusters in DMN in all groups).

The results obtained from ANCOVA using age, sex, and education as covariates clearly showed significant differences in functional connectivity of DMN between the aMCI, AD, and healthy subjects groups. Significant group differences of functional connectivity in DMN were observed in the left PCC, left MTG, right MFG, and bilateral parahippocampal gyrus (PHG) with hippocampus at $P_{\alpha} < 0.05$ (AlphaSim corrected, uncorrected $P < 0.01$ at a cluster size of at least 125 voxels; see Fig 2 (a) and Table 2 for a detailed list of the regions). Then, as shown in Fig 2 (b–d) and Table 3, we performed the *post hoc* two-sample *t* tests between pairs of groups in voxel-wise fashion with corrected $P_{\alpha} < 0.05$. Lower functional connectivity was found in patients with AD and aMCI compared with healthy subjects in the left PCC and left PHG. In addition, the functional connectivity in the left MTG and right PHG decreased in patients with AD compared with healthy subjects. Conversely, the increased functional connectivity in right MFG was found in patient with AD compared with patient with aMCI and healthy subjects.

Relationship between functional connectivity of DMN and MMSE scores—To evaluate the relationship between differences in functional connectivity of DMN and cognitive ability in the patients, we performed multiple regression analysis with age, sex, and education as covariates. In the aMCI group, as shown in Fig 3 (a), there was a significant negative correlation in the bilateral medial frontal gyrus and significant positive correlation in the cerebellum with corrected $P_{\alpha} < 0.05$. In the AD group, as shown in Fig 3 (b), there were significant positive correlations in the left IPL, left fusiform gyrus including MTG and left PCC. There were also significant negative correlations in the right MFG, inferior frontal gyrus, and cerebellum (corrected $P_{\alpha} < 0.05$). These results are presented in Table 4.

Discussion

Our major findings were as follows: 1) both patients with aMCI and AD showed decreased functional connectivity in the left PCC and left PHG compared with healthy subjects. Furthermore, patient with AD had significantly decreased functional connectivity in the left MTG and right PHG. There was no significant decrease in functional connectivity in patients with AD compared with patient with aMCI; 2) patients with AD exhibited significantly increased functional connectivity in the right MFG compared with patients with aMCI and healthy subjects 3) in patients with aMCI, there were significant positive correlations in right cerebellum and negative correlations in bilateral medial frontal gyrus between functional connectivity of DMN and MMSE scores; and 4) patients with AD exhibited significant positive correlations in left IPL, left fusiform gyrus including MTG and left PCC and negative correlations in right MFG, left cerebellum and right inferior frontal gyrus between functional connectivity of DMN and MMSE scores. Taken together, our findings suggested that an increased functional connectivity in the right MFG might compensate for the loss of function in the PCC, MTG and PHG via compensatory mechanisms in cortico-cortical connections.

Decreased functional connectivity of DMN in patients with AD and aMCI

We found that patients with aMCI and AD had significantly decreased functional connectivity in the left PCC and left PHG including hippocampus compared with healthy subjects. These results are consistent with those of previous studies (Greicius *et al.*, 2004; Bai *et al.*, 2009; Qi *et al.*, 2010; Binnewijzend *et al.*, 2012), which showed that the connectivity of DMN, including the PCC and PHG, decreased in patients with AD compared with healthy individuals (Greicius *et al.*, 2004; Binnewijzend *et al.*, 2012) and that patients with aMCI exhibited decreased connectivity compared with healthy individuals, in the same regions (Bai *et al.*, 2009; Qi *et al.*, 2010). Previous studies demonstrated that the PCC/precuneus plays a pivotal role in the DMN (Fransson & Marrelec, 2008) and that the PCC is the region that shows metabolic abnormalities in early AD (Minoshima *et al.*, 1997). Thus, our findings replicated previous results suggesting that patients with AD or aMCI might have decreased functional connectivity in DMN, especially in the PCC and PHG.

We also showed that functional connectivity in the left MTG was significantly different between patients with AD and healthy subjects. A previous study showed that there were structural changes in the temporal lobe between healthy subjects and AD patient group (Pennanen *et al.*, 2004; Im *et al.*, 2008). With structural changes in the temporal lobe, our results confirm a notable decrease in functional change in the temporal area in patients with AD.

We also showed that functional connectivity in the left PHG decreased in both patients with aMCI and AD compared with healthy subjects. On the contrary, the functional connectivity in the right PHG only decreased in the patients with AD compared with healthy subjects. In the patients with aMCI, the reduced functional connectivity in the left PHG can be related to the reduced functional connectivity between the left PHG and left PCC reported earlier (Sorg *et al.*, 2007; De Vogelaere *et al.*, 2012). Taken together, the decreased functional connectivity in the left PCC, left MTG and PHG with hippocampus could be a biomarker for identifying patients with aMCI and AD.

Increased functional connectivity of DMN in patients with AD and aMCI

Our second major finding was the presence of increased connectivity in the right MFG for patients with AD compared with patients with aMCI and healthy subjects. The MFG has been identified as an important region in the DMN (Buckner *et al.*, 2008). In this region, some studies also found increased connectivity based on RS functional connectivity in patients with AD (Wang *et al.*, 2006; Wang *et al.*, 2007; Zhang *et al.*, 2009). A study of hippocampus-related connectivity during RS showed increased connectivity between the left hippocampus and the right dorsolateral prefrontal cortex in patients with AD (Wang *et al.*, 2006). Another ROI study found that patients with AD had increased positive correlations within the prefrontal, parietal and occipital lobes (Wang *et al.*, 2007). A PCC-related connectivity analysis also showed increased connectivity in the left frontal and parietal cortices (Zhang *et al.*, 2009). In addition to the RS fMRI analysis, the increased event-related functional activity in prefrontal regions was also demonstrated in a PET based study (Grady *et al.*, 2003). In normal aging, healthy elderly individuals exhibit increased activity in the frontal regions compared with healthy young individuals (Cabeza *et al.*, 2002; Logan *et al.*, 2002). Although many areas might exhibit increased connectivity, this has been reported mainly in the frontal region (see the Supporting Information Table S2 for reviews of previous studies on frontal regions in AD, aMCI, and various other categories).

Recently, it was shown that decreased and increased functional connectivity of DMN coexists in patients with aMCI compared with healthy individuals (Qi *et al.*, 2010). Those authors suggested that the increased connectivity observed in patients might be due to a

compensatory mechanism for the loss of cognitive function. This interpretation of this increased connectivity of DMN in patients with AD was reported in several studies, although it was not found in the analysis of connectivity using ICA. From the point of view of compensatory mechanisms, these findings suggest that increased functional connectivity in the right MFG may reflect compensatory recruitment after loss of functional connectivity in the left PCC, left MTG, and bilateral PHG including hippocampus.

Alternatively, the increased functional connectivity in the MFG may reflect an early aberrant excitatory response to Amyloid beta (A β) (Mormino *et al.*, 2011). In elderly individuals, the task-related fMRI study reported a failure to deactivate the medial prefrontal regions during successful episodic memory encoding (Sperling *et al.*, 2009). Those authors proposed that A β deposition might cause aberrant modulation in the frontal region, and it is possible that persistent activation reflects the aberrant spiking activity that has been observed in an in vitro model and may be excitotoxic (Palop *et al.*, 2007). It is possible that compensatory responses may lead to downstream reduction in activity as neurons undergo excitotoxicity (Mormino *et al.*, 2011).

Relationship between functional connectivity of DMN and MMSE scores

We found significant positive and negative correlations between functional connectivity in patients with AD and aMCI and the MMSE scores. In the AD group, we found that the functional connectivity in left PCC, left IPL and left fusiform gyrus including MTG correlated significantly with MMSE scores. The correlation in PCC was also reported in an RS fMRI analysis using a regional coherence method (He *et al.*, 2007). It was reported that the resting metabolism in the PCC/precuneus measured using fluorodeoxyglucose positron emission tomography (PET) was correlated with disease progress evaluated using MMSE scores (Salmon *et al.*, 2000; Herholz *et al.*, 2002; Buckner *et al.*, 2005). This result supports the contention that AD-related changes in the DMN in the left PCC were associated with cognitive score, in particular with MMSE scores. The functional connectivity in left PCC and left MTG was correlated significantly with MMSE scores. Taken together, the functional connectivity in the left PCC and left MTG should reflect the progression of the disease in patient with AD.

In patients with AD, we found significant negative correlations between functional connectivity in the right MFG, left cerebellum and right inferior frontal gyrus and MMSE scores. The functional connectivity in frontal regions also exhibited negative correlations with MMSE scores in aMCI. Although several hypotheses about the increased connectivity in patients with AD and aMCI exist, our finding demonstrated that the negative correlations between the functional connectivity of frontal regions and MMSE scores provided further support for the compensatory hypothesis in frontal regions.

We performed additional correlation analysis using MMSE scores from pooling AD and aMCI patients, because aMCI refers to a transitional state between the cognition of normal aging and dementia. However, the results did not show much difference from the analysis using each patient (see Supporting Information Table S3, S4 and Figure S1 and S3).

Methodological considerations

Several previous DMN analysis studies used a masking procedure (Greicius *et al.*, 2004; Rombouts *et al.*, 2005; Sorg *et al.*, 2007; Wang *et al.*, 2007). Using a masking procedure, authors have shown only decreased connectivity of DMN determined by the specific mask in patients with AD or aMCI. However, other studies did not use a masking procedure. A whole-brain analysis was performed in these cases (Wang *et al.*, 2006; He *et al.*, 2007; Zhang *et al.*, 2009; Qi *et al.*, 2010), which revealed not only decreased, but also increased

connectivity of DMN in patients with AD or aMCI. Based on these results, the difference between analyses using a masking procedure and a whole-brain analysis is a sensitive and important issue. In this study, we assumed the existence of increased connectivity in patients with AD or aMCI. Therefore, we performed the whole-brain analysis.

ICA potentially decomposes DMN co-activations into more than one component and physiological noise may be included in the component. Because of these problems, two expert researchers (JHC and HSK) carefully selected the components for each individual subject via visual inspection. As a result, although we did not check every component, there was no splitting of DMN connectivity among the five components exhibiting the greatest differences between template and component.

Other groups also studied the comparison of functional connectivity of DMN in healthy subjects, MCI, and AD (Rombouts et al., 2005; Binnewijzend et al., 2012). However, it was not adopting a resting-state DMN but deactivation states during visual encoding task and non-spatial working memory task (Rombouts et al., 2005). In addition, another study did not show differences between three groups (Binnewijzend et al., 2012). Some chose the ROIs based on the statistical results themselves, which leads to biased estimates (Kriegeskorte et al., 2009). We performed additional ROI-based analyses to confirm the voxel-based analysis results (see the Supporting Information Table S4, Figure S2, S3 and S4). While both analyses are almost the same, there are some differences in the findings. We believe that the differences are mainly due to the methodological issues such as smoothness or signal to noise ratio (SNR) in ROI masks. The principal explanation for the differences in left MTG and right MFG may come from the methodological differences.

The strengths or limitations of our study

The strengths of our study are its prospective setting. We adopted the standardized MRI imaging protocol and standardized phenotyping of cognitive impairments. However, there are some limitations. First, because we did not perform pathological studies, we were not able to exclude the possible inclusion of patients with other degenerative pathologies. Second, because of cross-sectional nature of our study, we could only suggest the differences in functional connectivity between healthy individuals, aMCI, and AD. A longitudinal study would have been better suited to investigate the progression of functional connectivity based on a compensation hypothesis. Therefore, a longitudinal study is required for better analysis of functional connectivity. Third, our results might be confounded by gray matter loss. To explore this issue further, we performed voxel-based morphometry analysis (See the Supporting Information Figure S5). It would be ideal to quantify functional connectivity differences while controlling for the gray matter loss, but that analysis is rather difficult, since there are notable resolution differences between EPT and T1 images. We leave the important problem of exploring relationship between functional connectivity and gray matter density as future work. Nevertheless, our study is noteworthy because it is the first one to the best of our knowledge suggesting that an increased functional connectivity in the MFG might compensate for the loss of function in the PCC, MTG, and PHG including hippocampus via compensatory mechanisms in cortico-cortical connections.

Conclusion

The present study demonstrated that brain connectivity in the DMN was significantly different in patients with AD or aMCI compared with healthy subjects. The decreased functional connectivity in the left PCC and PHG with hippocampus could be a biomarker for identifying patients with aMCI and AD. The patients with AD exhibited significantly increased functional connectivity in the right MFG. In addition, our results showed that the functional connectivity in left PCC, left MTG and right MFG might reflect the progression

of disease in patient with AD. Taken together, an increase in connectivity in the MFG may compensate for the loss of function in the PCC and PHG including hippocampus via compensatory mechanisms that occur in cortico-cortical connections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AD	Alzheimer's disease
Aβ	amyloid beta
ADL	activities of daily living
aMCI	amnesic subtype of MCI
ANCOVA	analysis of covariance
DMN	default mode network
EPI	echo planar imaging
fMRI	functional magnetic resonance imaging
FWHM	full-width-at-half-maximum
ICA	independent component analysis
IPL	inferior parietal lobule
MCI	mild cognitive impairment
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components
MFG	middle frontal gyrus
MMSE	mini-mental state examination
MPRAGE	magnetization-prepared gradient echo
MTG	middle temporal gyrus
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
PCC	posterior cingulate cortex
PET	positron emission tomography
PHG	parahippocampal gyrus
ROI	region of interests
RS	resting-state

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Fig 1. The functional connectivity of default mode network (DMN) in healthy subjects, and patients with amnesic mild cognitive impairment (aMCI) or Alzheimer's disease (AD)
Brain regions showed significant functional connectivity of DMN (a) in healthy subjects, (b) patients with aMCI (c) and patients with AD. The significance threshold was set at uncorrected $P < 0.001$ ($|t| > 3.46$ (healthy subjects), > 3.61 (aMCI), and > 3.58 (AD)) with a cluster size $> 312 \text{ mm}^3$. The images are oriented with the anterior side placed at the top and the left side placed to the right. The red and blue color represents positive and negative functional connectivity respectively.

Fig 2. Brain regions exhibiting significant differences in functional connectivity of DMN
 (a) Brain regions showed significant differences in functional connectivity of DMN between healthy subjects, patients with aMCI and patients with AD ($P_{\alpha} < 0.05$ (uncorrected $P < 0.01$, $F > 4.78$, 1000mm^3 , AlphaSim corrected)). The following were results of the *post hoc* two-sample t tests between pairs of the healthy subjects, patient with AD and aMCI groups in voxel-wise analysis. The significant differences in brain regions was found (b) in patients with aMCI compared with healthy subjects, (c) in patients with AD compared with patients with aMCI (d) and in patients with AD compared with healthy subjects ($P_{\alpha} < 0.05$). The images are oriented with the anterior side placed at the top and the left side placed to the right.

Fig 3. Brain regions exhibiting significant correlations between functional connectivity of DMN and mini-mental state examination (MMSE) scores in voxel wise analysis

The brain regions which were significantly correlated by multiple regression analysis between the functional connectivity of DMN and MMSE scores (a) in the patients with aMCI, and (b) in the patients with AD. The significance threshold was set at corrected $P_{\alpha} < 0.05$ (uncorrected $P < 0.01$, > 2.75 (aMCI), and > 2.73 (AD) with a cluster size $> 1000 \text{ mm}^3$). The images are oriented with the anterior side placed at the top and the left side placed to the right.

Table 1

Demographic and clinical findings of healthy subjects, and patients with amnesic mild cognitive impairment (aMCI) or Alzheimer's disease (AD).

	Healthy Subjects	aMCI	AD
Number of Subjects	62	34	37
MMSE Score	28.6±1.93	27.1±2.1	16.8±6.9
Age	68.5±8.0	68.4±7.9	72.8±8.2
Sex (M/F)	17/45	18/16	10/27
Education	10.9±5.2	11.5±5.2	10.9±5.3

Demographic and clinical finding of healthy subjects, and patients with aMCI or AD Data for age, Education and MMSE, mini-mental state examination, score: mean ± SD Data for sex, M: male, F: female

Significant differences were noted in MMSE scores ($P<0.0001$). The p -value was obtained by one-way analysis of variance test.

Brain regions with significant differences in functional connectivity of default mode network (DMN) between healthy subjects and in patients with aMCI or AD.

Table 2

Brain regions	R/L	Coordinates (mm)			Peak F values	Voxels
		x	y	z		
<i>Healthy subjects vs aMCI vs AD</i>						
PCC	L	-10	-46	30	9.85	373
MTG	L	-52	-4	-16	12.3	303
MFG	R	42	10	42	11.5	144
PHG	R	34	-34	-14	10.4	129
PHG	L	-26	-38	-6	10.8	126

PCC: posterior cingulate gyrus, MTG: middle temporal gyrus, MFG: middle frontal gyrus, PHG: parahippocampal gyrus. Threshold: corrected $P_q < 0.05$ (uncorrected individual voxel height threshold of $P < 0.01$, $F > 4.776$ with a minimum cluster size of $1,000\text{mm}^3$)

Table 3

Results of post hoc two-sample t tests between pairs of groups of the healthy subjects, patient with AD and aMCI groups in voxel-wise analysis.

Brain Regions	R/L	Coordinates (mm)			Peak t value	Voxels
		x	y	z		
<u>Healthy subject vs aMCI</u>						
Precuneus	L	-4	-62	38	3.46	244
PHG	L	-20	-36	-4	3.96	84
PCC	L	-16	-42	14	3.11	7
<u>aMCI vs AD</u>						
MFG	R	44	14	50	-3.66	84
<u>Healthy subject vs AD</u>						
PCC	L	-10	-46	30	4.17	355
MTG	L	-52	-4	-16	4.91	295
MFG	R	42	10	42	-4.75	137
PHG	R	34	-34	-14	4.55	124
PHG	L	-26	-38	-6	4.2	103

PHG: parahippocampal gyrus, MFG: middle frontal gyrus, PCC: posterior cingulate gyrus, MTG: middle temporal gyrus. Positive values: Healthy subjects > aMCI, aMCI > AD, and Healthy subjects > AD; Negative values: aMCI > Healthy subjects, AD > aMCI, and AD > Healthy subjects; Threshold: corrected $P_q < 0.05$

Table 4

Results of multiple regression analysis between functional connectivity of DMN and mini-mental state examination (MMSE) score in patients with aMCI or AD in voxel wise analysis.

Brain regions	R/L	Coordinates (mm)			Peak t value	Voxels
		x	y	z		
<u>aMCI</u>						
Medial frontal gyrus	L	0	-8	58	-4.21	194
Medial frontal gyrus	R	10	0	60	-4.9	123
Cerebellum	R	52	-58	-48	4.43	122
<u>AD</u>						
MFG	R	42	38	30	-4.07	272
IPL	L	-48	-78	46	4.02	252
Cerebellum	L	0	-72	-22	-4.75	226
Inferior frontal gyrus	R	54	34	-2	-4.21	211
Fusiform gyrus	L	-38	-12	-32	4.67	207
PCC	L	-6	-48	28	3.65	131

MFG: middle frontal gyrus, PCC: posterior cingulate gyrus, IPL: inferior parietal lobule. Threshold: $P_{\text{q}} < 0.05$ (uncorrected individual voxel height threshold of $P < 0.01$ with a minimum cluster size of 1,000mm³)