Altered intrinsic brain activity in patients with CSF1R-related leukoencephalopathy

Jingying Wu1,2 · Yikang Cao3 · Mengting Li5 · Binyin Li2 · Xize Jia4 · Li Cao1,2

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Abstract
CSF1R-related leukoencephalopathy is an adult-onset white matter disease with high disability and mortality, while little is known about its pathogenesis. This study introduced amplitude of low-frequency fluctuations (ALFF) and regional homogeneity (ReHo) based on resting-state functional magnetic resonance imaging (rsfMRI) to compare the spontaneous brain activities of patients and healthy controls, aiming to enhance our understanding of the disease. RsfMRI was performed on 16 patients and 23 healthy controls, and preprocessed for calculation of ALFF and ReHo. Permutation tests with threshold free cluster enhancement (TFCE) was applied for comparison (number of permutations = 5,000). The TFCE significance threshold was set at $P_{FWE} < 0.05$. In addition, 10 was set as the minimum cluster size. Compared to healthy controls, the patient group showed decreased ALFF in right paracentral lobule, and increased ALFF in bilateral insula, hippocampus, thalamus, supramarginal and precentral gyrus, right inferior, middle and superior frontal gyrus, right superior and middle occipital gyrus, as well as left parahippocampal gyrus, fusiform, middle occipital gyrus and angular gyrus. ReHo was decreased in right supplementary motor area, paracentral lobule and precentral gyrus, while increased in right superior occipital gyrus and supramarginal gyrus, left parahippocampal gyrus, hippocampus, fusiform, middle occipital gyrus and angular gyrus, as well as bilateral middle occipital gyrus and midbrain. These results revealed altered spontaneous brain activities in CSF1R-related leukoencephalopathy, especially in limbic system and motor cortex, which may shed light on underlying mechanisms.

Keywords CSF1R-related leukoencephalopathy · Amplitude of low-frequency fluctuation · Regional homogeneity · Limbic system · Motor cortex

Abbreviations
CSF1R Colony-stimulating factor 1 receptor
MRI Magnetic resonance imaging
DWI Diffusion-weighted imaging
FLAIR Fluid attenuated inversion recovery
AD Alzheimer’s disease
MS Multiple sclerosis
SPECT Single photon emission computered tomography
rsfMRI Resting-state functional magnetic resonance imaging
ALFF Amplitude of low-frequency fluctuations
ReHo Regional homogeneity
TFCE Threshold free cluster enhancement
BOLD Blood oxygenation level-dependent signals
PD Parkinson’s disease
CADASIL Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
SIVD Subcortical ischemic vascular dementia
Introduction

The inherited leukoencephalopathy is a heterogeneous group of rare white matter diseases (Kohler et al., 2018) which affects only 1 in 50,000 (Heim et al., 1997) people. Compared to those young-onset ones, it is even rarer for adult-onset phenotypes such as CSF1R-related leukoencephalopathy, making it difficult to collect large samples for clinical studies, largely limiting the exploration on their mechanisms, diagnosis and treatment.

Despite its rarity, studies on CSF1R-related leukoencephalopathy are urgently needed, since it mainly affects adults of around 40 years old (Tian et al., 2019) who are usually the main supporters for families, and is associated with high morbidity and mortality considering most patients will get bedridden, severe dementia or dying within 4–6 years after diagnosis (Konno et al., 2017; Sundal et al., 2015). Besides, although hematopoietic stem cell transplantation could control the progression of this fatal disease (Eichler et al., 2016; Gelfand et al., 2020; Mochel et al., 2019; Tipton et al., 2021), its high risk and high cost are big obstacles for many families, thus exploration on its pathogenesis might shed new light on better treatment. Additionally, as a representative microgliopathy, advanced understanding on this disease will be significant for other microglia-related disease such as multiple sclerosis (MS), Alzheimer’s disease (AD) and Parkinson’s disease (PD) (Li & Barres, 2018).

Recent studies using single photon emission computered tomography (SPECT) (Daida et al., 2017; Kitani-Morii et al., 2014; Terasawa et al., 2013) and [18F]-fluorodeoxyglucose positron emission tomography (PET) (Kim et al., 2015; Nicholson et al., 2013) has already demonstrated diffuse cortical hypometabolism predominantly in fronto-parietal areas in several cases, indicating the relationship between brain function and CSF1R-related leukoencephalopathy. Considering the radioactivity and expensiveness of SPECT and PET, our previous study which conducted on the basis of an alternative technique, resting-state functional magnetic resonance imaging (rsfMRI), has also revealed some functional abnormalities among patients (Zhan et al., 2020), implying the potential of brain function changes as an entry point to explore the pathogenesis of CSF1R-related leukoencephalopathy.

In recent years, rsfMRI, as a non-invasive functional imaging technique, has shown its enormous potential in studying neural mechanisms of neurological dysfunctions (Biswal, 2012; Biswal et al., 1995), with high acceptance to patients and good reproducitity (Zou et al., 2015). The amplitude of low-frequency fluctuations (ALFF) and regional homogeneity (ReHo) are two commonly calculated local indices based on rsfMRI (Disner et al., 2018; Pan et al., 2017). ALFF is calculated as the mean square root of power spectrum in a low-frequency range (0.01–0.08 Hz) at each voxel based on blood oxygenation level-dependent (BOLD) signals (Zang et al., 2007). As a promising method for detecting the regional intensity of spontaneous activity, ALFF has been applied in the exploration of brain disorders including AD (Liu et al., 2014a, b; Yang et al., 2018), PD (Hou et al., 2014; Wang et al., 2020; Yue et al., 2020), MS (Liu et al., 2011), subcortical ischemic vascular dementia (SIVD) (Liu et al., 2014a, b) and moyamoya disease (Lei et al., 2014). On the other hand, ReHo is a measurement for the similarity of the time series of a given cluster. It could reflect local synchronization of spontaneous brain activity and indicate the changes of temporal neuronal activity in specific regional areas (Zang et al., 2004). The ReHo method has also been widely used for the investigation of neural activity changes in neurologic diseases, such as AD (Tu et al., 2020; Zhang et al., 2012), PD (Liu et al., 2019; Yue et al., 2020), MS (Wu et al., 2016; Zhu et al., 2020) and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (Orsolini et al., 2020; Su et al., 2019). The combination of ALFF and ReHo might offer us a comprehensive view of regional spontaneous activities in patients with CSF1R-related leukoencephalopathy, and provide potential regions of interest for further functional analysis. However, so far, no study has been conducted to explore the characteristics of local brain activity in this disease.

We hypothesize that patients with CSF1R-related leukoencephalopathy would exhibit abnormal temporal variability of brain activity compared to healthy controls. Two rsfMRI metrics, ALFF and ReHo, were employed to evaluate the variability of intrinsic brain activities, which might be useful for expanding our understanding of the disease.

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**Abbreviations**

- MMSE: Mini-Mental State Examination
- MoCA: Montreal Cognitive Assessment
- TR: Repetition time
- TE: Echo time
- FOV: Field of view
- GM: Gray matter
- WM: White matter
- CSF: Cerebrospinal fluid
- MNI: Montreal Neurological Institute
- FWHM: Full-width-half-maximum
- FFT: Fast Fourier transform
- KCC: Kendall’s coefficient of concordance
- DPABI: Data Processing and Analysis for Brain Imaging
- SMA: Supplementary motor area
- DKI: Diffusion kurtosis imaging
- ALFF: Amplitude of low-frequency fluctuations
- ReHo: Regional homogeneity
- CSF1R: C-C Chemokine Ligand 1 Receptor
- CSF: Cerebrospinal fluid
- FDG: Fluorodeoxyglucose
Patients and methods

Participants

The study was endorsed by the ethics committee of Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China, and was registered at http://www.chictr.org.cn (No. ChiCTR1800015295). All participants or their guardians provided written informed consent. 23 participants with CSF1R-related leukoencephalopathy and 24 healthy controls were preliminary enrolled in the study. Diagnosis of CSF1R-related leukoencephalopathy were made by at least two experienced neurologists, namely Dr. Li Cao, Dr. Binyin Li and Dr. Jingying Wu, according to Konno et al. criteria (2018). Mini-Mental State Examination (MMSE)-Chinese version and Montreal Cognitive Assessment (MoCA)-Beijing version was used for neuropsychological evaluations, and MRI severity was roughly evaluated according to the score system created by Sundal et al. (2012).

Imaging data acquisition

Imaging data were acquired using a 3.0 Tesla Philips Ingenia MRI scanner with a dStream HeadSpine coil. rsfMRI imaging was carried out using gradient echo planar imaging with the following parameters: 39 continuous axial slices, repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, resolution = 3.5 mm × 3.5 mm × 3.5 mm, thickness = 3.5 with no interslice gap mm, flip angle = 90 degrees, the scan time = approximately 8 min. High-resolution T1-weighted images were obtained using three-dimensional brain volume imaging sequences with an acquired voxel size of 1 × 1 × 1 mm³ as the following parameters: 192 continuous sagittal slices, TR = 7 ms, TE = 3 ms, field of view (FOV) = 256 × 256 mm, flip angle = 7 degrees, slice thickness = 1.0 mm with no interslice gap, matrix size = 256 × 256, and bandwidth = 241 Hz. During the scan, the participants were placed in a supine position with their head fixed and earplugs worn to reduce noise, and were instructed to lie quietly with their eyes closed but to stay awake without performing any other tasks.

fMRI preprocessing

The rsfMRI data were preprocessed using Resting-State fMRI Data Analysis Toolkit plus V1.24 (RESTplus V1.24) (Jia et al., 2019) (http://www.restfmri.net/forum/REST) running in MATLAB2018a(MathWorks, Natick, MA, USA). The preprocessing steps consisted of removal of the first 10 time points for magnetization stabilization, slice time correction, realignment, and spatial normalization. An individual T1-weighted image was co-registered to the mean functional image and then the T1-weighted image was segmented into gray matter (GM), white matter (WM) signal, and cerebrospinal fluid (CSF) signal. The EPI images were spatially normalized to the Montreal Neurological Institute (MNI) space with a resolution of 3 mm × 3 mm × 3 mm. Smoothing was completed using a 6-mm full-width-half-maximum (FWHM) Gaussian kernel to decrease spatial noise. After removing the linear trend of the time course, nuisance regression removed covariates including Friston-24 head motion (Friston et al., 1996; Yan et al., 2013) and global mean signal. Scans of 7 patients were excluded because their head motion exceeded 3 mm or rotation exceeded 3 degrees and 1 control was excluded due to incomplete cortical coverage. Finally, band-pass filter (0.01–0.08 Hz) was applied only in ReHo.

ALFF and ReHo calculation

We use RESTplus V1.24 software to calculate ALFF and ReHo. The calculation of ALFF was based on fast Fourier transform (FFT). Using FFT, each time course was converted to frequency domain and the power spectrum was then obtained. The square root was calculated at each frequency of the power spectrum and then averaged across the filtered band (0.01–0.08 Hz) at each voxel as the ALFF value. For standardization purpose, the ALFF of each voxel was divided by the global mean ALFF value (Zang et al., 2007). For ReHo, it was assigned to a given voxel by calculating the Kendall’s coefficient of concordance (KCC) of the time series of this voxel with those of its nearest 27 neighboring voxels (Zang et al., 2004). To reduce the influence of individual variations in the KCC value, ReHo map normalizations were performed by dividing the KCC among each voxel by the averaged KCC of the whole brain of all the participants in this group. Note that the spatial smoothing (FWHM = 6 mm) was performed after ReHo calculation.

Statistical analysis

Two-sample t-tests were performed to examine the age and education level differences between the two groups and categorical variable like sex was analyzed by Pearson Chi-square test. Data Processing and Analysis for Brain Imaging (DPABI V4.3) software (Yan et al., 2016) (http://rfmri.org/dpabi) was used for the statistical analyses of the ALFF and ReHo results. A permutation test with a threshold free cluster enhancement (PT TFCE, number of permutations = 5,000) method was used in the two-sample t-tests to compare differences in ALFF and ReHo between
patients and controls. Our TFCE significance threshold was set at $P_{FWE} < 0.05$. In addition, we set 10 as the minimum cluster size. Clusters located in cerebellum were excluded due to incomplete cerebellar coverage of some subjects.

Results

Demographic, clinical and genetic findings

23 patients with $CSF1R$-related leukoencephalopathy and 24 healthy controls were preliminary enrolled, while 6 patients were excluded due to excessive head motion and 1 control was excluded for incomplete cortical coverage. Demographic characteristics of 16 patients (8 males and 8 females; mean age, $42.00 \pm 8.35$ years) and 23 healthy controls (7 males and 16 females; mean age, $41.61 \pm 16.25$ years) are demonstrated in Table 1. There was no significant difference showed ($p > 0.05$) in gender, age and education level. Detailed clinical, radiological and genetic features of the patient group are listed in Table 2. The average age of onset was $40.06 \pm 8.25$ years (range 29–60 years) with the average duration of $1.94 \pm 1.00$ years (range 1–5 years). All those patients suffered from progressive cognition decline, and the average scores of MMSE and MoCA were $18.13 \pm 7.86$ and $13.13 \pm 7.40$ respectively. A severity scoring system based on Sundal et al. study (2012) was used to roughly evaluate the MRI changes of each patient, with an average score of $24.38 \pm 7.20$. 8 novel (c.2498C $>$ G/p. T833R, c.834C $>$ A/p.C278*, c.2567A $>$ C/p.Y856S, c.2473G $>$ C/p.E825Q, c.2426A $>$ C/p.Y809S, c.2442+1G $>$ A, c.2680_2691del/p.894_897del, c.2537G $>$ T/p.W846L) and 4 reported (c.2026C $>$ T/p.R676*, c.2381 T $>$ C/p.I794T, c.2342C $>$ T/p.A781V, c.2534 T $>$ C/p.L845P) mutations of $CSF1R$ gene were identified using whole exome sequencing and Sanger sequencing. All the mutations were interpreted to be pathogenic or likely pathogenic following the American College of Medical Genetics and Genomics Standards and Guidelines (ACMG).

Alterations of IBA changes between patients and healthy controls

Group differences in ALFF

As shown in Fig. 1, ALFF decreased in the patient group in right paracentral lobule, and increased in bilateral insula, hippocampus, thalamus, supramarginal and precentral gyrus, right inferior, middle and superior frontal gyrus, right superior and middle occipital gyrus, as well as left parahippocampal gyrus, fusiform, middle occipital gyrus and angular gyrus compared to healthy controls. Those regions in frontal and occipital lobe, as well as angular gyrus, were overlapped with abnormalities revealed in T2 and DWI sequence. The significant differences in ALFF between the two groups are shown in Table 3 and Fig. 1A. The average signal of each cluster in patients with different mutations were demonstrated in Fig. 1B.

Group Differences in ReHo

Compared to the healthy controls, ReHo value of the patient group was found decreased in right supplementary motor area (SMA), paracentral lobule and precentral gyrus, and increased in right superior occipital gyrus and supramarginal gyrus, left parahippocampal gyrus, hippocampus, fusiform, middle occipital gyrus and angular gyrus, as well as bilateral middle occipital gyrus and midbrain. Those regions in frontal and occipital lobe, as well as angular gyrus, were overlapped with hyperintensity in T2 and DWI sequence. Detailed information about the two groups is listed in Table 3 and Fig. 2A. The average signal of each cluster in patients with different mutations were demonstrated in Fig. 2B.

Discussion

$CSF1R$-related leukoencephalopathy is a rare hereditary white matter disease with high disability and mortality (Konno et al., 2017; Sundal et al., 2015). However, little is known about the mechanisms underlying this disease. Previous studies have illustrated the potential changes in brain function of patients, which might contribute to deepen our understanding (Zhan et al., 2020). Therefore, in this study, we introduced ALFF and ReHo based on rsfMRI to compare the spontaneous brain activities of patients with $CSF1R$-related leukoencephalopathy to those of healthy controls. We demonstrated abnormality in extensive regions involving limbic system, sensorimotor area and visual language cortex. These findings may

### Table 1 The demographics of patients with $CSF1R$-related leukoencephalopathy and healthy controls

| Characteristics         | Patients | Controls | P value  |
|-------------------------|----------|----------|----------|
| Gender(Male/Female)     | 8/8      | 7/16     | 0.2200*  |
| Age(years)              | $42.00 \pm 8.35$ | $41.61 \pm 16.25$ | 0.6112*  |
| Education(years)        | $13.13 \pm 2.75$ | $15.35 \pm 4.30$ | 0.0682*  |

*aChi-square test  
*bTwo-sample t-test
Table 2  Clinical, radiological and genetic characteristics of patients with CSF1R-related leukoencephalopathy

| Patient ID | Gender^a | Onset/Duration (year) | Clinical Featuresb | MRI Scale Scorec | Distribution of MRI abnormalitiesd | MMSE/ MoCA^e | CSF1R Mutationf | ACMGg |
|------------|----------|----------------------|-------------------|-----------------|-----------------------------------|-------------|-----------------|--------|
|            |          |                      |                   | T W A T1 T2 DWI |                     |             |                 |        |
| T3893      | F        | 35/2                 | Cl, PD, BS, Ps    | 22 18 4         | FL, PL, TL, CC, CST       | FL, TL, CC  | 19/15           | c.2381 T>C/p.I794T | P      |
| T4033      | F        | 30/1                 | Cl, PD, BS, Ps    | 24 17 6         | FL, TL, CC, CST           | FL, TL      | 14/11           | c.2026C>T/p. R676X* | P      |
| T4543      | M        | 40/2                 | Cl, Ps            | 10 7 3          | FL                  | FL, CST     | 28/25           | c.2381 T>C/p.I794T | P      |
| T4557      | F        | 30/1                 | Cl, PD, BS, Ps    | 27 20 6         | FL, PL, TL, CC, CST      | FL, PL, TL, CC | 12/10           | c.2026C>T/p. R676X* | P      |
| T4829      | M        | 38/3                 | Cl, Ps            | 27 20 6         | FL, PL, TL, CC          | FL, PL, TL, OL, CC | 23/15           | c.2342C>T/p.A781V | LP     |
| T4873      | F        | 29/2                 | Cl, PD, Ps        | 34 24 9         | FL, PL, TL, OL, CC, CST  | FL, TL, CC  | 6/2             | c.2534T>C/p. L845P | LP     |
| T5555      | M        | 41/2                 | Cl, PD            | 35 27 7         | FL, TL, CC, CST          | FL, TL, CC  | 27/24           | c.2498C>G/p.T833R | LP     |
| T5678      | F        | 47/2                 | Cl, PD, Ps        | 35 27 7         | FL, PL, TL, CC           | FL, PL, TL, CC | 5/1             | c.834C>A/p.C278X* | LP     |
| T5830      | F        | 37/2                 | Cl, PD            | 27 20 6         | FL, PL, TL, CC, CST      | FL, TL      | 22/21           | c.2567A>C/p.Y856S | LP     |
| T5959      | F        | 46/2                 | Cl, PD, BS, Ps    | 25 17 7         | FL, PL, CC, CST          | FL, PL, TL, OL, CC | 27/17           | c.2473G>C/p.E825Q | LP     |
| T6116      | F        | 45/1                 | Cl                | 24 17 7         | FL, PL, TL, CC           | FL, TL, CC  | 26/18           | c.2442+1G>A      | LP     |
| T6164      | M        | 37/1                 | Cl, PD            | 14 10 4         | FL, TL, CC, CST          | FL, TL      | 20/15           | c.2426A>C/p.Y899S | LP     |
| T6167      | M        | 60/2                 | Cl, Ps            | 15 11 4         | FL, PL, TL, CC           | FL, PL, TL, CC | 15/12           | c.2381T>C/p.I794T | P      |
| T6515      | M        | 38/1                 | Cl, PD, BS, Ps    | 28 20 8         | FL, PL, TL, OL, CC, CST  | FL, PL, TL, TL, CC | 5/1             | c.2680_2691del/p.894_897del | LP     |
| T6555      | M        | 51/2                 | Cl, PD            | 21 16 5         | FL, PL, TL, CC, CST      | FL, TL, CC  | 21/14           | c.2381 T>C/p.I794T | P      |
| T6988      | M        | 41/2                 | Cl, PD            | 21 16 5         | FL, PL, TL, CC, CST      | FL, TL, CC  | 20/9            | c.2537G>T/p.W846L | LP     |

^aF female, M Male

^bCI cognitive impairment, PD parkinson’s like symptoms, BS bulbar symptoms like swallow difficulty and/or choking, Ps psychiatric symptoms

^cT Total score, W score of white matter, A score of atrophy

^dFL frontal lobe, PL parietal lobe, TL temporal lobe, OL occipital lobe, CC corpus callosum, CST corticospinal tract

^eMMSE Mini Mental State Examination, MoCA Montreal Cognitive Assessment

^fTranscript: NM_005211

^gACMG American College of Medical Genetics and Genomics, LP likely pathogenic, P pathogenic *nonsense mutation, which means one of the twenty amino acids specified by the genetic code is changed to a chain-terminating codon
provide insights for the dysfunctional brain patterns of CSF1R-related leukoencephalopathy.

The most distinctive pattern in CSF1R-related leukoencephalopathy is the increased ALFF and ReHo in limbic system, especially bilateral parahippocampus gyrus and hippocampus, indicating the increased spontaneous neural activities in these areas. Limbic system is a group of interconnected cortical and subcortical structures that functionally dedicated to the activity of three distinct networks (Catani et al., 2013). The first network includes hippocampal-diencephalic circuit and the parahippocampal-parietal circuit, which contributes to memory and spatial orientation (Aggleton, 2008; Vann et al., 2009) respectively. Altered function of parahippocampus gyrus and hippocampus found in patients with CSF1R-related leukoencephalopathy is consistent with the fact that cognitive decline, especially memory loss, is the most common symptom of this disease (Konno et al., 2017). Secondly, limbic system also formed the dorsomedial part of default-mode network (DMN) (Raichle et al., 2001), and increased ALFF was also found in parts of this network such as bilateral insula. The dorsomedial DMN is closely associated with self-referential judgment and recollection of prior experience (Raichle, 2015), thus the alteration of this subnetwork is most likely correlated to neuropsychiatric symptoms such as depression and anxiety (Whitfield-Gabrieli & Ford, 2012; Zhou et al., 2020), which is also the common initial features of CSF1R-related leukoencephalopathy. As for the third network, the temporo-amygdala-orbitofrontal network is responsible for the integration of visceral and emotional states with cognition and behavior (Catani et al., 2013), and regions like fusiform gyrus and orbitofrontal cortex were also identified in our results.

The intrinsic brain activities of motor system are extensively affected, including motor cortex such as precentral gyrus, paracentral lobule and SMA (Ebbesen & Brecht, 2017), as well as another motor center midbrain. The abnormality of spontaneous neural activity will lead to imbalance of motor function (Ebbesen & Brecht, 2017), hence, patients with CSF1R-related leukoencephalopathy behave various motor symptoms such as asymmetric parkinsonism, spasticity and dystonia (Konno et al., 2017). One of the remarkable decreased areas in ReHo was in SMA. SMA is active before internally generated movement, or even when a motor plan is activated by “object affordance”, that is the facilitation or speeding up of behavioral responses to an object (Nachev et al., 2008). Deficits in the SMA is relative to difficulty in gait initiation or execution (Della Sala et al., 2002; Richard et al., 2017), which resemble the features of gait disturbance in patients with CSF1R-related leukoencephalopathy (Konno et al., 2017).

In addition, CSF1R-related leukoencephalopathy is related to parietooccipital cortex which is responsible for visuospatial learning, memory and language (Tumati et al., 2019), including middle occipital gyrus, supramarginal gyrus, angular and superior occipital gyrus. Thalamus, as the central brain structure crucially involved in cognitive, emotional, sensorimotor functions (Boelens Keun et al.,

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**Fig. 1** Changes of ALFF in patients with CSF1R-related leukoencephalopathy. (A) The patients group showed increased ALFF in left dorsolateral superior frontal gyrus, left postcentral gyrus, left precentral gyrus, right precuneus, as well as bilateral insula, parahippocam-
Table 3  Brain regions with significantly differences in ALFF and ReHo between patients with CSF1R-related leukoencephalopathy and healthy controls

| Indices | Cluster | Peak (MNI) | Size | Peak intensity |
|---------|---------|------------|------|----------------|
|         |         | x  y   z   |      |                |
| ALFF    | Cluster1| -24 -21 -6 | 2936 | 7.0038         |
|         | SupraMarginal_L | 91 |
|         | Insula_L | 84 |
|         | Precentral_R | 70 |
|         | Hippocampus_R | 61 |
|         | Insula_R | 54 |
|         | Hippocampus_L | 51 |
|         | ParaHippocampal_L | 49 |
|         | SupraMarginal_R | 34 |
|         | Precentral_L | 33 |
|         | Fusiform_L | 31 |
|         | Thalamus_L | 23 |
|         | Thalamus_R | 23 |
|         | Cluster2 | 30 42 -9 | 41  | 4.7864 |
|         | Frontal_Inf_Orb_R | 23 |
|         | Frontal_Mid_Orb_R | 12 |
|         | Cluster3 | 15 60 6 | 130 | 4.4829 |
|         | Frontal_Mid_Orb_R | 42 |
|         | Frontal_Sup_R | 32 |
|         | Frontal_Sup_Medial_R | 31 |
|         | Frontal_Sup_Orb_R | 24 |
|         | Cluster4 | -33 -72 12 | 62  | 6.0288 |
|         | Occipital_Mid_L | 39 |
|         | Cluster5 | 30 63 12 | 14  | 4.5200 |
|         | Frontal_Sup_R | 11 |
|         | Cluster6 | 27 -66 27 | 17  | 3.3870 |
|         | Occipital_Sup_R | 9 |
|         | Occipital_Mid_R | 6 |
|         | Cluster7 | -39 -66 33 | 30  | 4.9467 |
|         | Angular_L | 19 |
|         | Cluster8 | 9 -21 78 | 15  | -5.1466 |
|         | Paracentral_Lobule_R | 9 |
| ReHo    | Cluster1 | -18 -6 -30 | 42  | 4.4896 |
|         | Parahippocampal_L | 34 |
|         | Hippocampus_L | 6 |
|         | Cluster2 | -18 -27 -24 | 50  | 4.4476 |
|         | Fusiform_L | 17 |
|         | ParaHippocampal_L | 14 |
|         | Cluster3 | 15 -18 -12 | 19  | 4.3443 |
|         | Midbrain | 15 |
|         | Cluster4 | -33 -75 15 | 136 | 6.3760 |
|         | Occipital_Mid_L | 88 |
|         | Angular_L | 36 |
|         | Cluster5 | 39 -66 27 | 223 | 5.4917 |
|         | Occipital_Mid_R | 57 |
|         | Occipital_Sup_R | 18 |
|         | Cluster6 | 48 -30 30 | 18  | 6.3979 |
|         | SupraMarginal_R | 8 |
|         | Cluster7 | 9 -9 78 | 25  | -5.2986 |
was also identified and might contribute to extensive involvement of brain functions in this disease. Notably, some of abnormal brain regions were overlapped with abnormalities in T2 and DWI sequences, especially those regions in frontal, temporal and occipital cortex, which further confirmed the importance of these regions. However, limbic system, especially hippocampus, parahippocampal and insular gyrus, was exclusive to structural results, suggesting the potential of these regions for further functional analysis. 

Besides, our results were mostly overlapped with abnormal regions revealed in the only other article using rsfMRI in CSF1R-related leukoencephalopathy (Zhan et al., 2020). In this article, the authors revealed impaired functional connectivity (FC) between the bilateral hippocampus and their contralateral caudate nucleus, and enhanced FC between the precuneus, paracentral lobules, and inferior frontal gyrus in patients. These regions were also identified in our results except caudate nucleus. MS patients who share mostly clinical and radiological features with patients with CSF1R-related leukoencephalopathy, also exhibit similar pattern of brain function (Liu et al., 2011, 2012, 2015, 2016; Wu et al., 2016; Yin et al., 2018; Zheng et al., 2021; Zhou et al., 2014; Zhu et al., 2020), especially in insula and parietooccipital cortex. However, nor ALFF or ReHo was abnormal in hippocampus, parahippocampus and motor cortex in MS. These

| Indices | Cluster | Peak (MNI) | Size | Peak intensity |
|---------|---------|------------|------|---------------|
| Supp_Motor_Area_R | 9 |
| Paracentral_Lobule_R | 5 |
| Precentral_R | 4 |

\(^a\) ALFF amplitude of low-frequency fluctuations, \(^b\) ReHo regional homogeneity

Fig. 2 Changes of ReHo in patients with CSF1R-related leukoencephalopathy. (A) The ReHo value of the patient group increased in right superior occipital gyrus, right precentral gyrus, left angular gyrus, as well as bilateral parahippocampal gyrus, hippocampus, middle occipital gyrus, supramarginal gyrus and extra-nuclear, while decreased in bilateral supplementary motor area and paracentral lobule (\(P_{TWE} < 0.05\)). The t-values are color-coded. (B) The average signals of each cluster in patients with different mutations.

\(^c\) MNI Montreal Neurological Institute
differences might be helpful for differentiation between two diseases.

As for other sequences, there was only one study using diffusion kurtosis imaging (DKI) to study this disease (Zhan et al., 2020), and reduced diffusivity was detected in midline cortex structures. Additionally, two studies focusing on T1, T2 and PD maps (Granberg et al., 2016; Mangeat et al., 2020) calculated the average signals of grey matter (GW) and white matter (WM) of the whole brain, and showed lower fraction of GW and WM compared to healthy controls. The morphological atrophy was then located in thalamus and hippocampus (Zhan et al., 2020). All the mentioned regions were identified in our results, and might be valuable for investigation on mechanisms.

Moreover, patients with different CSF1R mutations exhibited different patterns of local brain activity. Analysis between brain regions and genes might be helpful since its potential for expanding understanding of pathogenesis has been confirmed in AD and PD (Bi et al., 2020a, b, 2021).

This study had several limitations. Limited by the rarity of the disease, the sample size of the study is relatively small, thus further studies with a larger sample are needed for verification. The rarity also resulted in large heterogeneity of our patients. Although our results would still be helpful to discover brain regions involved in underlying mechanisms, prospective cohort studies on asymptomatic carriers would be of greater help for determining critical regions participating in pathogenesis.

Conclusion

In this study, we have investigated the spontaneous brain activity of CSF1R-related leukoencephalopathy compared to healthy controls through ALFF and ReHo based on rsfMRI. We have found some unique alternations in the disease in limbic system, sensorimotor system and language areas of the brain. These results suggest a special dysfunctional brain pattern, which may indicate the neural circuit mechanisms underlying CSF1R-related leukoencephalopathy.

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Authors’ contributions Author contributions included conception and study design (JW, YC, XJ and LC), data collection or acquisition (JW, BL), statistical analysis (YC, ML and LC), interpretation of results (JW, BL, YC, ML and LC), drafting the manuscript work or revising it critically for important intellectual content (JW, YC, BL, YJ, ML and LC) and approval of final version to be published and agreement to be accountable for the integrity and accuracy of all aspects of the work (All authors).

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Availability of data and material All data and material of the study are available from the corresponding author by request.

Code availability All data were processed with the legal edition of RESTplus V1.24. All code generated or used during the study are available from the corresponding author by request.

Declarations

Ethics approval The study was endorsed by the ethics committee of Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China, and was registered at http://www.chictr.org.cn (No.ChicTR1800015295).

Consent to participate All participants or their guardians provided written informed consent.

Consent for publication All participants or their guardians approved for publication with written consent.

Conflicts of interest None of the authors have a conflict of interest to declare.

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