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# Abnormal brain functional connectivity coupled with hypoperfusion measured by Resting-State fMRI: An additional contributing factor for cognitive impairment in patients with Alzheimer's disease

Zhang Bing[a,1], Hua Rui[b,1], Qing Zhao[a,1], Ni Ling[a], Zhang Xin[a], Zhao Hui[c], Liu Renyuan[c], Lu Jiaming[a], Wu Sichu[a], Xu Yun[c], Zhu Bin[a,*], Wan Suiren[b,*], Sun Yu[d,e,*]

[a] Department of Radiology, Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China
[b] The Laboratory for Medical Electronics, School of Biological Sciences and Medical Engineering, Southeast University, Nanjing, China
[c] Department of Neurology, Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China
[d] International Laboratory for Children's Medical Imaging Research, School of Biological Sciences and Medical Engineering, Southeast University, Nanjing, China
[e] Institute of Cancer and Genomic Sciences, University of Birmingham, UK

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ABSTRACT

The contribution of hypoperfusion to abnormal functional connectivity in Alzheimer's disease (AD) and mild cognitive impairment (MCI) remains unclear. In this study, we investigated the potential association between brain perfusion and functional connectivity (FC), and its effects on the cognitive impairment among AD, MCI, and normal controls (NC). One-time acquisition of resting-state functional magnetic resonance imaging (rs-fMRI) was used to study brain perfusion and FC. Compared to the NC, the perfusion in the left temporal lobe showed significantly lower in AD, and bilateral hypoperfusion in the frontal lobe showed in MCI. Using these hypoperfusion areas as seed regions, we found that FC between the left inferior temporal gyrus and medial frontal-cingulate regions in AD patients was significantly lower than that in NCs. The FC between the right medial superior frontal gyrus and left parietal lobe in MCI patients was significantly higher than that in NCs. Additionally, the FC between the right medial superior frontal gyrus and the left superior parietal gyrus were found to be correlated significantly and negatively with mini-mental state examination (MMSE) scores in MCI patients. In conclusion, hypoperfusion may affect cognitive states via abnormal FC as an additional factor contributing to cognitive impairment.

## 1. Introduction

Previous studies demonstrated abnormal brain perfusion as a typical feature of Alzheimer's diseases (AD) (Tang et al., 2012). For example, decreased perfusion in AD was reported in the posterior cingulate cortex (PCC) (Dai et al., 2009; Iizuka and Kameyama, 2017) and the parietal lobe (Benedictus et al., 2017; Binnewijzend et al., 2013; Hansson et al., 2009; Leeuwis et al., 2017). Hypoperfusion refers to a lack of blood supply to organ microcirculation, and usually manifests as a decrease in blood flow velocity in the middle cerebral artery (Ruitenberg et al., 2005) which can affect metabolic, anatomic and cognitive function adversely (de la Torre, 2000). The areas of hypoperfusion were found to be associated with abnormal structural connectivity (Lacalle-Aurioles et al., 2016), metabolic changes (Lee et al., 2011), and cognitive impairment (Leeuwis et al., 2017).

Brain functional connectivity was also reported simultaneously with brain perfusion in different groups. For example, an integrated pseudocontinuous arterial spin labeling (pCASL) magnetic resonance imaging (MRI) and resting-state functional MRI (rs-fMRI) study demonstrated that the functional connectivity (FC) alterations in medial prefrontal areas were associated with the cerebral blood flow (CBF) level in middle cingulate cortices and the PCC in mild AD after 12-week donepezil treatment (Li et al., 2012). The effect of CBF alterations towards functional brain network reorganization in patients with mild cognitive impairment (MCI) was evaluated through arterial spin labeling (ASL) and blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) (Lou et al., 2016). The result showed probable contribution of hypoperfusion towards eigenvector centrality reductions. And eigenvector centrality is a term in graph theory which measures the influence of a node in a network. Notably, a

* Corresponding authors.
*E-mail addresses:* gobincdr@163.com (B. Zhu), srwan@seu.edu.cn (S. Wan), sunyu@seu.edu.cn (Y. Sun).
[1] These three authors contributed equally to this article.

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significant spatial correlation between the relative CBF (rCBF) and functional connectivity strength (FCS) has been reported in the default mode network and executive control network for normal control (NC) subjects (Liang et al., 2013). Therefore, we hypothesize there is a potential association between brain hypoperfusion and brain functional connectivity abnormality in AD and even in MCI patients.

Brain perfusion (computed tomography perfusion imaging [CTPI], dynamic susceptibility contrast [DSC] imaging, and positron emission tomography computed tomography [PET-CT]) is commonly measured through contrast media that is a kind of exogenous media replace blood to track perfusion invasively, and this limits their applicability in elderly patients with AD. Recently, Lv et al. (2013) proposed the time-shift analysis (TSA) method for evaluating brain perfusion without contrast media, which prevents the adverse effects of contrast media in patients who undergo this technique and has been applied in previous clinical study successfully (Christen et al., 2015). As a non-invasive method based on BOLD signals, TSA assessments allow measurement of brain perfusion and synchronized brain spontaneous activity simultaneously in one scan. Therefore, rs-fMRI preserves its advantages in perfusion and brain functions that are essential in clinical practice in elderly patients and retrospective studies. Our study investigated the association among perfusion, functional connectivity and cognition using the TSA method without perfusion data.

There are three goals in the current study: (1) demonstrate the feasibility of TSA method to detect brain perfusion non-invasively in patients with AD; (2) investigate the potential association between hypoperfusion and FC abnormality in brain; (3) investigate the correlation between FC abnormality and behavioral performance and its impact on cognitive impairment. The analytic plan of our study is shown in Fig. 1. We proposed a hypothesis that hypoperfusion may

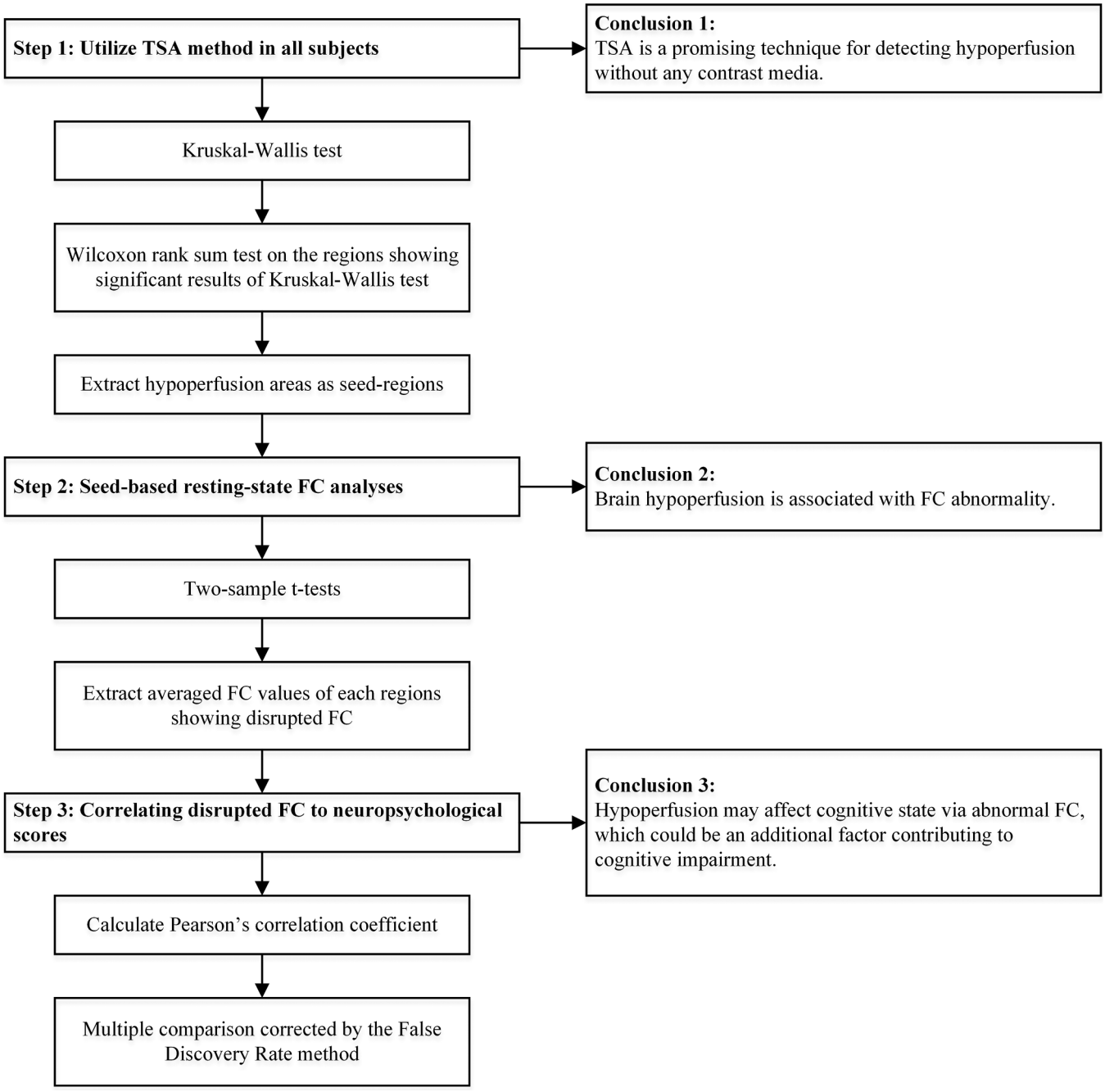


**Fig. 1.** The analytic plan of our study.

affect cognitive state via abnormal FC, which could be an additional factor contributing to cognitive impairment.

## 2. Material and methods

### 2.1. Participants

One hundred and six subjects were recruited from the memory clinic of Affiliated Drum Tower Hospital of Nanjing University Medical School. After a detailed explanation of the study procedures, written informed consent was obtained from all subjects or their proxies, and the protocol for this study was approved by the Ethical Review Board of the Affiliated Drum Tower Hospital of Nanjing University Medical School. All subjects underwent a series of standardized clinical assessments to define clinical status, including the mini-mental state examination (MMSE) (Folstein et al., 1975). All subjects had neither history of major neurological or psychiatric disorders nor demyelinating lesions in the deep white matter except AD and MCI. Subjects with histories of brain injury, alcohol abuse, or drug abuse were excluded.

The AD patients ($n = 31$) met the NINCDS-ADRDA Alzheimer's Criteria (McKhann et al., 1984). MCI patients ($n = 43$) were diagnosed on the basis of Petersen's criteria (Petersen, 2004), including: (a) patients had memory complaints confirmed by an informant; (b) the objective memory impairments were not affected by age or education levels; (c) patients had normal or near-normal cognitive states and daily life activities were not affected; and (d) patients did not meet the criteria for a diagnosis of dementia. The NC group ($n = 32$) had neither cognitive complaint nor structural abnormalities detectable on a conventional MRI scan, and scored normally on MMSE. Followed by MRI data quality control screening, 18 subjects were excluded for head motion, and 88 subjects (21 AD and 35 MCI patients and 32 NCs) were ultimately included in the analysis. Details of the demographic data and neuropsychological tests are presented in Table 1.

### 2.2. MRI data acquisition

MRI data were acquired on a 3-Tesla MR scanner (Achieva 3.0 T TX dual Medical Systems; Philips Medical Systems, Eindhoven, Netherlands) in the Affiliated Drum Tower Hospital of Nanjing University Medical School. All subjects were placed in an eight-channel phased array head coil and fitted with foam padding to reduce head motion; a pair of earplugs was used to reduce scanner noise. Participants were instructed to close their eyes, remain still and calm, and to not fall asleep. T1-weighted sequences were used to acquire structural images of the brain, and gradient-echo echo-planar (GRE-EPI) sequences were obtained to measure the BOLD signal of the brain. The parameters of the sequences are presented in Table 2.

### 2.3. Data preprocessing

Functional MRI data were preprocessed using Data Processing Assistant for Resting State fMRI Advanced edition (DPARSFA Chao-Gan and Yu-Feng, 2010, http://www.restfmri.net) and Resting-State fMRI Data Analysis Toolkit (REST Song et al., 2011, http://www.restfmri.net) in MATLAB (The MathWorks, Inc., USA). The pipeline included the following steps: (a) removal of the first 10 time points; (b) slice timing; (c) head motion correction, and excluding subjects based on 2 mm and 2°; (d) regressing out nuisance variables including Friston 24 head motion parameters, white matter signal and cerebrospinal fluid signal; (e) normalization to the Montreal Neurological Institute (MNI) space; (f) smoothing with an isotropic Gaussian kernel with full width at half maximum of 6 mm; (g) removal of the linear trend; and (h) filtering of the band pass (0.01–0.08 Hz).

### 2.4. Time-shift analysis

TSA method was initially used in patients with acute stroke to identify hypo-perfused areas (Lv et al., 2013). The author proposed the hypothesis that the slowing blood flow in an area of embolism will lead to a temporal delay of BOLD signal, thus the time delay can be used to evaluate the CBF dynamics. Their results demonstrated that the time delay is sensitive to perfusion deficits and provide comparable information to that of conventional perfusion magnetic resonance imaging.

The mean time signal of the whole brain was used as the reference signal, called *Y*, and the time course of each voxel within the brain was extracted and then shifted from −3 TR to 3 TR (from −6 s to +6 s),

**Table 1**
Demographic and clinical characteristics of the participants.

| | AD ($N = 21$) | MCI ($N = 35$) | NC ($N = 32$) | *p* value All groups | AD vs. NC | MCI vs. NC |
|---|---|---|---|---|---|---|
| Ethnicity | Chinese | Chinese | Chinese | | | |
| Age (years) | 73.05 ± 13.68 | 74.94 ± 13.23 | 70.63 ± 11.86 | 0.944 (0.393) [a] | 0.671 (0.504) [b] | 1.373 (0.173) [b] |
| Male, n (%) | 10 (47.6) | 21 (60) | 21 (65.6) | 1.720 (0.423) [c] | 1.693 (0.193) [c] | 0.226 (0.634) [c] |
| Education (years) | 12.29 ± 2.87 | 12.67 ± 2.78 | 14.67 ± 1.97 | 2.658 (0.090) [a] | −2.016 (0.055) [b] | −1.827 (0.080) [b] |
| MMSE | 17.53 ± 5.71 | 25.35 ± 2.42 | 28.89 ± 1.26 | 63.388 (<0.001) [a] | −11.248 (<0.001) [b] | −3.991 (<0.001) [b] |
| MoCA | 11.60 ± 4.24 | 21.91 ± 2.09 | 27.04 ± 1.99 | 160.249 (<0.001) [a] | −17.899 (<0.001) [b] | −6.677 (<0.001) [b] |

Data are presented as mean ± standard deviation. AD, Alzheimer's disease; MCI, mild cognitive impairment; NC, normal control; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment. Significant differences in age, education level, MMSE score as well as MoCA score among the AD, MCI, and NC groups were evaluated by one-way analysis of variance (ANOVA) at $p < 0.05$, and [a]denotes *F* value. Multiple comparisons were corrected by the Fisher's Least Significant Difference (LSD) method, and [b]denotes *LSD-t* value. Significant differences in gender among the AD, MCI, and NC groups were evaluated by Pearson chi-square test at $p < 0.05$, and [c]denotes $\chi^2$ value.

**Table 2**
Parameters of the sequences.

| Sequence name | TR (ms) | TE (ms) | Matrix | FOV ($mm^2$) | FA (°) | Slice thickness (mm) | Volumes |
|---|---|---|---|---|---|---|---|
| $T_1$WI | 9.8 | 4.6 | 256 × 256 | 256 × 256 | 8 | 1 | 1 |
| GRE-EPI | 2000 | 30 | 64 × 64 | 192 × 192 | 90 | 4 | 230 |

$T_1$WI, T1 weighted imaging; GRE-EPI, gradient-echo echo-planar imaging; TR, repetition time; TE, echo time; FOV, field of view; FA, flip angle.

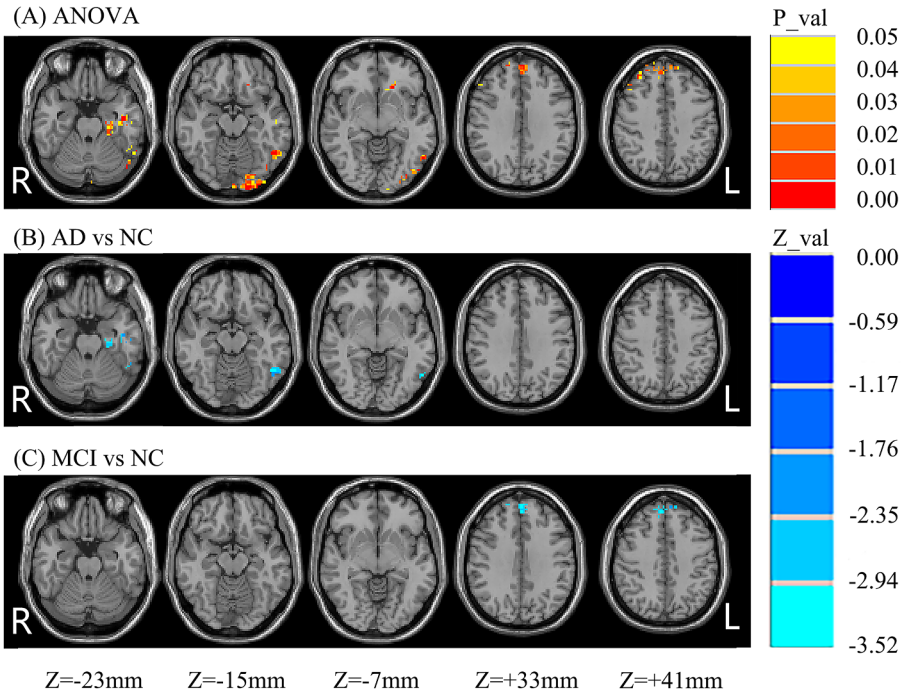


**Fig. 2.** Brain regions showing significant time delay in intergroup comparisons, indicating hypoperfusion areas. (A) Significant intergroup differences among the Alzheimer's disease (AD), mild cognitive impairment (MCI), and normal control (NC) groups were found in the left inferior temporal gyrus, left hippocampus, left parahippocampal gyrus and bilateral medial superior frontal gyrus ($p < 0.05$, alpha < 0.01, cluster size > 67). (B) Significant hypoperfusion was found in the left inferior temporal gyrus, left hippocampus, and left parahippocampal gyrus in the AD group in comparison with the NC group ($p < 0.05$, alpha < 0.01, cluster size > 133). (C) Significant bilateral hypoperfusion was found in the medial superior frontal gyrus in the MCI group in comparison with the NC group ($p < 0.05$, alpha < 0.01, cluster size > 116).

called $X_i$. The correlation coefficients between the shifted time course of each voxel and the reference time course were calculated.

$$R_i = \frac{dot(X_i', Y')}{m}$$

where $X_i' = \frac{X_i - repmat(mean(X_i,2),m)}{repmat(std(X_i,2),m)}$, $Y' = \frac{Y - repmat(mean(Y,2),m)}{repmat(std(Y,2),m)}$, m denotes the number of time points in BOLD signals, and $i$ denotes the index of time shift, range from $-3$ to 3.

Finally, each voxel was assigned a value based on the time shift required for the maximal correlation coefficient to the reference time course, yielding a time-shift map for each individual. Thus, negative values indicate temporal delay in BOLD signal. The areas with negative values corresponded to the hypo-perfused areas.

According to the statistical analysis of TSA between patients and normal controls, brain regions showing significantly intergroup difference will be treated as regions of abnormal perfusion. Hypoperfusion given by negative values will be used as regions of interest (ROIs) in the following FC analysis.

### 2.5. Seed-based functional connectivity analysis

Seed-based resting-state FC analysis was used to investigate perfusion-related brain functional abnormalities. Firstly, regions showing significantly different TSA findings in AD patients and NC subjects were extracted. Regions spreading spatially across large areas were segmented into smaller seed regions based on the anatomical automatic labeling (AAL) templates. FC is derived through Pearson correlation between brain regions. FC maps were evaluated for each seed using the DPARSFA toolkit. Regions showing significant intergroup differences in FC maps were extracted and used as the ROIs for correlation analysis in the following step. The procedure above is then used between MCI patients and NC subjects.

### 2.6. Correlating disrupted FC to neuropsychological scores

The average FC values of each region showing significant intergroup differences were extracted. The correlations between these FC values and MMSE scores were then derived across subjects, in order to explore the potential association between abnormal functional connectivity and cognitive deficits among these subjects. The correlation coefficients were derived as

$$r = \frac{\sum_{j=1}^{n}(FC_j - \overline{FC_j})(MMSE_j - \overline{MMSE_j})}{\sqrt{\sum_{j=1}^{n}(FC_j - \overline{FC_j})^2}\sqrt{\sum_{j=1}^{n}(MMSE_j - \overline{MMSE_j})^2}}$$

where $n$ denotes the number of subjects in corresponding group.

### 2.7. Statistical analysis

One-way analysis of variance (ANOVA) was performed among the AD, MCI, and NC groups to evaluate the age, education level, MMSE score, and Montreal Cognitive Assessment (MoCA) score. Post-hoc multiple comparisons were performed by Fisher's Least Significant Difference (LSD) method. Pearson's chi-square test was performed to evaluate the gender differences among the three groups.

To evaluate the intergroup differences of brain perfusion, a Kruskal–Wallis test was performed with the TSA maps of the AD, MCI, and NC groups, and a Wilcoxon rank sum test was performed between each patient group and NC on the regions showing significant results in the Kruskal–Wallis test. Multiple comparison correction was performed using AlphaSim evaluations in AFNI (http://afni.nimh.nih.gov), where the threshold was set as $p < 0.05$ and the cluster level at $p < 0.01$. The between-group differences in FC were evaluated by two-sample $t$-tests with an AlphaSim correction ($p < 0.05$, cluster level $p < 0.01$). The correlation between FC and MMSE was evaluated by Pearson correlation coefficients, and multiple comparison was corrected by the False Discovery Rate (FDR) method. A $p$ value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. Demographics and cognitive assessment

Demographic data and the findings of cognitive assessments are listed in Table 1. Gender, age, and education showed no significant intergroup differences ($p > 0.05$). MMSE and MoCA scores were significantly lower in both the AD and MCI groups than in the NC group

($p < 0.001$) as expected.

### 3.2. Hypoperfusion analysis by the TSA method between groups

Based on the overall results for the patient groups and NC group, significant intergroup differences were found in the left inferior temporal gyrus (ITG), left hippocampus (HIP), left parahippocampal gyrus (PHG) and bilateral medial superior frontal gyrus ($p < 0.05$, alpha < 0.01, AlphaSim corrected; Fig. 2(A)). Since negative values indicates hypoperfusion in the TSA method, significant hypoperfusion appeared in several brain regions including the left ITG, left HIP, and left PHG in the AD group in comparison with the NC group ($p < 0.05$, alpha < 0.01, AlphaSim corrected; Fig. 2(B), Table 3). Significant bilateral hypoperfusion was found in the medial superior frontal gyrus in the MCI group in comparison with the NC group ($p < 0.05$, alpha < 0.01, AlphaSim corrected; Fig. 2(C), Table 3). Detailed comparisons of TSA values among the three groups were given as boxplots in Fig. 3.

### 3.3. Functional connectivity analysis using the areas showing significant hypoperfusion as seeds

The regions showing significant hypoperfusion in intergroup comparison were used as seeds in FC analysis. Based on the TSA results, a comparison of the AD and NC groups indicated two separate clusters. One cluster can be further divided into three regions based on AAL templates: the middle part of the left ITG, left HIP, and left PHG, in which the second cluster is the posterior part of the left ITG, as is given in Table 3. The four regions were used as seeds to compare FC between AD and NC groups, in which only one seed, the middle part of left ITG,

**Table 3**
Brain regions with a significant time delay between patient groups and NC.

| Brain regions | Peak MNI coordinates (x y z) | | | Z value | p value | AAL | Voxels |
|---|---|---|---|---|---|---|---|
| **AD versus NC** | | | | | | | |
| Left temporal lobe | −27 | −24 | −27 | −3.5052 | 0.0002 | 89 (ITG.L_mid) | 64 |
| | | | | | | 37 (HIP.L) | 19 |
| | | | | | | 39 (PHG.L) | 30 |
| Left inferior temporal gyrus | −57 | −51 | −18 | −3.4889 | 0.0002 | 89 (ITG.L_post) | 103 |
| **MCI versus NC** | | | | | | | |
| Bilateral medial superior frontal gyrus | −3 | 54 | 33 | −3.5038 | 0.0002 | 23 (SFGmed.L) | 79 |
| | | | | | | 24 (SFGmed.R) | 79 |

ITG.L_mid, middle part of left inferior temporal gyrus; HIP.L, left hippocampus; PHG.L, left parahippocampal gyrus; ITG.L_post, posterior part of left inferior temporal gyrus; SFGmed.L, left medial superior frontal gyrus; SFGmed.R, right medial superior frontal gyrus.

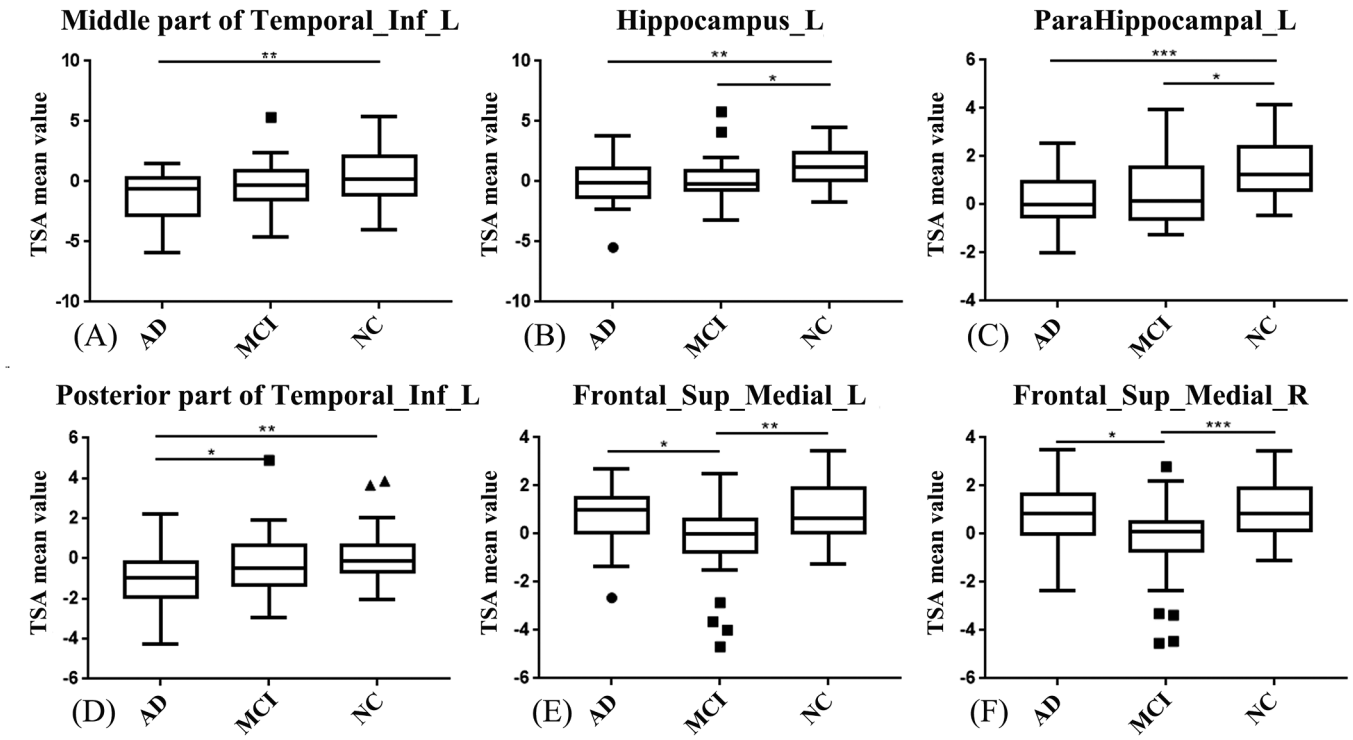


**Fig. 3.** Boxplots of time-shift analysis (TSA) values in the Alzheimer's disease (AD), mild cognitive impairment (MCI), and normal control (NC) groups. The abscissa is the name of the group and the ordinate is the TSA mean value. The titles indicate the regions used to calculate the TSA mean value. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. (A) The TSA mean value in the middle part of the left inferior temporal gyrus was significantly lower in the AD group in comparison with the NC group. (B) The TSA mean value in the left hippocampus was significantly lower in the AD group in comparison with the NC group. (C) The TSA mean value in the left parahippocampal region was significantly lower in the AD group in comparison with the NC group. (D) The TSA mean value in the posterior part of the left inferior temporal gyrus was significantly lower in the AD group in comparison with the NC group. (E) The TSA mean value in the left medial superior frontal gyrus was significantly lower in the MCI group in comparison with the NC group. (F) The TSA mean value in the right medial superior frontal gyrus was significantly lower in the MCI group in comparison with the NC group.

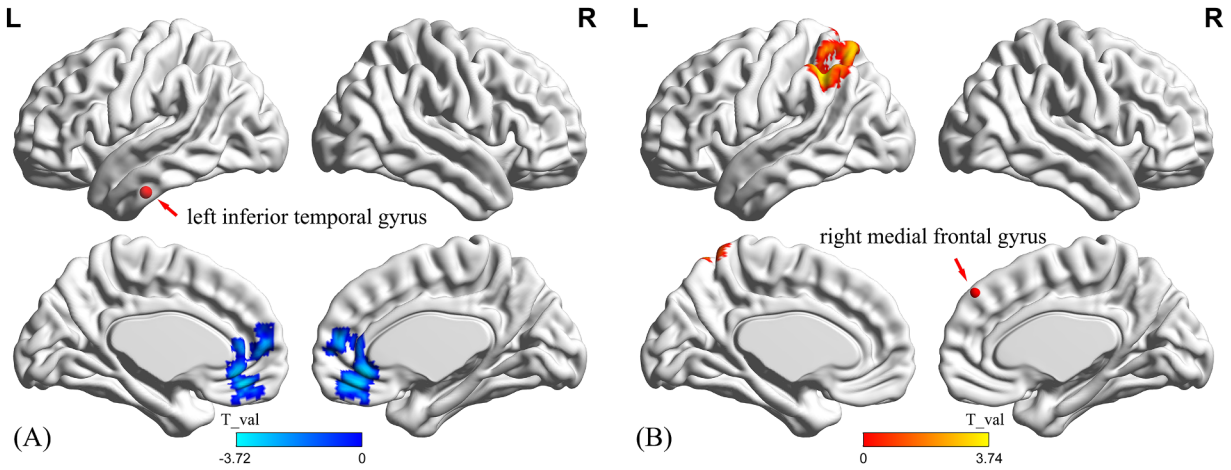


**Fig. 4.** Brain regions showing functional connectivity (FC) abnormality in each patient groups when using significant hypoperfusion areas as seed regions. The red arrows indicate the seed regions. (A) In comparison with the normal control (NC) group, the Alzheimer's disease (AD) group shows a significant reduction in FC between left inferior temporal gyrus and bilateral medial frontal-cingulate regions when the seed region is the middle part of the left inferior temporal gyrus. (B) In comparison with the NC group, the mild cognitive impairment (MCI) group shows a significant increment in FC between right medial frontal gyrus and left parietal lobe when the seed region is the right medial superior frontal gyrus.

showed significant difference. FC between the middle part of left ITG and bilateral medial frontal-cingulate region was found significantly lower in the AD group ($p < 0.05$, alpha $< 0.01$, cluster size $> 260$; Fig. 4(A)). Two seeds were used for comparing MCI and NC groups, which are the left medial superior frontal gyrus and the right medial superior frontal gyrus (Table 3). FC between the left parietal lobe and the right medial superior frontal gyrus was found significantly higher in the MCI group ($p < 0.05$, alpha $< 0.01$, cluster size $> 365$; Fig. 4(B)), while there was no significant difference when the left medial superior frontal gyrus was used as a seed.

### 3.4. Correlation between FC and MMSE scores

Pearson's correlation analysis between FC values and MMSE scores was performed for regions showing significant intergroup differences in FC. The average FC values between the right medial superior frontal gyrus and the left superior parietal gyrus (SPG) were found to be correlated significantly and negatively with MMSE scores in the MCI group, ($p < 0.05$, $r = -0.5888$, Fig. 5(B)). The average FC values between the right medial superior frontal gyrus and the left inferior parietal gyrus (IPL) showed negatively correlated trend, but not get through the FDR correction ($p > 0.05$, $r = -0.4443$, Fig. 5(A)). Moreover, no significant correlations were found between other FC values or MMSE scores (Table 4).

## 4. Discussion

There are three major findings of the current study. Firstly, the TSA assessments showed hypoperfusion not only in the left HIP and left PHG that are generally regarded as the most likely regions to suffer damage, but also in the left ITG and bilateral medial superior frontal gyrus. Secondly, we found that the areas of hypoperfusion showed abnormal FC with other brain regions. The left ITG showed decreased perfusion and decreased FC with other regions in AD patients, while the right medial superior frontal gyrus showed decreased perfusion and increased FC with other regions in MCI patients. Thirdly, the FC between the right medial superior frontal gyrus and left SPG showed significant negative correlation with MMSE scores in the MCI group. In conclusion, the hypoperfusion may affect the cognitive state via abnormal FC, which could be an additional factor contributing to cognitive impairment. Moreover, the time-shift analysis methodology is a promising technique for detecting hypoperfusion without any contrast media.

Previous studies have highlighted the incidence of hypoperfusion in AD patients (Chen et al., 2011; Ding et al., 2014; Ones et al., 2012). In our study, the TSA results revealed the progression of brain hypoperfusion from the normal elderly control group to the MCI group and the AD group. Specifically, typical patterns of perfusion alterations were found in the left ITG, left HIP, and left PHG, which indicated a trend of progressively increasing damage from the NC group to the MCI group and further to the AD group. This progression has been confirmed by other accounts of cerebral hypoperfusion in the temporal lobe in people with clinically diagnosed AD (Chen et al., 2011). A significant difference was found between the MCI and NC patients in both sides of the medial superior frontal gyrus, a finding consistent with previous study reporting that the left frontal lobe is affected from the onset of disease (Ones et al., 2012). Our results are consistent with the findings of previous studies (Ding et al., 2014; Hansson et al., 2009; Tang et al., 2012), and indicate the spatial specificity of hypoperfusion in AD progression. Therefore, current results demonstrate that TSA is a promising method in perfusion studies on AD.

Our results also show that the abnormal FC might be associated with hypoperfusion. In the current study, regions showing hypoperfusion also showed significantly different FC patterns between groups. In the AD group, regions showed hypoperfusion also showed decreased FC with other brain regions, indicating the effect of hypoperfusion on functional dysconnectivity. In addition, our results showed increased FC between the seed region and the left IPL as well as the seed region and the left SPG in the MCI group compared with those in the NC group, while the seed region, right medial superior frontal gyrus, showed decreased perfusion. These results may indicate the presence of hyperconnectivity among functional networks when hypoperfusion are present in the brain. A previous study (Hillary et al., 2015) proposed that hyperconnectivity is a fundamental response to neurological disruption, and there is a shift to diminished connectivity as degeneration progresses, which are consistent with our study. Another study (Delli Pizzi et al., 2019) proposed that hyperconnectivity may represent a compensatory strategy against the progression of cognitive impairment. However, the hyperconnectivity in our study cannot be a compensatory strategy according to our third finding that the hyperconnectivity showed significant negative correlation with MMSE scores which is discussed below. In short, our results indicated functional dysconnectivity appeared in regions with hypoperfusion for AD patients, while hyperconnectivity appeared in regions with hypoperfusion for MCI patients. These findings imply a possible association between FC abnormality and brain hypoperfusion.

In our study, abnormal FC was significantly correlated with MMSE

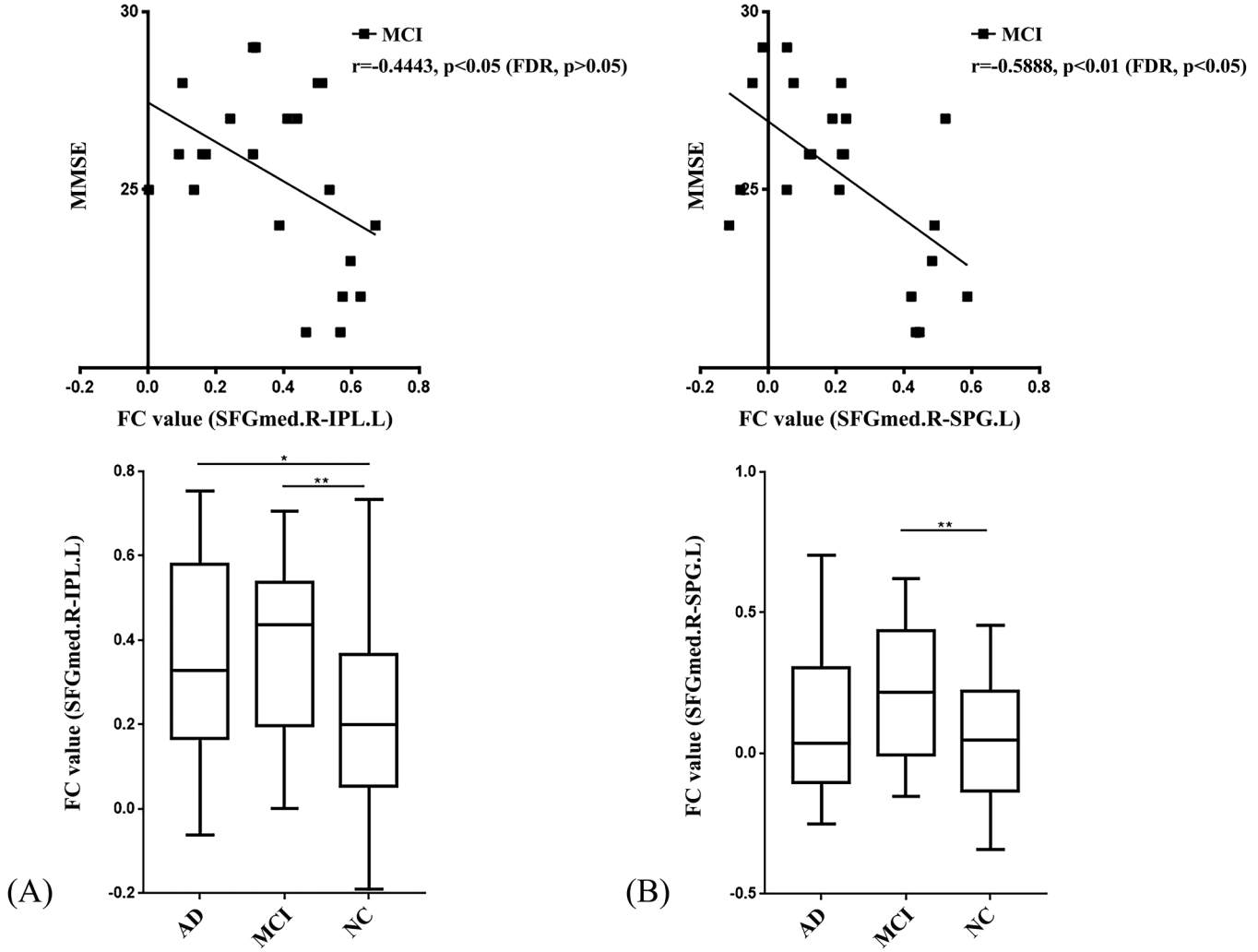


**Fig. 5.** Significant negative correlation or negative correlation trend between functional connectivity (FC) values and mini-mental state examination (MMSE) scores in the mild cognitive impairment (MCI) group. The correlation coefficient is considered significant at $p < 0.05$. (A) Negative correlation trend between MMSE scores and the FC between the right medial superior frontal gyrus and left inferior parietal gyrus in the MCI group. Average FC between the right medial superior frontal gyrus and left inferior parietal gyrus was significantly higher in the MCI group than that in the NC group. (B) Significant negative correlation between MMSE scores and the FC between the right medial superior frontal gyrus and left superior parietal gyrus in the MCI group. Average FC between the right medial superior frontal gyrus and left superior parietal gyrus was significantly higher in the MCI group than that in the NC group.

**Table 4**
Correlation between FC values and MMSE scores in the different groups.

| ROIs/Cluster | Pearson's correlation (r) | | |
|---|---|---|---|
| | AD | MCI | NC |
| **Middle part of left inferior temporal gyrus** | | | |
| Left medial frontal-cingulate regions↓ | 0.1714 | −0.0845 | −0.1447 |
| Right medial frontal-cingulate regions↓ | 0.1533 | −0.1535 | −0.1047 |
| **Right medial superior frontal gyrus** | | | |
| Left inferior parietal gyrus↑ | −0.1443 | **−0.4443** | 0.1723 |
| Left superior parietal gyrus↑ | 0.0499 | **−0.5888*** | 0.0909 |

*Note:* The two seed clusters are marked in bold (i.e., middle part of left inferior temporal gyrus, right medial superior frontal gyrus), followed by their respective target clusters. ↑ denotes increased FC between seed cluster and target cluster, while ↓ denotes decreased FC between seed cluster and target cluster. Correlations between the average FC values in the target clusters and the MMSE scores were calculated. Multiple comparisons were corrected by the False Discovery Rate (FDR) method with considering twelve correlations (3 group and 4 regions). The two correlation coefficients marked in bold (i.e., −0.4443, −0.5888) denote significant correlation trend before FDR correction, while * denotes $p < 0.05$ after FDR correction.

scores, indicating that hypoperfusion might contribute to cognitive impairment via abnormal FC. For example, the average FC values between the right medial superior frontal gyrus and left SPG showed significant negative correlation with MMSE scores in the MCI group, indicating that the hyperconnectivity between the right medial superior frontal gyrus and left SPG might be associated with cognitive impairment. The higher FC values, the lower MMSE scores. The average FC values between the right medial superior frontal gyrus and left IPL showed negative correlation trend with MMSE scores. This correlation was not significant, because it does not get through the FDR correction. We guess that the heterogeneity of amnestic mild cognitive impairment (aMCI) and non-amnestic MCI (non-aMCI) might influence the significance. However, it also indicated the same inference that the hyperconnectivity associated with hypoperfusion might contribute to cognitive impairment to some degree. In the AD group, although the correlation between average FC value and the MMSE scores was not significant, it still showed an extremely weak positive correlation trend, indicating that dysconnectivity associated with hypoperfusion might contribute to cognitive impairment. Given that the abnormal FC was associated with hypoperfusion, we proposed a hypothesis that hypoperfusion might be an additional factor contributing to cognitive impairment.

There are a few limitations in the current study. Firstly, although the TSA technique yielded perfusion data under non-invasive conditions, the data were obtained as discrete leading to limited resolution. Secondly, aMCI and non-aMCI have not been discriminated in the current retrospective study, so there may be heterogeneity in our MCI group and affect the significance of the conclusion. Thirdly, this is a cross-sectional study and the current results about the hypoperfusion progression need to be verified with further longitudinal research.

In conclusion, the study showing reasonable brain hypoperfusion demonstrates that TSA is effective in detecting hypoperfusion under non-invasive conditions in patients with AD. Cognitive impairment is found associated with abnormal FC that is associated with brain hypoperfusion, suggesting hypoperfusion as a potential additional factor contributing to cognitive decline.

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## References

Benedictus, M.R., Leeuwis, A.E., Binnewijzend, M.A., Kuijer, J.P., Scheltens, P., Barkhof, F., van der Flier, W.M., Prins, N.D., 2017. Lower cerebral blood flow is associated with faster cognitive decline in Alzheimer's disease. Eur. Radiol. 27, 1169–1175.

Binnewijzend, M.A., Kuijer, J.P., Benedictus, M.R., van der Flier, W.M., Wink, A.M., Wattjes, M.P., van Berckel, B.N., Scheltens, P., Barkhof, F., 2013. Cerebral blood flow measured with 3D pseudocontinuous arterial spin-labeling MR imaging in Alzheimer disease and mild cognitive impairment: a marker for disease severity. Radiology 267, 221–230.

Chao-Gan, Y., Yu-Feng, Z., 2010. DPARSF: a MATLAB toolbox for "pipeline" data analysis of resting-state fMRI. Front. Syst. Neurosci. 4, 13.

Chen, W., Song, X., Beyea, S., D'Arcy, R., Zhang, Y., Rockwood, K., 2011. Advances in perfusion magnetic resonance imaging in Alzheimer's disease. Alzheimers. Dement. 7, 185–196.

Christen, T., Jahanian, H., Ni, W.W., Qiu, D.Q., Moseley, M.E., Zaharchuk, G., 2015. Noncontrast mapping of arterial delay and functional connectivity using resting-state functional MRI: a study in Moyamoya patients. J. Magn. Reson. Imaging. 41, 424–430.

Dai, W., Lopez, O.L., Carmichael, O.T., Becker, J.T., Kuller, L.H., Gach, H.M., 2009. Mild cognitive impairment and Alzheimer disease: patterns of altered cerebral blood flow at MR imaging. Radiology 250, 856–866.

de la Torre, J.C., 2000. Critically attained threshold of cerebral hypoperfusion: the CATCH hypothesis of Alzheimer's pathogenesis. Neurobiol. Aging 21, 331–342.

Delli Pizzi, S., Punzi, M., Sensi, S.L., Alzheimer's Disease Neuroimaging, I., 2019. Functional signature of conversion of patients with mild cognitive impairment. Neurobiol. Aging 74, 21–37.

Ding, B., Ling, H.W., Zhang, Y., Huang, J., Zhang, H., Wang, T., Yan, F.H., 2014. Pattern of cerebral hyperperfusion in Alzheimer's disease and amnestic mild cognitive impairment using voxel-based analysis of 3D arterial spin-labeling imaging: initial experience. Clin. Interv. Aging 9, 493–500.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. 12, 189–198.

Hansson, O., Buchhave, P., Zetterberg, H., Blennow, K., Minthon, L., Warkentin, S., 2009. Combined rCBF and CSF biomarkers predict progression from mild cognitive impairment to Alzheimer's disease. Neurobiol. Aging 30, 165–173.

Hillary, F.G., Roman, C.A., Venkatesan, U., Rajtmajer, S.M., Bajo, R., Castellanos, N.D., 2015. Hyperconnectivity is a fundamental response to neurological disruption. Neuropsychology 29, 59–75.

Iizuka, T., Kameyama, M., 2017. Cholinergic enhancement increases regional cerebral blood flow to the posterior cingulate cortex in mild Alzheimer's disease. Geriatr. Gerontol. Int. 17, 951–958.

Lacalle-Aurioles, M., Navas-Sanchez, F.J., Aleman-Gomez, Y., Olazaran, J., Guzman-De-Villoria, J.A., Cruz-Orduna, I., Mateos-Perez, J.M., Desco, M., 2016. The disconnection hypothesis in Alzheimer's disease studied through multimodal magnetic resonance imaging: structural, perfusion, and diffusion tensor imaging. J. Alzheimers Dis. 50, 1051–1064.

Lee, J.S., Im, D.S., An, Y.S., Hong, J.M., Gwag, B.J., Joo, I.S., 2011. Chronic cerebral hypoperfusion in a mouse model of Alzheimer's disease: an additional contributing factor of cognitive impairment. Neurosci. Lett. 489, 84–88.

Leeuwis, A.E., Benedictus, M.R., Kuijer, J.P.A., Binnewijzend, M.A.A., Hooghiemstra, A.M., Verfaillie, S.C.J., Koene, T., Scheltens, P., Barkhof, F., Prins, N.D., van der Flier, W.M., 2017. Lower cerebral blood flow is associated with impairment in multiple cognitive domains in Alzheimer's disease. Alzheimers Dement. 13, 531–540.

Li, W., Antuono, P.G., Xie, C., Chen, G., Jones, J.L., Ward, B.D., Franczak, M.B., Goveas, J.S., Li, S.J., 2012. Changes in regional cerebral blood flow and functional connectivity in the cholinergic pathway associated with cognitive performance in subjects with mild Alzheimer's disease after 12-week donepezil treatment. NeuroImage 60, 1083–1091.

Liang, X., Zou, Q., He, Y., Yang, Y., 2013. Coupling of functional connectivity and regional cerebral blood flow reveals a physiological basis for network hubs of the human brain. Proc. Natl. Acad. Sci. USA 110, 1929–1934.

Lou, W., Shi, L., Wong, A., Chu, W.C., Mok, V.C., Wang, D., 2016. Changes of Cerebral Perfusion and Functional Brain Network Organization in Patients with Mild Cognitive Impairment. J. Alzheimers. Dis. 54, 397–409.

Lv, Y., Margulies, D.S., Cameron Craddock, R., Long, X., Winter, B., Gierhake, D., Endres, M., Villringer, K., Fiebach, J., Villringer, A., 2013. Identifying the perfusion deficit in acute stroke with resting-state functional magnetic resonance imaging. Ann. Neurol. 73, 136–140.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., Stadlan, E.M., 1984. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34, 939–944.

Ones, T., Midi, I., Dede, F., Tuncer, N., Erdil, T.Y., Onultan, O., Ceylan, S., Inanir, S., Turoglu, H.T., 2012. Initial mini-mental state and cerebral perfusion in Alzheimer's disease. Clin. Neuroradiol. 22, 219–226.

Petersen, R.C., 2004. Mild cognitive impairment as a diagnostic entity. J. Intern. Med. 256, 183–194.

Ruitenberg, A., den Heijer, T., Bakker, S.L., van Swieten, J.C., Koudstaal, P.J., Hofman, A., Breteler, M.M., 2005. Cerebral hypoperfusion and clinical onset of dementia: the Rotterdam study. Ann Neurol 57, 789–794.

Song, X.W., Dong, Z.Y., Long, X.Y., Li, S.F., Zuo, X.N., Zhu, C.Z., He, Y., Yan, C.G., Zang, Y.F., 2011. REST: a toolkit for resting-state functional magnetic resonance imaging data processing. PloS One 6, e25031.

Tang, Z., Pi, X., Chen, F., Shi, L., Gong, H., Fu, H., Qu, Z., 2012. Fifty percent reduced-dose cerebral CT perfusion imaging of Alzheimer's disease: regional blood flow abnormalities. Am. J. Alzheimers. Dis. Other. Dement. 27, 267–274.