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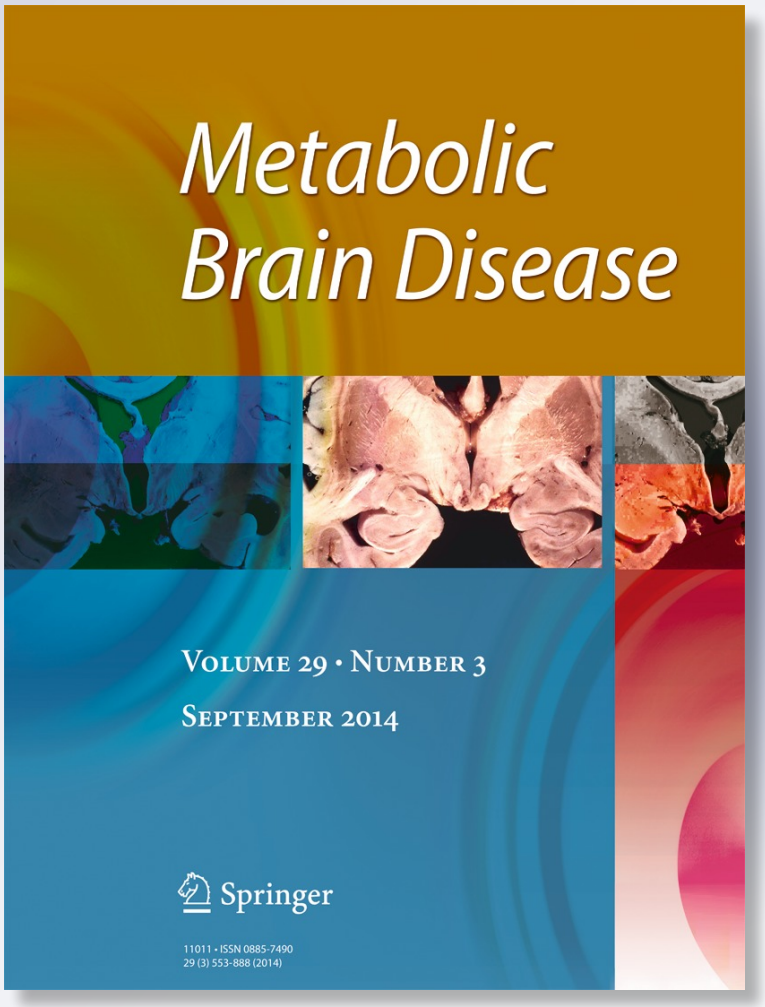
Metabolic Brain Disease

ISSN 0885-7490
Volume 29
Number 3

Metab Brain Dis (2014) 29:777-786
DOI 10.1007/s11011-014-9568-6



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RESEARCH ARTICLE

Altered brain functional connectivity in hemodialysis patients with end-stage renal disease: a resting-state functional MR imaging study

Gang Zheng · Jiqiu Wen · Liping Zhang · Jianhui Zhong · Xue Liang · Wenwei Ke · Xiang Kong · Tiezhu Zhao · Yong He · Xinian Zuo · Song Luo · Long Jiang Zhang · Guang Ming Lu

Received: 13 February 2014 / Accepted: 12 May 2014 / Published online: 21 May 2014

Abstract The changes of whole brain functional connectivity in hemodialysis (HD) patients with end-stage renal disease (ESRD) are still unclear, which may be associated with multiple factors, such as elevated neurotoxins, anemia, and side effects of hemodialysis. Resting-state functional magnetic resonance imaging (rs-fMRI) data of 71 patients (43 males, 28 females; mean age, 33.4±9.4 years) and 43 age- and gender-matched healthy volunteers (29 males, 14 females; mean age, 30.6±8.8 years) were acquired. Neuropsychological tests including number connection test type A (NCT-A), digit symbol test (DST), line-tracing test (LTT), serial-dotting test (SDT), self-rating depression scale (SDS) and self-rating anxiety scale (SAS) were used to evaluate cognitive and psychiatric conditions in all subjects. Blood biochemistry tests including serum creatinine levels, blood urea, hematocrit, and Ca^{2+} level were taken in HD patients. Forty-two connections significantly different between HD patients with ESRD and controls were found (all $P<0.05$, Bonferroni corrected) and identified as connectivities of interests (COIs), among which 39 connections (92.9 %) were markedly decreased in patients. Of the 39 weaker connections, 24 were related to the frontal lobe regions. Widespread weakening of cortical and subcortical network connectivity in ESRD patients was more directly related with neuropsychological impairments and anemia rather than serum creatinine level, blood urea and dialysis duration. In particular, impairments in the medial prefrontal lobe could play an important role in mediating psychological dysfunctions.

Keywords End-stage renal disease · Hemodialysis · Resting-state fMRI · Functional connectivity

Abbreviations

ESRD	End-stage renal disease
HD	Hemodialysis
NCT-A	Number connection test type A
DST	Digit symbol test
LTT	Line-tracing test
SDT	Serial-dotting test

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SDS Self-rating depression scale
SAS Self-rating anxiety scale
COI Connectivity of interests

Introduction

End-stage renal disease (ESRD) is characterized by a glomerular filtration rate of<15 mL/min/1.73 m^2, or the point at which permanent renal replacement therapy is needed (Kim et al. 2011). ESRD is becoming a substantial public health problem worldwide. In the United States, approximately 8 % of the population has chronic renal disease and 571,000 patients receive treatment for ESRD (Collins et al. 2011). In ESRD patients undergoing hemodialysis, the prevalence of cognitive impairment has been estimated at 30–60 % (Bugnicourt et al. 2013). With life expectancy increasing and the population aging in developed and even some developing countries, the cognitive disorder burden associated with ESRD will be further worsened (Bugnicourt et al. 2013). However, cognitive dysfunction and dementia is often overlooked in ESRD patients, and the cause and pathophysiology of cognitive disorders in ESRD patients remain unclear.

The brain disorders of ESRD patients might be directly or indirectly associated with many risk factors, such as elevated neurotoxins, anemia, and side effects of hemodialysis. The uremic state of ESRD is characterized by the retention of solutes that are toxic in high concentration, such as creatinine and urea Meyer and Hostetter (2007). Secondary to chronic renal failure, anemia is usually present. A marked increase in the prevalence of anemia could develop with the decrease of creatinine clearance (Hsu et al. 2002). To remove toxins in the blood, hemodialysis is used as the routine renal replacement therapy for ESRD patients. However, long-term hemodialysis could lead to blood ion concentrations that may alter brain functions. For example, changes in calcium among chronic hemodialysis patients resulted in vascular calcification (Kirschbaum 2004). Vascular calcification could disturb the delivery of blood and might lead to insufficient oxygen delivery. All the above factors potentially impair brain function in ESRD patients. However, it is still unclear which factors are more closely related to brain dysfunction in patients with ESRD.

Multiple risk factors tend to induce widespread brain disorders in ESRD patients. For example, creatinine and urea could be delivered throughout the brain. Also, low hemoglobin and high calcium of blood could impair all brain regions coincided with the blood delivery. Furthermore, previous medical imaging studies supported widespread brain disorders in ESRD patients. For example, Fazekas et al. (Fazekas et al. 1996) found decreased cerebral blood flow in the frontal cortex and thalamus and increased cerebral blood flow in the occipital lobe in hemodialysis patients using single photon emission computed tomography (SPECT), which correlated with cognitive test results. Voxel-based morphometry MR can be used to investigate focal differences in brain anatomy using statistical parametric mapping to locate brain atrophy. Recently, Zhang et al. reported that gray volumes of ESRD patients were significantly decreased in the bilateral occipital lobes, precuneus and markedly increased right extranuclear, right caudate, and right thalamus (Zhang et al. 2013). Arterial spin labeling MR perfusion results showed that ESRD patients' CBFs were significantly decreased in left posterior middle temporal gyrus and a left inferior hypothalamic area bordering the fourth ventricle (Prohovnik et al. 2007). Diffusion tensor imaging found widespread white matter alterations reflected by increased axial diffusivity, radial diffusivity, and mean diffusivity values and decreased anisotropy values, mostly in the corpus callosum, bilateral sagittal stratum, and pons (Hsieh et al. 2009; Chou et al. 2013). Recently, Liang et al. found decreased regional homogeneity values in widespread cortical regions in ESRD patients based on blood oxygen level dependent resting-state functional MRI (rs-fMRI) with ReHo analysis (Liang et al. 2013). These findings regionally uncovered the neuropathological mechanisms of ESRD. The brain interconnects a large amount of neurons and neural fibers, and the risk factors of renal diseases might also impair brain functions without selection. However, brain dysfunctions of ESRD patients in full brain level and their corresponding risk factors are still unclear.

Full brain functional connectivity analysis has been widely used in fMRI studies, for neurological disorders such as Alzheimer's disease (AD), epilepsy, schizophrenia, hepatic encephalopathy, and others (Xie and He 2012; Madhavan et al. 2013; Venkataraman et al. 2012; Rocca et al. 2010; Zhang et al. 2012). In this paper, our hypothesis was that ESRD patients could have altered brain functional connectivity, which may be associated with their cognitive and psychiatry situations. We further assumed that these disrupted functional connectivity were related with the blood toxins, the prevalence of anemia, and ion concentrations secondary to hemodialysis. Our goal was to use rs-fMRI to assess changes in whole brain functional connectivity in ERSD patients undergoing hemodialysis, and to look for the relationship between these alterations, and neuropsychological tests and blood biochemistry tests.

Materials and methods

Subjects

This retrospective study was approved by the local institutional review board and was conducted in compliance with Health

Insurance Portability and Accountability Act from Jan 2012 to May 2013. All subjects provided written informed consent before the fMRI or neuropsychological evaluation. A battery of neuropsychiatric tests, including number connection test type A (NCT-A), digit symbol test (DST), line-tracing test (LTT), and the serial-dotting test (SDT), was used to evaluate neurocognitive functions (Ferenci et al. 1998). Additionally, the self-rating anxiety scale (SAS) and self-rating depression scale (SDS) were given to all subjects before MRI studies to evaluate their anxiety and depression state (Kimmel et al. 2007; Cukor et al. 2006; Theofilou 2011). In this study, 71 ESRD patients undergoing hemodialysis (43 males, 28 females, 33.4±9.4 years old) were recruited from inpatients. The inclusion criteria for recruitment of the ESRD patients were as follows: patients who could finish fMRI examinations without any MRI contraindication and with head motion less than 1 mm, and patients without artificial teeth or other foreign bodies in the head causing significant artifacts. All patients had no other diseases affecting brain functions, such as drug abuse and trauma. Blood biochemistry tests, including serum creatinine levels, blood urea, hematocrit, and Ca^{2+} levels, were measured in HD patients within 24 h before MR examinations. The patient demographics and clinical data are summarized in Table 1. Forty-three age- and gender-matched healthy subjects (29 males, 14 females, 30.6±8.8 years old) were recruited from local communities. All healthy subjects had no other diseases affecting brain functions. Abdominal ultrasound scans revealed no abnormal findings for all healthy subjects. Blood biochemistry tests were not available in healthy subjects.

Magnetic resonance imaging

MR examinations were performed with a clinical 3T whole-body scanner (TIM Trio, Siemens Medical Solutions, Erlangen, Germany) using a standard birdcage head transmit/receive coil. The head was positioned with soft pads inside the coil to reduce head movement. A total of 250 volumes of axial EPI images were obtained with the following parameters: field-of-view (FOV)=240 mm×240 mm, matrix size=64×64×30, flip angle=90°, TR=2,000 ms, TE=30 ms, slice thickness=4 mm with distance factor=10 %. For each subject, magnetization-prepared, rapid acquisition gradient echo image sequence with isotropic resolution of 1 mm was used to obtain high-resolution, 3D T1 weighted anatomical images for spatial normalization. Axial T2- FLAIR sequence was used to exclude brain anatomical lesions with the following parameters: 25 axial slices, thickness=4 mm, slice gap=1.2 mm, image matrix=232×256, field of view (FOV)=220 mm×220 mm, repetition time (TR)=9,000 ms, echo time (TE)=93 ms, flip angle=130°, inversion time (TI)=2,500 ms. During the MRI scans, all subjects were instructed to rest with their eyes closed and heads still.

Data preprocessing

To obtain the regional mean time series of each subject for computing functional connectivity, BOLD fMRI data were preprocessed with the toolbox Data Processing Assistant for Resting-State fMRI (DPARSF, http://www.restfmri.net/forum/DPARSF) based on SPM8 (Statistical Parametric Mapping, http://www.fil.ion.ucl.ac.uk/spm/). The beginning ten volumes of each dataset were discarded for reaching T1 equilibrium. The remaining 240 measures were first processed for slice timing and realigned to the middle volume to correct for inter scan head motions. Secondly, the functional scans were spatially normalized to the Montreal Neurological Institute template and resampled to 3×3×3 mm^3. Thirdly, the BOLD signal was de-trended to correct a linear trend, and then band filtered (0.01–0.08 Hz) to reduce both low-

Table 1 Demographical, clinical, and biochemical data of the ESRD patients and healthy subjects

Variable	ESDR patients	Healthy controls	*P* value
Age (years)	33.4±9.4	30.6±8.8	0.116
Sex (Male/Female)	43/28	29/14	NS
NCT-A (s)	44.14±15.97	34.95±11.85	**<0.001**
DST (score)	51.59±13.14	62.65±13.62	**<0.001**
LTT (s)	59.82±24.46	48.60±15.67	**0.006**
SDT (s)	53.45±15.50	43.91±9.72	**<0.001**
SAS (score)	35.70±9.00	29.16±5.61	**<0.001**
SDS (score)	33.13±8.50	26.49±5.07	**<0.001**
Serum creatinine level (in mol/L)	825.1±284.8	N/A	N/A
Blood urea (in mmol/L)	20.91±8.35	N/A	N/A
Hematocrit (%)	28.2±6.5	N/A	N/A
Ca^{2+} (in mmol/L)	2.20±0.29	N/A	N/A
Dialysis duration (month)	7.49±10.40	N/A	N/A

Values are mean ± SD or number of patients;

Abbreviation NCT-A number connection test type A, *DST* digit symbol test, *LTT* Line tracing test, *SDT* Serial dotting test, *SAS* self-rating anxiety scale, *SDS* self-rating depression scale, *NS* no statistical significance, *N/A* not applicable data

frequency drift and high-frequency physiological noise. Fourthly, nuisance covariates including cerebrospinal fluid signals, global mean signals, white matter signals and head motion parameters were regressed out from the fMRI data. Finally, the mean time series of ninety regions of interests (ROI) defined by the Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer et al. 2002) were estimated for each individual by averaging the fMRI time series over all voxels in each ROI.

To assess the functional connectivity of each subject (Biswal et al. 1995), the Pearson correlation coefficient (CC) between every pair of 90 regional time series was computed by the following formula:

$$r_{ij} = \frac{< v(x_i,t)v(x_j,t) > - < v(x_i,t) >< v(x_j,t) >}{\sigma(v(x_i,t))\sigma(v(x_j,t))} \quad (1)$$

where $v(x_i,t)$ is the time course of the *ith* region, $\sigma(v(x,t)) = \sqrt{< v^2(x,t) > - < v(x,t) >^2}$ is the corresponding mean square deviation of the time course, and r_{ij} is the value of CC, which is referred to as the functional connectivity in the following. A Fisher's r-to-z transform is used on the CC value of each subject to improve the normality of the CCs according to the following formula:

$$Z = 0.5 * \log[(1+r)(1-r)] \quad (2)$$

where Z is the z-score of *r*. For more details about the data processing, please see Fig. 1.

Statistical analysis

Statistical analyses were performed using the software SPSS (version 13.0, SPSS Inc. Chicago, Ill., USA). A student *t* test for independent samples was used to compare the differences in age and neuropsychological tests scores between the patients and controls. A chi-square test was used to compare the differences in gender between both groups. One-way analysis of covariance (ANCOVA), with age and gender as nuisance covariates, was applied to compare the functional connectivity differences between patients and controls. Since ANCOVA was calculated in all 4005 connections, a correction for multiple comparisons was strictly necessary. A Bonferroni correction was performed to correct multiple comparisons, and a corrected *P* value less than 0.05 was considered significant. Significantly different connectivities were chosen as connectivity of interests (COIs) for further analyses and were displayed by BrainNet Viewer (Version 1.0 RC1, http://www.nitrc.org/projects/bnv/). Correlations between neuropsychological tests and CCs of COIs were calculated among all subjects. Since biochemistry tests were not available for all healthy subjects, correlations between these tests and CCs of COIs were computed in ESRD patients. The significance level of correlations was set at a *P* value<0.05 after correction for multiple comparisons using the false discovery rate (FDR) (Benjamini and Hochberg 1995).

Results

No significant differences were found in age and gender between the ESRD patients and controls. There were significant differences in NCT-A, DST, LTT, SDT, SDS and SAS between the ESRD patients and controls (Table 1). In ESRD patients, the hematocrit and serum creatinine levels both positively correlated with dialysis duration (P=0.005, r=0.329 and P=0.0015, r=0.369, respectively). There was positive correlation between blood urea and serum creatinine concentration (P=0.0015, r=0.370). The blood Ca^{2+} level was

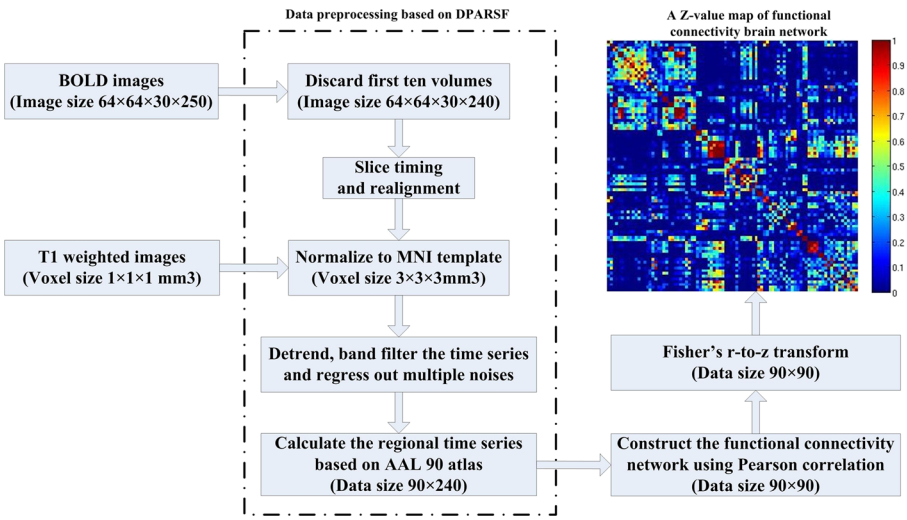

Fig. 1 The data processing procedures for the construction of brain functional connectivity network. Note: DPARSF stands for Data Processing Assistant for Resting-State fMRI

positively related with hemotocrit (P=0.008, r=0.314). Correlation between neuropsychological tests and blood biochemistry tests was only found between blood urea and SAS (P=0.012, r=0.295).

Forty-two connections between every two ROIs were significantly different (all P<0.05, Bonferroni corrected) in ESRD patients compared with controls (Figs. 2, 3 and 4; Tables 2, 3, 4, 5, and 6) and were defined as COIs for further analysis. Among these COIs, 39 (92.9 %) connections, either

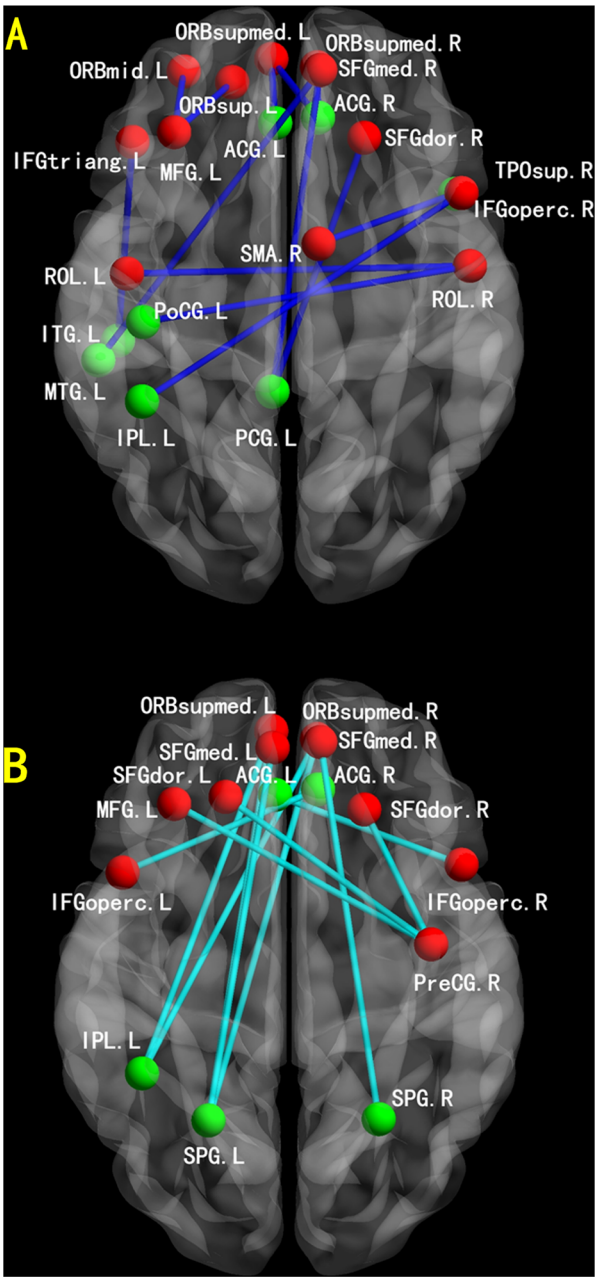

Fig. 2 Decreased functional connectivity related with frontal lobes in ESRD patients. **a**. Decreased positive connectivity in ESRD patients (*Blue* edges); **b**. Decreased negative connectivity in ESRD patients (*Light blue* edges). Notes: Red nodes = frontal lobe ROIs; Green nodes = non frontal lobe ROIs; R = Right; L = Left

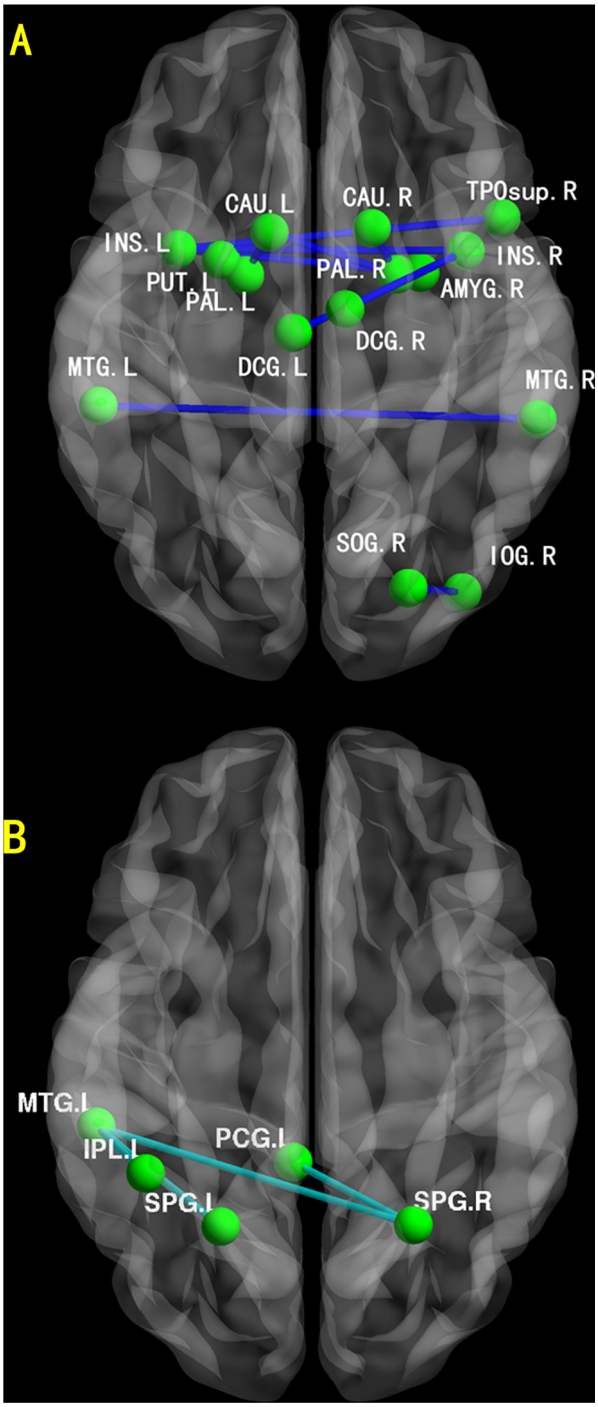

Fig. 3 Decreased functional connectivity unrelated with frontal lobes in ESRD patients. **a**. Decreased positive connectivity in ESRD patients (*Blue* edges); **b**. Decreased negative connectivity in ESRD patients (*Light blue* edges). Notes: Green nodes = non frontal lobe ROIs; R = Right; L = Left

negative or positive, were weaker in ESRD patients than in the controls (Figs. 2 and 3; Tables 2, 3, 4, and 5), and only 3 positive ones were stronger in patients (Fig. 4; Table 6).

Among the 39 connections weaker in ESRD patients, 24 COIs (61.5 %, 24/39; Fig. 2; Tables 2 and 3), including 13

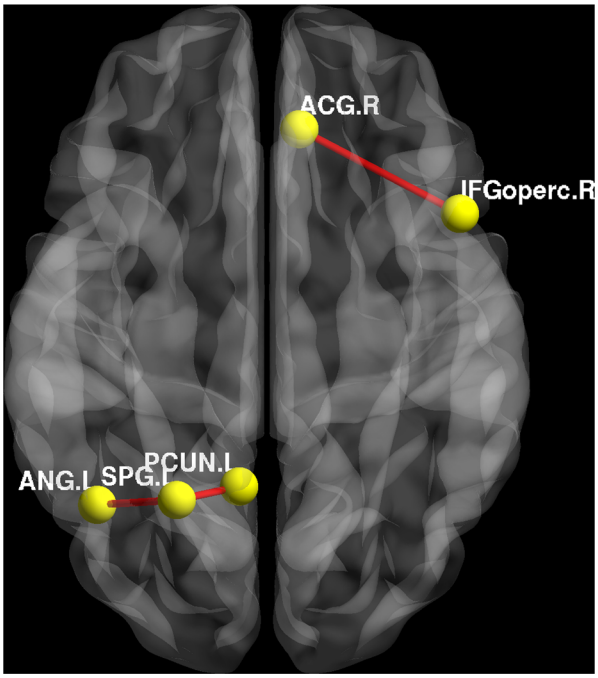

Fig. 4 Elevated functional connectivity in ESRD patients. Notes: R = Right; L = Left

positive and 11 negative connections, were related to the front lobe regions. The decreased positive connections were listed and shown in Table 2 and Fig. 2a, respectively. The decreased negative connections related to the front lobe were shown in Fig. 2b and Table 3.

Fifteen weaker COIs of ESRD patients were not related to front lobe regions, which were mainly between the insula, partial lobes, temporal lobes and basal ganglia (Fig. 3, Tables 4 and 5). Five positive weaker COIs were found between the right insula and bilateral median cingulate gyri, the left insula and right amygdala, the left insula and right temporal pole part of the superior temporal gyrus, and between the left and right insula. Four positive connectivities, found in the edges between the left caudate and bilateral pallidums, the right caudate and right pallidum, and between the left putamen and left pallidum, were weaker in ESRD patients. The remaining two weaker positive COIs in ESRD patients were found between the right superior occipital gyrus and right Inferior occipital gyrus and between the left and right middle temporal gyrus (Fig. 3a and Table 4). The four negative weaker COIs of the ESRD patients were between the right superior

Table 2 Decreased positive connectivity related with frontal lobes in ESRD patients compared with controls

COIs	Z-scores of COIs in controls	Z-scores of COIs in ESRD patients	ANCOVA *P* values§	CCs between neuropsychological test scores and Z-scores of COIs						CCs between hematocrit and Z-scores of COIs in ESRD patients
				NCT-A	DST	LTT	SDT	SDS	SAS	
SFGdor.R-PCG.L	0.253±0.290	0.037±0.237	**0.033**	−0.033	0.140	−0.041	−0.123	−0.047	−0.086	0.108
ORBsup.L-MFG.L	0.264±0.247	0.009±0.263	**0.011**	−0.121	**0.228***	−0.215	−0.206	−0.175	**−0.223***	0.117
MFG.L-ORBmid.L	0.605±0.209	0.302±0.275	**<0.001**	−0.170	**0.299****	**−0.283***	**−0.276***	−0.234	**−0.289***	0.106
IFGoperc.R-IPL.L	0.404±0.185	0.217±0.215	**0.026**	−0.147	**0.211***	−0.074	−0.150	−0.247	**−0.226***	0.249
IFGtriang.L-ITG.L	0.369±0.241	0.099±0.275	**0.004**	−0.244	**0.360****	−0.154	−0.237	−0.149	**−0.275***	0.145
ROL.L-ROL.R	1.266±0.312	0.870±0.358	**<0.001**	−0.196	**0.233***	−0.097	−0.190	−0.163	**−0.213***	0.297
ROL.R-PoCG.L	0.629±0.279	0.371±0.245	**0.004**	−0.117	**0.210***	−0.110	−0.202	−0.112	**−0.208***	0.190
SMA.R-TPOsup.R	0.232±0.216	−0.006±0.236	**0.004**	−0.038	0.160	−0.018	−0.110	−0.164	**−0.262***	0.192
SFGmed.R-PCG.L	0.535±0.240	0.283±0.271	**0.003**	−0.113	**0.215***	−0.069	−0.209	−0.078	**−0.256***	0.176
SFGmed.R-MTG.L	0.441±0.244	0.180±0.277	**0.005**	−0.166	**0.250***	−0.120	−0.225	−0.081	**−0.236***	0.048
ORBsupmed.L-ACG.L	0.544±0.260	0.281±0.295	**0.028**	−0.106	0.123	−0.019	−0.081	−0.202	**−0.257***	0.027
ORBsupmed.L-ACG.R	0.407±0.259	0.089±0.282	**<0.001**	−0.061	0.106	−0.041	−0.170	−0.189	**−0.241***	0.116
ORBsupmed.R-ACG.R	0.519±0.271	0.227±0.282	**0.003**	−0.047	0.102	−0.030	−0.095	−0.177	**−0.218***	0.231

COI Connectivity of interests, *ESRD* End-stage renal disease, *CC* Correlation coefficient, *LTT* Line-tracing test, *SDT* Serial-dotting test, *SDS* Self-ratingdepression scale, *SAS* Self-rating anxiety scale, *R* Right, *L* Left, *SFGdor* Superior frontal gyrus, *PCG* Posterior cingulate gyrus, *ORBsup* Orbital part of superior frontal gyrus, *MFG* Middle frontal gyrus, *ORBmid* Orbital part of middle frontal gyrus, *IFGoperc* Opercular part of inferior frontal gyrus, *IPL* Inferior parietal gyrus, *IFGtriang* Triangular part of inferior frontal gyrus, *ITG* Inferior temporal gyrus, *ROL* Rolandic operculum, *PoCG* Postcentralgyrus, *SMA* Supplementary motor area, *TPOsup* Temporal pole part of the superior temporal gyrus, *SFGmed* Medial part of superior frontal gyrus, *MTG* Middle temporal gyrus, *ORBsupmed* orbital part of middle frontal gyrus, *ACG* Anterior cingulate gyrus

§ ANCOVA $P<0.05$, Bonferroni-corrected

*: Correlation $P<0.05$

**: Correlation $P<0.01$, FDR corrected

Table 3 Decreased negative connectivity related with frontal lobes in ESRD patients compared with controls

COIs	Z-scores of COIs in controls	Z-scores of COIs in ESRD patients	ANCOVA *P* values§	CCs between neuropsychological test scores and Z-scores of COIs						CCs between hematocrit and Z-scores of COIs in ESRD patients
				NCT-A	DST	LTT	SDT	SDS	SAS	
PreCG.R-SFGdor.L	−0.389±0.200	−0.176±0.244	**0.028**	0.172	**−0.256***	0.083	0.140	0.132	**0.205***	**−0.305***
PreCG.R-SFGdor.R	−0.303±0.255	−0.031±0.279	**0.003**	0.123	**−0.194***	0.106	0.183	0.033	0.136	**−0.357***
PreCG.R-MFG.L	−0.310±0.222	−0.073±0.256	**0.008**	0.197	**−0.244***	0.086	0.111	0.104	0.137	**−0.326***
IFGoperc.L-ACG.R	−0.229±0.247	0.031±0.289	**0.011**	0.158	**−0.280***	0.045	0.181	−0.046	0.091	−0.082
IFGoperc.R-ACG.L	−0.221±0.220	0.014±0.262	**0.008**	0.201	**−0.255***	0.013	0.109	0.032	0.169	−0.235
SFGmed.L-SPG.L	−0.431±0.251	−0.205±0.261	**0.042**	0.222	**−0.230***	0.147	0.162	0.176	**0.217***	0.122
SFGmed.R-SPG.R	−0.482±0.243	−0.243±0.274	**0.031**	0.201	**−0.185***	0.033	0.171	0.146	**0.303****	−0.114
ORBsupmed.L-SPG.L	−0.290±0.211	0.008±0.278	**<0.001**	0.193	**−0.278***	0.066	0.152	0.191	**0.277****	0.071
ORBsupmed.L-IPL.L	−0.374±0.245	−0.103±0.266	**0.003**	0.202	**−0.235***	0.034	0.106	0.229	**0.331****	−0.202
ORBsupmed.R-SPG.L	−0.326±0.203	−0.074±0.262	**0.002**	0.174	**−0.265***	0.063	0.076	0.198	**0.251***	−0.074
ORBsupmed.R-IPL.L	−0.400±0.222	−0.169±0.229	**0.006**	0.194	**−0.238***	−0.002	0.064	0.221	**0.366*****	**−0.317***

COI Connectivity of interests, *ESRD* End-stage renal disease, *CC* Correlation coefficient, *NCT-A* Number connection test type A, *DST* Digit symbol test, *LTT* Line-tracing test, *SDT* Serial-dotting test, *SAS* Self-rating anxiety scale, *SDS* Self-ratingdepression scale, *R* Right, *L* Left, *PreCG* Precentralgyrus, *SFGdor* Superior frontal gyrus, *MFG* Middle frontal gyrus, *IFGoperc* Opercular part of inferior frontal gyrus, *ACG* Anterior cingulate gyrus, *SFGmed* Medial part of superior frontal gyrus, *SPG* Superior parietal gyrus, *ORBsupmed* Orbital part of middle frontal gyrus, *IPL* Inferior parietal gyrus

§ ANCOVA $P<0.05$, Bonferroni-corrected

*: Correlation $P<0.05$

**: Correlation $P<0.01$

***: Correlation $P<0.001$, FDR corrected

parietal gyrus and left posterior cingulate gyrus, the bilateral superior parietal gyri and left middle temporal gyrus, and between the left Inferior parietal gyrus and left middle temporal gyrus (Fig. 3b and Table 5).

Table 4 Decreased positive connectivity unrelated with frontal lobes in ESRD patients compared with controls

COIs	Z-scores of COIs in controls	Z-scores of COIs in ESRD patients	ANCOVA *P* values§	CCs between neuropsychological test scores and Z-scores of COIs						CCs between hematocrit and Z-scores of COIs in ESRD patients
				NCT-A	DST	LTT	SDT	SDS	SAS	
INS.L-INS.R	1.296±0.291	0.935±0.338	**<0.001**	−0.189*	0.110	−0.124	**−0.216***	−0.148	−0.186	0.260
INS.L-AMYG.R	0.277±0.203	0.072±0.208	**0.001**	0.029	−0.016	−0.051	−0.122	−0.032	−0.119	0.209
INS.L-TPOsup.R	0.403±0.231	0.151±0.246	**0.001**	−0.005	−0.021	−0.069	**−0.209***	−0.143	−0.226	0.127
INS.R-DCG.L	0.437±0.220	0.182±0.239	**0.001**	−0.146	0.236	−0.189	**−0.289****	−0.241	−0.221	0.036
INS.R-DCG.R	0.546±0.246	0.287±0.257	**0.005**	−0.094	0.230	−0.085	**−0.236***	−0.142	−0.142	0.084
SOG.R-IOG.R	0.541±0.342	0.273±0.307	**0.047**	0.001	0.041	0.055	−0.048	−0.139	−0.181	0.149
CAU.L-PAL.L	0.554±0.216	0.283±0.270	**0.001**	−0.202	**0.313***	**−0.245***	**−0.253***	−0.208	−0.133	0.209
CAU.L-PAL.R	0.434±0.211	0.191±0.238	**0.002**	−0.184	**0.281***	**−0.261***	**−0.253***	−0.210	−0.122	0.175
CAU.R-PAL.R	0.425±0.196	0.183±0.259	**0.005**	−0.230	**0.337***	**−0.266***	**−0.293****	−0.169	−0.089	0.286
PUT.L-PAL.L	1.308±0.198	1.108±0.232	**0.040**	−0.217	**0.267***	−0.129	−0.080	−0.236	−0.166	0.236
MTG.L-MTG.R	0.744±0.221	0.480±0.260	**0.001**	−0.221	0.115	−0.184	−0.133	−0.059	−0.122	0.080

COI Connectivity of interests, *ESRD* End-stage renal disease, *CC* Correlation coefficient, *NCT-A* Number connection test type A, *DST* Digit symbol test, *LTT* Line-tracing test, *SDT* Serial-dotting test, *SDS* Self-ratingdepression scale, *SAS* Self-rating anxiety scale, *R* Right, *L* Left, *INS* Insular lobe, *AMYG* Amygdala, *TPOsup* Temporal pole part of the superior temporal gyrus, *DCG* Middle cingulumgyrus, *CAU* caudate, *PAL* Pallidum, *PUT* Putamen, *MTG* Middle temporal gyrus

§ ANCOVA $P<0.05$, Bonferroni-corrected

*: Correlation $P<0.05$

**: Correlation $P<0.01$, FDR corrected

Table 5 Decreased negative connectivity unrelated with frontal lobes in ESRD patients compared with controls

COIs	Z-scores of COIs in controls	Z-scores of COIs in ESRD patients	ANCOVA *P* values[§]	CCs between neuropsychological test scores and Z-scores of COIs						CCs between hematocrit and Z-scores of COIs in ESRD patients
				NCT-A	DST	LTT	SDT	SDS	SAS	
PCG.L-SPG.R	−0.427±0.244	−0.191±0.238	**0.007**	0.106	−0.080	0.080	0.192	0.096	0.170	−0.154
SPG.L-MTG.L	−0.300±0.289	−0.004±0.240	**<0.001**	0.111	**−0.228***	0.119	0.176	0.084	0.166	0.224
SPG.R-MTG.L	−0.427±0.285	−0.158±0.223	**0.001**	0.159	**−0.205***	0.064	0.168	0.069	0.217	0.105
IPL.L-MTG.L	−0.165±0.210	0.058±0.253	**0.033**	**0.316****	**−0.296****	0.112	0.191	0.070	0.161	−0.026

COI Connectivity of interests, *ESRD* End-stage renal disease, *CC* Correlation coefficient, *NCT-A* Number connection test type A, *DST* Digit symbol test, *LTT* Line-tracing test, *SDT* Serial-dotting test, *SDS* Self-ratingdepression scale, *SAS* Self-rating anxiety scale, *R* Right, *L* Left, *PCG* Posterior cingulate gyrus, *SPG* Superior parietal gyrus, *MTG* Middle temporal gyrus, *IPL* Inferior parietal gyrus

§ ANCOVA $P<0.05$, Bonferroni-corrected

*: Correlation $P<0.05$

**: Correlation $P<0.01$, FDR corrected

Three positive connections were higher in ESRD patients compared to healthy controls. These connections were between the right opercular part of the Inferior frontal gyrus and the right anterior cingulate gyrus, the left superior parietal gyrus and left angular gyrus, and the left superior parietal gyrus and left precuneus (Fig. 4 and Table 6).

The Z-scores of 29 COIs were associated with alterations in DST scores (Tables 2,3,4,5, and 6); 19 COIs related with frontal lobes were correlated with SAS scores (Tables 2 and 3); 8 COIs were correlated with SDT scores (Tables 2 and 4); 4 COIs related with LTT scores (Tables 2 and 4); one connection was correlated with NCT-A (Table 5). No correlations was found between COIs and SDS scores. In ESRD patients, 4 negative connections related with frontal lobes were negatively correlated with their hematocrit levels (Table 3). There was no correlation between serum creatinine level, blood urea, blood Ca^{2+} level, dialysis duration and all COIs found between ESRD patients and controls (all $P>0.05$, FDR corrected).

Discussion

This study showed that functional connectivity was predominantly decreased in ESRD patients undergoing hemodialysis, especially in the frontal lobe, which was associated with neurocognitive dysfunction. Additionally, we found that anxiety and decreased hematocrit levels appeared to be factors affecting neurocognitive function and functional connectivity of ESRD patients.

Predominantly decreased functional connectivity in the frontal lobe in ESRD patients is an important finding in this study. We found 42 abnormal functional connectivities in ESRD patients compared with healthy controls, more than half of which (57.1 %) were related to the prefrontal lobe. The prefrontal lobe is utilized in planning complex cognitive behavior, personality expression, decision-making, and moderating social behavior. Thus, functional disconnection of the prefrontal lobe in ESRD patients could play an important role in mediating their neurocognitive dysfunction. Our findings

Table 6 Increased connectivity in ESRD patients compared with controls

COIs	Z-scores of COIs in controls	Z-scores of COIs in ESRD patients	ANCOVA *P* values[§]	CCs between neuropsychological test scores and Z-scores of COIs						CCs between hematocrit and Z-scores of COIs in ESRD patients
				NCT-A	DST	LTT	SDT	SDS	SAS	
IFGoperc.R-ACG.R	−0.112±0.212	0.133±0.236	**<0.001**	0.203	**−0.233***	0.001	0.176	0.004	0.163	−0.266
SPG.L-ANG.L	−0.117±0.249	0.130±0.255	**0.006**	0.075	**−0.181***	0.048	0.134	0.042	0.148	0.160
SPG.L-PCUN.L	0.238±0.178	0.445±0.243	**0.012**	0.158	**−0.253***	0.161	0.118	0.131	0.176	0.023

COI Connectivity of interests, *ESRD* End-stage renal disease, *CC* Correlation coefficient, *NCT-A* Number connection test type A, *DST* Digit symbol test, *LTT* Line-tracing test, *SDT* Serial-dotting test, *SDS* Self-ratingdepression scale, *SAS* Self-rating anxiety scale, *R* Right, *L* Left, *IFGoperc* Opercular part of inferior frontal gyrus, *ACG* Anterior cingulate gyrus, *SPG* Superior parietal gyrus; *ANG* Augulargyrus

§ ANCOVA $P<0.05$, Bonferroni-corrected

*: Correlation $P<0.05$

**: Correlation $P<0.01$, FDR corrected

were also supported by the behavioral and neuroimaging studies (Kim et al. 2011). Previous behavioral studies have demonstrated that ESRD patients had neuropsychological deficits such as attention, speed, learning and memory, and decision-making, typical of medial prefrontal lobe dysfunctions (Burn and Bates 1998; Madan et al. 2007; Elias et al. 2009; Giang et al. 2011; Raphael et al. 2012). Neuroimaging studies also indicated the medial prefrontal lobe was often involved in the ESRD patients. ERSD patients had an abnormally distributed perfusion pattern, which is characterized by significantly decreased cerebral blood flow in the frontal cortex and thalamus and increased cerebral blood flow in the occipital lobe, which correlated with cognitive test results such as the Mini Mental State Examination and Mattis Dementia Rating Scale (Fazekas et al. 1996). Kamata et al. indicated frontal lobe atrophy in patients on hemodialysis for 10 years or more was significantly greater than in patients dialyzed for less than 10 years (Kamata et al. 2000). Our findings on the predominantly decreased functional connectivity of medial prefrontal lobe appeared specific for ESRD patients, which is different from other metabolic brain disease, such as hepatic encephalopathy. In one previous study of minimal hepatic encephalopathy, functional connectivity abnormalities of the basal ganglia-thalamocortical circuit were found, although widespread cortical and subcortical network connectivity changes were observed (Zhang et al. 2012). We did find the disrupted functional connection of the basal ganglia in ESRD patients; however, these abnormal functional connections are within basal ganglia rather than between basal ganglia and other cortical regions. In short, decreased prefrontal lobe connectivity could be the typical and specific metabolic brain disorders in patients with ESRD.

We found that altered functional connectivity in ESRD patients was associated with neuropsychological tests results, especially the DST. The DST is widely used to evaluate attention and psychomotor speed (Bajaj et al. 2009). By contrast, the NCT-A is a test of visual-spatial orientation and psychomotor speed. Our finding indicates that the DST appears to be more sensitive towards predicting the cognitive dysfunction in ESRD patients. The DST was correlated with 29 functional connectivities of ESRD patients, indicating functional connectivity abnormality could reflect neurocognitive deficits in ESRD patients. However, it is rather difficult to uncover the causes of neurocognitive dysfunction in this study because we also found that anxiety was associated with functional connectivity in these ESRD patients. This indicates that mood can have a negative role in cognitive dysfunction (Kimmel et al. 2007; Cukor et al. 2006; Theofilou 2011).

We found hematocrit rather than serum creatinine level, blood urea, and dialysis duration were associated with altered functional connectivity in ESRD patients. Decreased hematocrit can lead to decreased brain oxygen delivery, with a detrimental effect on brain metabolism (Pereira et al. 2005). Anemia in patients with ESRD has been associated with cognitive dysfunction, and neuropsychological and neurophysiological tests have shown improvement with the treatment of anemia in ESRD patients (Radić et al. 2010). Kamata et al. reported that there was a significant negative correlation between brain atrophy index and hematocrit (Kamata et al. 2000). Our patients had significantly decreased hematocrit relative to reference values. It is possible that decreased hematocrit caused low oxygen delivery and altered functional connectivity, contributing to cognitive dysfunction.

We acknowledge several limitations in our study. First, we did not investigate specifically whether the patients with and without cognitive dysfunction had different whole brain functional connectivity patterns because no CKD patients without ESRD was included in current study. However, we believe a difference is expected. Second, among the battery of neuropsychological tests we performed, none were global for the evaluation of cognitive function of ESRD patients. Detailed and comprehensive evaluation of cognitive function is needed. Third, although altered functional connectivity in ESRD patients was observed in this study, we believe explorations of the causes of altered functional connectivity are needed in future studies because of many coexist factors, such as anxiety and lower hematocrit level. Last, no blood tests were performed for our controls; thus, no available data were used for them.

In conclusion, our study indicated that functional connectivities were predominantly decreased in ESRD patients undergoing hemodialysis, especially for the frontal lobe, which was associated with neurocognitive dysfunction, anxiety, and decreased hematocrit. It is possible to improve ESRD patients' cognitive function through mitigating anxiety and treating anemia.

Conflict of interest The authors disclose no conflicts.

Funding Supported by grants from National Natural Science Foundation of China (grant No. 81101039 to G.Z., grants No. 81322020, 81230032, and 81171313 to L.J.Z.), the Program for New Century Excellent Talents in the University (NCET-12-0260 to L.J.Z.), and China Postdoctoral Science Foundation of Jiangsu Province (grant No. 1002083C to G.Z.).

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