Quantitative assessment of resting-state functional connectivity MRI to differentiate Amnestic Mild Cognitive Impairment, late-onset Alzheimer's disease from Normal subjects

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Background

Alzheimer's disease (AD) is a neurological disorder with brain network dysfunction. Investigation of the brain network functional connectivity (FC) alterations using resting-state functional MRI (rs-fMRI) can provide valuable information about the brain network pattern in early AD diagnosis.

Purpose

To quantitatively assess FC patterns of resting-state brain networks and graph theory metrics (GTMs) to identify potential features for differentiation of amnestic mild cognitive impairment (aMCI) and late-onset AD from normal.

Study Type

Prospective.

Subjects

14 normal, 16 aMCI, and 13 late-onset AD.

Field Strength/Sequence

3.0T; rs-fMRI: single-shot 2D-EPI & T1-weighted structure: MPRAGE.

Assessment

By applying bivariate correlation coefficient and Fisher transformation on the time series of pre-defined ROIs' pairs, correlation coefficient matrixes and ROI-to-ROI Connectivity (RRC) were extracted. By thresholding the RRC matrix (with a threshold of 0.15), a graph adjacency matrix was created to compute GTMs.

Statistical Tests

ROI-based analysis: parametric multivariable statistical analysis (PMSA) with a false discovery rate using (FDR)-corrected $p < 0.05$ cluster-level threshold together with post-hoc uncorrected $p < 0.05$ connection-level threshold.

Graph-theory analysis (GTA): p-FDR-corrected < 0.05.

One-way ANOVA and Chi-square tests were used to compare clinical characteristics.

Results

PMSA differentiated AD from normal, with a significant decrease in FC of default mode, salience, dorsal attention, frontoparietal, language, visual, and cerebellar networks. Furthermore, significant increase in overall FC of visual and language networks was observed in aMCI compared to normal. GTA revealed a significant decrease in global-efficiency ($28.05 < 45$), local-efficiency ($22.98 < 24.05$), and betweenness-centrality ($14.60 < 17.39$) for AD against normal. Moreover, a significant increase in local-efficiency ($33.46 > 24.05$) and clustering-coefficient ($25 > 20.18$) were found in aMCI compared to normal.

Data Conclusion

This study demonstrated resting-state FC potential as an indicator to differentiate AD, aMCI, and normal. GTA revealed brain integration and breakdown by providing concise and comprehensible statistics.

Keywords: Alzheimer's Disease, Mild Cognitive Impairment, fMRI, Functional Connectivity, Resting-State Networks, Graph Theory Analysis.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative, age-related, irreversible disease with dysfunction of brain networks. AD is the commonest form of dementia (60%–80% of all dementia

cases), with 5-7 million new diagnoses each year (1). This syndrome typically begins with episodic memory impairment and gradually has a destructive effect on the brain's regions responsible for thinking, learning, and behavior (2). It is, therefore important to understand the early stages, known as amnestic mild cognitive impairment (aMCI).

The two main pathologic markers of AD, amyloid-β, and tau, cause dysfunction and degeneration of neuronal cells and synapses and affect neuronal signaling in the brain (3). Functional MRI (fMRI) is reliable for studying connectivity-based brain dysfunction. The activity of neural networks is detected by spontaneous low-frequency fluctuations of the fMRI blood oxygen level-dependent (BOLD) signal. Functional connectivity (FC) measures the statistical correlation (temporal correlation) of the BOLD signal between two or more anatomically distinct regions, and it is assumed that the areas in which the signal is correlated form functional networks. In several studies, FC is considered an indicator for diagnosing AD-related cognitive decline (4, 5). However, studies on FC alteration in the resting-state brain network of AD and aMCI patients are limited, and findings are inconsistent (6). This inconsistency may be due to different data acquisition and analysis methods and considering different areas of the brain. Most studies have investigated FC in the default mode network (4, 7), although some studies have conducted a more comprehensive survey on resting-state networks (8, 9). However, there is a need for more studies on the role of resting-state networks in AD progression.

Thus, this exploratory study aimed to quantitatively investigate FC patterns in 8 resting-state brain networks in patients with aMCI, late-onset AD, and normal subjects. Furthermore, we want to see whether graph theory analysis and graph metrics could be useful in distinguishing between these groups.

MATERIALS AND METHODS

Subjects

The academic Medical Ethics Committee has approved this study. Three groups, including 14 normal, 16 aMCI, and 13 late-onset AD subjects, participated in this study. Participants or their authorized caregivers signed an informed consent form that was in accordance with the guidelines of the Medical Ethics Review Board.

A neurologist with 25 years of experience in aging and dementia, founder and director of cognitive neurology and neuropsychiatry in the academic department of psychiatry selected subjects through clinical history, physical examination, screening, laboratory tests, structural MRI findings, cognitive evaluation, and neuropsychological assessments such as Mini-Mental State Examination (MMSE) (10), Montreal Cognitive Assessment (MoCA) (10), and Functional Assessment Staging (FAST) (11). Exclusion criteria for all groups were: other neuropsychiatric and dementia diseases such as Parkinson's disease, mixed dementia, frontotemporal dementia, and Lewy bodies dementia, dependence on drugs and alcohol, history of head trauma, systemic diseases such as high blood pressure, anemia, cancer, and diabetes, lesions more than 5 mm in diameter or four lesions less than 5 mm in diameter in T2-weighted fluid-attenuated inversion recovery (FLAIR) images.

MRI Acquisition

Three-dimensional (3D) T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) and rs-fMRI protocols were performed on subjects using a 3.0 T MAGNETOM Prisma MRI scanner (Siemens Healthcare, Germany). Participants were told to keep their eyes open and not move during the scan. The scanning parameters of 3D T1-weighted sequence were as follows: TR/ TE/ TI: 1840/ 3.55/ 800 msec; flip angle: 7º; voxel size: 0.9 × 0.9 × 0.9 mm^3, number of slices: 176; FOV: 220 × 220 mm^2. The rs-fMRI sequence was acquired

using a two-dimensional single-shot echo-planar imaging sequence: TR/ TE: 3000/ 30 msec; flip angle: 90º; voxel size: 2.8 × 2.8 × 3mm^3; number of slices: 45 interleaved; FOV: 220 × 220 mm^2. For each subject, 100 volumes (each volume in 3 seconds) were acquired.

Pre-Processing

Image pre-processing for 3D T1-weighted MPRAGE and rs-fMRI data was performed using CONN's default pre-processing pipeline (www.nitrc.org/projects/conn, RRID:SCR_009550). Removal of non-brain tissue, spatial normalization to Montreal Neurological Institute (MNI) space, tissue segmentation to grey matter, white matter, and CSF, and resampling to 1 × 1 × 1 mm^3 for MPRAGE data were performed using the mentioned pipeline. For pre-processing of rs-fMRI T2*-weighted images realigned to the first scan, then co-registered on high-resolution 3D T1-weighted images and normalized to Montreal Neurological Institute (MNI) space and resampled at 2 × 2 × 2 mm^3. The normalized images were smoothed using a 4mm FWHM Gaussian kernel. The parameters of the head movement were checked for each subject and in criteria of ± 0.9 mm displacement and ± 5 signal changes were excluded (outlier identification). In the next step, denoising (temporal band-pass filtering (0.01-0.1 HZ) and linear regression of potential confounding factors in the BOLD signal) were applied.

Region of Interest (ROI) for Resting-State Functional Connectivity Analysis

32 ROIs from 8 resting-state network with coordinates from the CONN atlas file were selected: MPFC (medial prefrontal cortex), LP(L) (left lateral parietal), LP(R) (right lateral parietal) and PCC (posterior cingulate cortex) from default mode network (DMN), left lateral sensorimotor, right lateral sensorimotor and superior sensorimotor from sensorimotor network (SMN), medial visual, occipital visual, left lateral visual and right lateral visual from visual network (VN), ACC (anterior cingulate cortex), Alnsula(L) (left anterior insula), Alnsula(R) (right anterior insula),

RPFC(L) (left rostral prefrontal cortex), RPFC(R) (right rostral prefrontal cortex), SMG(L) (left supramarginal gyrus) and SMG(R) (right supramarginal gyrus) from salience network (SN), FEF(L) (left frontal eye field), FEF(R) (right frontal eye field), IPS(L) (left intraparietal sulcus) and IPS(R) (right intraparietal sulcus) from dorsal attention network (DAN), LPFC(L) (left lateral prefrontal cortex), PPC(L) (left posterior parietal cortex), LPFC(R) (right lateral prefrontal cortex) and PPC(R) (right posterior parietal cortex) from frontoparietal network (FPN), IFG(L) (left inferior frontal gyrus), IFG(R) (right inferior frontal gyrus), pSTG(L) (left posterior superior temporal gyrus) and pSTG(R) (right posterior superior temporal gyrus) from language network (LN), and anterior cerebellar and posterior cerebellar from cerebellar network (CN) (Table S1 and Figure S1).

Resting-State Analysis

Resting-state FC analyses was performed using CONN toolbox version 20b. In the first-level analyses tab of the CONN toolbox, ROI-to-ROI correlation analysis was defined by selecting 'Functional connectivity' in the analysis type section and 'ROI-to-ROI' connectivity in the connectivity type section. For each pair of ROIs, 'bivariate correlation' was selected for the functional connectivity measure type section, and 'no weighting' was selected for the type of weighting scan. In this ROI-based analysis, the matrix of correlation coefficients (r) and ROI-to-ROI Connectivity (RRC) matrix of Fisher-transformed correlation coefficients (Z) were extracted to determine the level of FC between each pair of (i, j) ROIs BOLD signal time series (R), CONN toolbox (www.nitrc.org/projects/conn, RRID:SCR_009550):

$$r(i,j) = \frac{\int R_i(t)R_j(t)dt}{\left(\int R_i^2(t)dt \int R_j^2(t)dt\right)^{1/2}} \quad (1)$$

$$Z(i,j) = \tanh^{-1}\big(r(i,j)\big) \quad (2)$$

STATISTATISTICAL ANALYSIS

ROI-ROI Analysis

In CONN toolbox, we used multivariate parametric statistics analysis with family-wise error control, starting by defining resting state networks of related ROIs and analyzing all connection sets between all ROI pairs. A multivariate parametric general linear model analysis of functional connectivity matrices produced a statistical matrix of T or F values showing differences in connectivity between groups among all pairs of selected ROIs. To threshold ROI-to-ROI parametric maps with family-wise error rate control, functional network connectivity with a false discovery rate (FDR) corrected $p < 0.05$ cluster-level threshold was utilized to examine all network-to-network connectivity sets for choosing those that were significant (representing multivariate effects larger than what could be excepted under the null hypothesis). In addition, a post-hoc uncorrected $p < 0.05$ height (connection-level) threshold was used to specify the individual connections pattern demonstrating the most considerable effects within each significant set.

Graph Theory Analysis (ROI-ROI Analysis)

At the second level, we used the CONN toolbox automated graph theory analysis algorithms to examine the connectivity in groups. For each subject, a graph adjacency matrix was extracted by thresholding the RRC matrix by an edge-defining threshold value (cost level) of 0.15, one-sided (relative threshold), and p- FDR-corrected < 0.05 threshold, two-sided (absolute threshold). Then, global-efficiency, local-efficiency, clustering-coefficient, and betweenness-centrality metrics were computed from the resulting graphs.

Demographic and Cognitive Data

The Statistical Package for Social Sciences (SPSS version 26, IBM Corporation, Armonk, NY, USA) was employed for comparing demographic variables via one-way ANOVA (with Tukey post hoc) and Chi-square tests for numerical and categorical variables, respectively. The significance level was set at $p \leq 0.05$.

RESULTS

The number of women in all three groups was more than that of men. According to statistical results provided in Table 1, MMSE, MoCA, and FAST scores varied significantly between late-onset AD, aMCI, and normal control groups. The lowest scores on MMSE and MoCA tests belonged to the AD group, and the highest scores were for the normal group. On the FAST scale, the normal group was successful, the aMCI subjects mostly scored 3, and the late-onset AD subjects mostly scored 4-5. Moreover, based on Table 1, no significant differences were found in age among the three groups under study ($p = 0.059$)

Results of "Between-Network" Comparisons

In "between-network" comparisons (parametric multivariate statistics: p-FDR corrected < 0.05 together with post-hoc p-uncorrected < 0.05): (a) AD group versus aMCI group, showed a significant decrease in FC strength of 6 ROI-ROI connections, the strongest negative correlation was at the [SN. RPFC (R)]-[FPN. PPC (R)] connection (b) compared to the normal group, the AD group showed a significant decrease in FC in 20 ROI-ROI connections: The strongest negative correlation was at the [SN. ACC]-[VN. visual medial] connection and the [SN. ACC]-[VN. visual lateral (R)] connection (Figure 1). There was no significant differences between the aMCI group and the normal group in FC strength in "between-network" comparisons (See absolute p-values in Table S10).

Results of "Within-Network" Comparison

Default Mode Network

In the assessment of the DMN in three groups, FC strength of all connections except LP(L)-MPFC, decreases from normal to aMCI and from aMCI to AD group (Table 2). The greatest decrement was observed in PCC-MPFC connection in order (AD vs Normal, AD vs aMCI, and aMCI vs Normal groups) by -54.67%, -38.59%, and -26.18%, and also in LP(R)-MPFC connection in order by -52.41%, -32.57% and -29.42% (Figure 2, Table S2).

Salience Network

The strength of RPFC (R) -ACC and RPFC (R) -RPFC (L) connection in comparison between aMCI - normal decreased by -23.64% and -27.84%, and in comparison, between AD and two other groups increased by +74.82% and +59.76% (in AD vs. aMCI group) and by +33.49% and +15.27% (in AD vs. normal group). AInsula (R) -RPFC (R) and AInsula (L) -AInsula (R) connections from normal to aMCI and AD have had a substantial alteration in the strength of FC so that from normal to AD 67.11% and 57.47% decrease, from normal to aMCI 56.31% and 13.46% decrement and from aMCI to AD 24.73 and 50.85% reduction was observed (Figure 2, Table 2 and Table S3).

Sensorimotor Network

In the SMN of the AD group, FC impairment in the form of a 34.89% increment in the sensorimotor lateral (L)-sensorimotor superior connection and a 33.50% decrement in the sensorimotor lateral (R)-sensorimotor superior connection was found. Also, in the aMCI group compared to the normal group, a reduction in the strength in the sensorimotor superior-sensorimotor lateral (R) connection by 24.51% and in the sensorimotor superior-sensorimotor lateral (L) connection by 0.58% was observed (Figure 3 and Table S4).

Visual Network

In the visual network of the AD group compared to the normal and aMCI groups, an overall decrease in FC was found: the strongest decrease in connectivity was observed in visual lateral (R)-visual occipital by 36.84% and 53.15% and in visual lateral (L)-visual occipital by 30.16% and 33.33% respectively. The most robust FC among three groups was seen in the aMCI group: in aMCI compared to the normal group, 34.80% increment in visual lateral (R)-visual occipital, 20.67% in visual occipital-visual medial, 19.88% in visual lateral (R)-visual medial, 9.56% in visual lateral (R)-visual lateral (L) and 4.77% in visual lateral (L)-visual occipital connections was found (Table 2, Table S5 and Figure S5).

Dorsal Attention Network

In the AD and aMCI groups versus the normal group, FC strength of FEF (L)-IPS (L) and FEF (L)-FEF (R) connections decreased the most in order by 50.05%, 45.27% in AD, and 33.58%, 35.28% in aMCI. In AD group, compared to the aMCI group, FEF (L)-IPS (R) with a 46.98% decrement in the FC strength had the highest strength reduction. In comparison between AD and aMCI with the normal group, 17.15% and 75.63% increment was found in IPS (L) –IPS (R). In AD versus normal and aMCI, 17.41% and 28.26% increment was found in FEF (R)-IPS (L) connection, respectively (Table 2, Table S6, and Figure S6).

Frontoparietal Network

In comparison between AD and aMCI with normal group, FC strength of the FPN nodes' connections decreased by 71.68% and 72.72% in PPC (R)-LPFC (R), 63.77% and -39.02% in LPFC (R)-LPFC (L), 61.59% and -55.59% in PPC (R)-LPFC (L) and 51.72% and 32.06% in PPC (L)-LPFC (R). Furthermore, in PPC (L)-LPFC (L) connection, a 17.86% and 52.57% increment was observed in AD and aMCI compared to the normal group. Comparing AD with aMCI group, the entire network showed decreased FC strength (Figure 3, Table 2, and Table S7).

Language Network

Comparing AD with the aMCI and normal group, the entire network showed a decrease in FC strength, and the greatest weakness in both comparisons was found in pSTG (L)-pSTG (R) and pSTG (R)-IFG(R) connections. In the aMCI group, compared to the normal group, the entire network showed increased FC strength: there was a 107.12% increase in pSTG (L)-IFG (R) connection, 63.74% in pSTG (L)-IFG (L), 35.19% in IFG (L)-IFG (R), 21.29% in pSTG (L)-pSTG (R) and 13.75% in pSTG (R)- IFG (R) (Table 2, Table S8 and Figure S8).

Cerebellar Network

FC strength of the cerebellar anterior- cerebellar posterior connection in the CN decreased from the normal group to the aMCI and AD groups, respectively. The reductions of FC in cerebellar anterior-cerebellar posterior connection from normal to AD and from aMCI to AD were approximately 50%, while that from normal to aMCI was less than 10% (Table 2, Table S9, and Figure S9).

Comparing the three groups, the average strength of FC in network connections was as follows: DMN (5.63 in AD < 7.64 in aMCI < and 8.71 in the normal group), CN (2.94 in AD < 5.90 in aMCI < and 6.63 in the normal group), DAN (6.73 in AD < 8.20 in aMCI < 8.36 in the Normal group), FPN (4.56 in AD < 7.66 in aMCI < 8.48 in the Normal group), LN (8.87 in aMCI > 6.28 in the Normal group), VN (9.59 in aMCI > 8.91 in the Normal group), SN (8.35 in AD < 10.18 in the Normal group), LN (4.29 in AD < 6.28 in the Normal group), and VN (7.24 in AD < 8.91 in the Normal group).

Results of Graph Theory Analysis

There was a significant decrease in global-efficiency (28.05 versus 45), local-efficiency (22.98 versus 24.05), and betweenness-centrality (14.60 versus 17.39) metrics in the AD group compared

to the normal group. In the aMCI group, compared to the normal group, a higher local-efficiency (33.46 versus 24.05) and a higher clustering-coefficient (25 versus 20.18) were found (Table 3).

DISCUSSION

This study analyzed resting-state brain networks and investigated the FC map in late-onset AD, aMCI, and normal subjects. We also examined whether graph theory metrics could differentiate between these three groups. We found significant differences in FC strength "between-within" resting-state brain networks of late-onset AD, aMCI, and normal subjects, and these findings are in agreement with cognitive assessments: in the comparison of three groups, along with a decrease in MMSE and MoCA score from normal to aMCI and AD and decreasing the patient's ability with the FAST scale, the strength of average FC in connections of DMN, CN, DAN, and FPN also decreased. In the aMCI group compared to the normal group, the average FC values in LN and VN were not associated with a decrease in MMSE, MoCA, and with the deterioration of FAST scores. Comparing AD with normal, the average FC values in SN, LN, and VN was in line with MMSE, MoCA, and FAST scores.

In the aMCI group compared to the normal group, in all studied networks except LN and VN, the number of weakened connections was higher than the number of strengthened connections. Higher FC strength in the aMCI group is probably due to the compensatory mechanism to prevent network degradation or disruption of the brain network. Functional hypoactivity and hyperactivity may reflect functional disconnection and compensation in response to early neurodegeneration change and direct or indirect pathological mechanisms (4). Thus, in the aMCI group, disruption in resting-state networks was found in both FC increase and decrease.

Examining the findings shows that in the AD group, compared to the normal group, the CN, FPN, DMN, LN, DAN, VN, SN and SMN showed the greatest decrease in FC, respectively. However,

as we reported, by comparing the AD group with the normal group, we also found an increase in FC in some connections in the SN, DMN, and SMN. Also, comparing AD with aMCI, the LN, CN, FPN, DMN, VN, and DAN showed the greatest decrease in FC, respectively, and the sensorimotor and SN showed the greatest increase in FC, respectively. Several studies have reported increased FC in some brain networks of AD and aMCI patients, which has been explain as a compensatory mechanism to prevent cognitive decline before AD deteriorates brain function (5, 12). Thus, in the AD group, network disruption is observed in both decreasing and increasing FC strength. In all studied networks, the number of attenuated connections was higher in the AD group than the number of robust connections, and the decrease in FC strength in AD was greater than in aMCI.

The results of the within-network comparison show that the pattern of decrease and increase in FC in connections of all resting-state networks studied is similar from aMCI to AD and from normal to AD, and in most connections. However, the percentage of FC decrease from normal to AD is more than aMCI to AD, but the percentage of FC increase from aMCI to AD is more than from normal to AD. This finding is consistent with previous studies (13, 14). Also, in comparing late-onset AD, aMCI, and normal subjects, "between-network" FC in the AD group compared to the normal group was weaker in 20 ROI-ROI connections, and in the AD group compared to the aMCI group was weaker in 6 ROI-ROI connections. This result shows that the number of robust connections in the AD group is less than in the normal and aMCI groups.

Default Mode Network

In both AD and aMCI groups, lower FC strength in nodes' connections of DMN was found compared to the normal group. The reduction of FC from normal to AD was more than from normal to aMCI. Hypoactivity of the DMN network in aMCI and AD have been reported in fMRI

studies (9, 15). Studies have shown that white matter integrity loss, grey matter atrophy, and metabolic impairments in the parietal lobe occur with conversion to AD (16, 17). Also, studies show that in AD, in addition to amyloid deposition in PCC, its metabolism decreases (18, 19). Due to the role of PCC, MPFC, and lateral parietal on episodic memory (20), impairment in this process in AD can be due to FC dysfunction in connections of these nodes in DMN as a result of reduced integration, gray matter atrophy, amyloid deposition, metabolic disorders and decrease in metabolism.

Sensorimotor Network

In comparing AD with normal, network disorder in the form increment in FC strength of sensorimotor superior-sensorimotor lateral (L) and decrement in sensorimotor superior-sensorimotor lateral (R) was observed. Also, impairment in aMCI compared to normal in the form of reduction in the whole network was found. Due to the role of the SMN in perceiving the world and setting goals (21), we can infer that impaired FC in SMN of the AD and aMCI groups compared to the normal group may be one of the reasons for the decline in attention levels and planning ability in AD or aMCI patients. Previous studies have shown that changes in functions such as olfactory, auditory, visual, and motor systems might precede the onset of cognitive decline due to neocortical tau deposition in this network, which worsens with the progression of AD (8, 21).

Dorsal Attention Network

Our study showed a lower FC and intrinsic activity in the DAN of patients with AD compared to the normal group. These findings are in line with previous studies (8, 22). Since DAN is involved in voluntary orientation and visual-spatial attention (23), the reduction of FC between the nodes

of this network and the disorders in this network can be the reason for the impairment of visual-spatial attention and perceptual-motor coordination in patients with AD.

Frontoparietal Network

In the FPN, comparing the AD and aMCI groups with the normal group, a significant decrease in the connection strength of the network was observed, and the decrease in FC and the number of reduced connections was greater in AD than in aMCI. This finding is consistent with previous studies showing a decline in FC of FPN in AD patients (24, 25) and a greater reduction in FC of FPN in AD comparing aMCI patients (8).

Cerebellar Network

Clinical evidence and neuroimaging studies have shown cerebellar alteration in AD (vermis and posterior cerebellar change in early stages of AD and anterior cerebellar change in more advanced stages of the disease) (26, 27). Previous studies have also reported cerebellar gray matter atrophy progression from early to late clinical stages of AD (28, 29). Based on the findings of our research, which showed a decrease in FC in the connection between nodes of this network from normal and aMCI to AD, this decrease in connection strength is possibly due to cerebral atrophy and loss of gray matter in this part of the brain, which affects the progression of AD.

Salience Network

Based on our findings, which showed a decrease in FC strength of nodes in this network from normal to aMCI and AD, and due to the role of the insular cortex in emotion and interpersonal experience (30), and the importance of the supramarginal gyrus in cognitive emotion regulation (31), emotional disorders such as depression and crying in AD may be due to the progression of breakdown in this network.

Visual Network

We found lower FC in the entire VN in the AD group compared to the normal group. Our finding is consistent with the study by Huang et al., which reported that FC of the VN in AD disrupted and decreased in a manner dependent on the severity of the disease (32). In this network, we found enhancement in the FC strength of the aMCI group compared to the other two groups. Higher FC strength in the aMCI group may show the efforts of the VN to prevent disruption. Based on these findings, we suggest that the VN is one of the important networks to differentiate the normal group from aMCI.

Language Network

Compared to the normal group, a reduction in FC strength was found throughout the LN in the AD group. Neuroimaging studies in AD patients have shown that language disorders are associated with atrophy or hypometabolism in the left IFG and temporal regions (33, 34). Also, impairments in phonological processing are associated with damage to the left pSTG (35). Possibly due to dysfunction and a lower FC between nodes of the network, a decrease in semantic and pragmatic processing may occur as the disease progresses. In the LN, the strongest FC was found in the aMCI group compared to the other two groups. This increment in connection strength could explain the efforts of the LN to prevent network breakdown, so this network may be important for differentiating normal subjects from aMCI patients.

Graph Theory Metrics

Global-efficiency and local-efficiency quantify the degree of network integration: greater local efficiency and global-efficiency indicate ease of functional connections between nodes and represent more efficient message transmission from any region to any other region (36, 37).

Clustering-coefficient (i.e., the tendency of a group of nodes to form dense interconnections) measures the degree of network segregation (36): a high clustering coefficient reflects a high level of segregation between local clusters, allowing specialized processing (36, 38). We found a reduction in the global and local efficiency of AD patients, which can be inferred as a decline in network integration of this group. This finding is in line with a previous study (39). In the aMCI group, higher local-efficiency and clustering-coefficient were found compared to the normal group (40), possibly indicating a compensatory mechanism.

Limitations

This study's data set was not large, and the cohorts were not gender-equal. Furthermore, in our functional imaging, the number of time points was 100, and more accurate functional information can be obtained with more time points. On the other hand, more data set is required to determine the accuracy and specificity of the functional features and if the extracted features could be used as biomarkers to differentiate late-onset AD, aMCI, and normal groups. We try to measure the accuracy and replicability of the findings in future studies with more data sets and more time points.

Conclusion

The FC map differed in the resting state networks of normal, aMCI, and late-onset AD groups. Our study demonstrated the usefulness of FC patterns as potential features for differentiation between these three groups. The FC analysis and graph theory analysis results also showed that network integrity was decreased in AD patients, and network disruption was beginning. The local-efficiency and clustering-coefficient metrics may be important parameters for differentiating aMCI from the normal group.

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TABLES

TABLE 1. The group's demographic (sex and gender) and cognitive information (MMSE, MoCA, and FAST).

Details of subjects	**Normal (n=14)**	**aMCI (n=16)**	**late-onset AD (n=13)**	**F/χ^2**	***P* Value**	**Post-hoc pairwise comparisons**
Sex (male-female)	6-8	6-10	5-8	0.100	0.950	-
Age (years)	71.57 ± 7.14	72.44 ± 7.11	77.77 ± 7.95	5.310	0.059	-
MMSE score	28.07 ± 0.83	25.06 ± 2.24	18.62 ± 1.39	117.200	< 0.00001	Normal vs. aMCI: 0.000032 Normal vs. AD: < 0.00001 aMCI vs. AD: < 0.00001
MoCA score	27.29 ± 1.14	22.19 ± 3.94	16.15 ± 3.53	41.906	< 0.00001	Normal vs. aMCI: 0.000219 Normal vs. AD: < 0.00001 aMCI vs. AD: 0.000024
FAST score	Norm	III	IV-V	101.437	< 0.00001	Normal vs. aMCI: < 0.00001 Normal vs. AD: < 0.00001

	aMCI vs. AD: 0.002669

Note: One-way ANOVA and Chi-square analyses were employed to test for group differences. The statistical significance level was set at $P < 0.05$. **Abbreviations:** aMCI: amnestic mild cognitive impairment, AD: Alzheimer's disease, MMSE: Mini-Mental State Examination (which is one of the most popular cognitive functions, consists of 20 items that cover different domains like instant memory, delayed recall, attention & computational power, orientation, object naming, reading comprehension, speech comprehension, language retelling, speech expression, and visual space ability). MoCA: Montreal Cognitive Assessment test (which investigates cognitive function in 7 domains, i.e., visuospatial/executive function, naming, memory, attention, language, abstraction, and delayed recall with a total of 30 points), FAST: Functional Assessment Staging (which is a reliable and valid assessment method for assessing the AD patients and includes 16 steps to assess the severity of dementia).

TABLE 2. The percentage of changes in functional connectivity strength in three groups: AD vs. aMCI, AD vs. normal group, and aMCI vs. normal group. + indicates an increase, and – indicates a decrease in the percent of functional connectivity strength.

Default Mode Network	**AD vs Normal**	**AD vs aMCI**	**aMCI vs Normal**
PCC -LP(R)	-30.40%	-29.30%	-1.55%
LP(L) -PCC	-34.18%	-15.62%	-22.00%
LP(L)-LP(R)	-26.44%	-24.26%	-2.89%
PCC -MPFC	-54.67%	-38.59%	-26.18%
LP(L)-MPFC	+4.58%	-18.77%	+26.18%
LP(R)-MPFC	-52.41%	-32.57%	-29.42%
Salience network	**AD vs Normal**	**AD vs aMCI**	**aMCI vs Normal**
RPFC (R) -ACC	+33.49%	+74.82%	-23.64%
RPFC (R) -RPFC (L)	+15.27%	+59.76%	-27.84%
ACC -RPFC (L)	-10.34%	-9.81%	-0.59%
AInsula (R) -RPFC (R)	-67.11%	-24.73	-56.31%
AInsula (L) -AInsula (R)	-57.47%	-50.85%	-13.46%
AInsula (L) -RPFC (L)	-25.00%	-40.93%	+26.97%
Sensorimotor network	**AD vs Normal**	**AD vs aMCI**	**aMCI vs Normal**
SensoriMotor.Lateral(L)-SensoriMotor.Superior	+34.89%	+35.69%	-0.58%
SensoriMotor.Lateral(R)-SensoriMotor.Superior	-33.50%	-11.90%	-24.51%
Visual network	**AD vs Normal**	**AD vs aMCI**	**aMCI vs Normal**

Visual.Lateral (R) - Visual.Lateral (L)	-21.52%	-28.36%	+9.56%
Visual.Lateral (L) - Visual.Occipital	-30.16%	-33.33%	+4.77%
Visual.Lateral (R) - Visual.Occipital	-36.84%	-53.15%	+34.80%
Visual.Lateral (L) - Visual.Medial	-25.98%	-1.52%	-24.84%
Visual.Lateral (R) - Visual.Medial	+20.47%	+0.49%	+19.88%
Visual.Medial - Visual.Occipital	+14.82%	-4.35%	+2.67%
Dorsal attention network	**AD vs Normal**	**AD vs aMCI**	**aMCI vs Normal**
IPS(L) -IPS(R)	+17.15%	-33.30%	+75.63%
FEF(L)-IPS (L)	-50.05%	-24.80%	-33.58%
FEF(L)-FEF (R)	-45.27%	-15.43%	-35.28%
FEF(R)-IPS (R)	-13.74%	+26.90%	-32.02%
FEF (R)-IPS (L)	+17.41%	+28.26%	-8.46%
FEF (L)-IPS (R)	-43.18%	-46.98%	+7.17%
Frontoparietal network	**AD vs Normal**	**AD vs aMCI**	**aMCI vs Normal**
PPC(L)-PPC (R)	-13.70%	-20.94%	+9.17%
PPC(L)-LPFC (L)	+17.86%	-22.75%	+52.57%
PPC(R)-LPFC (L)	-61.59%	-55.59%	-13.51%

PPC(L)-LPFC (R)	-51.72%	-32.06%	-28.93%
PPC(R)-LPFC (R)	-71.68%	-72.72%	+3.82%
LPFC(R)-LPFC (L)	-63.77%	-39.02%	-40.60%
Language network	**AD vs Normal**	**AD vs aMCI**	**aMCI vs Normal**
pSTG (L) -pSTG (R)	-46.99%	-56.29%	+21.29%
pSTG (L) -IFG (L)	-1.46%	-39.82%	+63.74%
pSTG (R) -IFG (R)	-41.46%	-48.54%	+13.57%
pSTG (L) -IFG (R)	-26.41%	-64.47%	+107.12%
IFG (L) -IFG (R)	-33.58%	-50.87%	+35.19
Cerebellar network	**AD vs Normal**	**AD vs aMCI**	**aMCI vs Normal**
Cerebellar.Anterior - Cerebellar.Posterior	-53.85%-	-50.17%	-7.38%

TABLE 3. Graph theory metrics in late-onset AD, aMCI, and normal subjects.

Graph-theory metrics	**Global-efficiency**	**Local-efficiency**	**Clustering-coefficient**	**Betweenness-centrality**
late-onset AD	28.05	22.98	20.55	14.60
aMCI	26.31	33.46	25.00	10.81
Normal	45.00	24.05	20.18	17.39

Global-efficiency: the mean inverse distances between a node and all other nodes in the graph. Local-efficiency: the number of connections between a node and its neighboring nodes. Local efficiency indicates direct functional connections and shows the effective ability of the network to compensate for localized failure in a single node. Betweenness-centrality: the number of shortest paths between any two pairs of nodes that pass through the node of interest. Clustering-coefficient: represent the degree of connectivity strength among a node and its neighboring nodes, i.e., the degree to which nodes tend to cluster together.

FIGURES

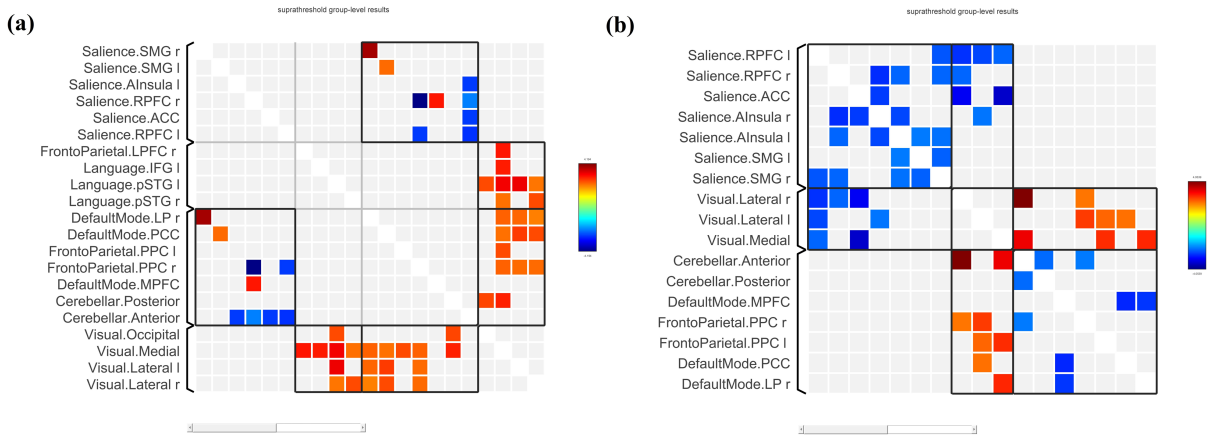

FIGURE 1. ROI-ROI connection matrices extracted from ROI-to-ROI connectivity analysis (parametric multivariate statistics use p-FDR corrected < 0.05 to select significant network-network connectivity set together with post-hoc p-uncorrected < 0.05 to select the significant connection.) (a) Alzheimer's disease group versus Amnestic Mild Cognitive Impairment group showed a significant decrease in functional connectivity at 6 ROI-ROI connections (blue colors) (b) Alzheimer's disease group versus normal group, showed a significant decrease in functional connectivity at 20 ROI-ROI connections (blue colors). Weaker connections are shown in darker blue tones. Stronger connections are shown in darker red tones.

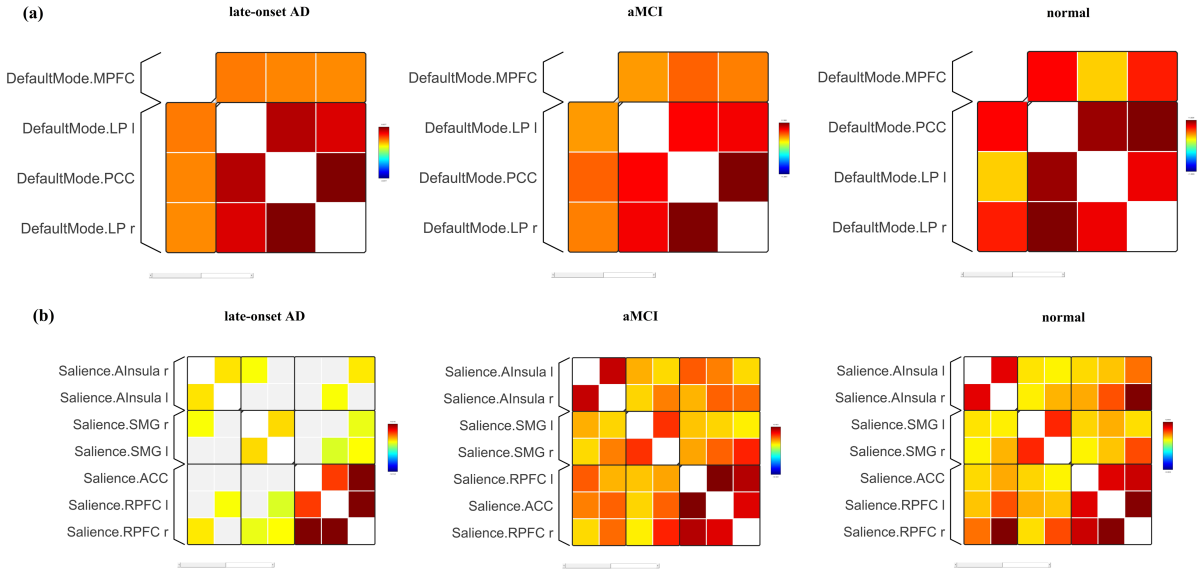

FIGURE 2. Functional connectivity (ROI-to-ROI connectivity) analysis (parametric multivariate statistics; cluster threshold: $p < 0.05$ cluster-level p-FDR corrected; connection threshold: $p < 0.05$ p-uncorrected): "within-network" comparison in Alzheimer's disease (AD), Amnestic Mild Cognitive Impairment (aMCI), and normal groups in default mode network (a) and salience network (b). Stronger connections are shown in more red tones.

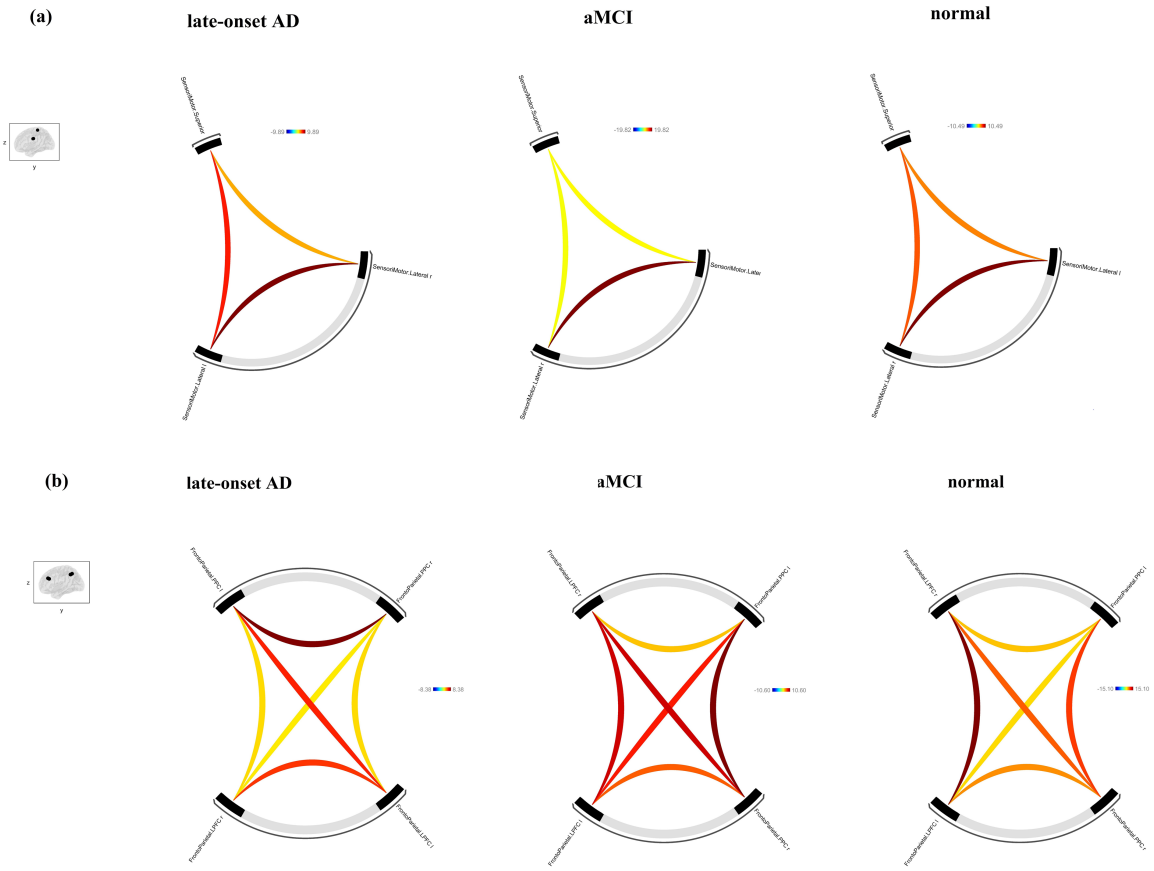

FIGURE 3. Functional connectivity (ROI-to-ROI connectivity) analysis (parametric multivariate statistics; cluster threshold: $p < 0.05$ cluster-level p-FDR corrected; connection threshold: $p < 0.05$ p-uncorrected): "within-network" comparison in Alzheimer's disease (AD), Amnestic Mild Cognitive Impairment (aMCI), and normal groups in sensorimotor network (a) and frontoparietal network (b). Stronger connections are shown in more red tones.