Decreased mean kurtosis in the putamen is a diagnostic feature of minimal hepatic encephalopathy in patients with cirrhosis

Takuro Sato 1), Kei Endo 1) Keisuke Kakisaka 1), Yuji Suzuki 1), Yohei Kooka 1), Kei Sawara 1), Kenji Ito 2), Makoto Sasaki 2), Yasuhiro Takikawa 1)

1) Division of Hepatology, Department of Internal Medicine, Iwate Medical University, Morioka, Japan

2) Division of Ultrahigh Field MRI, Institute for Biomedical Sciences, Iwate Medical University, Morioka, Japan

Address for correspondence:

Keisuke Kakisaka, MD, PhD

School of Medicine, Iwate Medical University

19-1 Uchimaru, Morioka 0208505, Japan

Tel.: +81-19-651-5111

Fax: +81-19-652-6664

E-mail: keikaki@iwate-med.ac.jp

Short title: Decreased MK in the putamen in patients with MHE

Word count: 2892

Keywords: diffusion kurtosis imaging; DKI; minimal hepatic encephalopathy; putamen.

Abbreviation: area under the ROC (AUROC), serum albumin levels (Alb), caudate nucleus (CN), diffusion kurtosis imaging (DKI), diffusion tensor imaging (DTI), fractional anisotropy, (FA), globus pallidus (GP), hepatic encephalopathy (HE), mean diffusivity (MD), model of end stage liver disease (MELD), minimal encephalopathy (MHE), mean kurtosis (MK), ammonia (NH3), neuropsychological testing (NPT), prothrombin time international ratio (PT-INR), putamen (Put), region of interest (ROI), tract-based spatial statistics (TBSS), thalamus (TH) , and total bilirubin (T-Bil)

Author contributions.

Takuro Sato: drafting the manuscript, final approval of the article

Kei Endo: Acquisition of data, final approval of the article

Keisuke Kakisaka: Design of the research, analysis, drafting the manuscript, final approval of the article

Yuji Suzuki: Acquisition of data, final approval of the article

Yohei Kooka: Acquisition of data, final approval of the article

Kei Sawara: Acquisition of data, final approval of the article

Kenji Ito: Acquisition of data, final approval of the article

Makoto Sasaki: drafting the manuscript, final approval of the article

Yasuhiro Takikawa: drafting the manuscript, final approval of the article

Acknowledgement

Financial support: This study was supported by Grant-in-Aid for Strategic Medical Science Research (S1491001) from MEXT of Japan, and JSPS KAKENHI (grant number JP18K07980 for KK and JP15K19346 for KS). The authors report no personal conflicts of interest.

Abstract

Background & Aims: To prevent development of overt hepatic encephalopathy, early intervention for minimal hepatic encephalopathy (MHE) based on accurate diagnosis is essential. This study evaluated to investigate whether magnetic resonance diffusion kurtosis imaging (DKI) and diffusion tensor imaging (DTI) could detect brain microstructure abnormalities in MHE. The aim was to confirm whether brain microstructure abnormalities detected by magnetic resonance imaging were used as diagnosis of MHE.

Methods: Thirty-two subjects were prospectively examined with a 3-T MR scanner. Tract-based spatial statistics and region of interest analyses of diffusion imaging were performed to compare mean kurtosis (MK), fractional anisotropy (FA), and mean diffusivity (MD) values between patients with/without minimal hepatic encephalopathy. Diagnostic performance for detection of MHE was assessed with the receiver operating characteristic analysis.

Results: Ten subjects were diagnosed as MHE by neuropsychological testing. After exclusion of unsuitable subjects, we analyzed 9 subjects with MHE and 14 subjects without MHE. The patients with MHE had reduced MK in widespread white matter. We found significant decreases in MK in the caudate nucleus, putamen, globus pallidus,

and/or thalamus in the subjects with MHE. The MK in the putamen showed the best diagnostic performance to differentiate the subjects with MHE from those without MHE (cut-off value, 0.74; sensitivity, 0.89; specificity, 0.86).

Conclusions: DKI detects changes in the cerebral white matter and basal ganglia regions of the patients with MHE more sensitively than DTI. MK values in the putamen can be a useful marker for diagnosing MHE from cirrhotic patients without MHE.

Introduction

Hepatic encephalopathy (HE) is a neuropsychiatric and cognitive disorder that occurs due to decompensated cirrhosis (1). Symptomatic HE is termed as ‘overt’ HE, which decreases quality of life and increases mortality in patients with cirrhosis (2). Minimal hepatic encephalopathy (MHE) increases risk of progression to overt HE (3). Since symptoms of MHE comprise attention and visuo-motor coordination deficits, sensitive examination methods that assess psychometric performance are required to identify this condition (4, 5). Thus, MHE is defined as a condition characterized by subtle abnormalities, which are detected only by using specific neuropsychometric and/or neurophysiological tools in cirrhosis patients with otherwise normal neurological examination results (4).

For accurate diagnosis of MHE, the following parameters need to be evaluated: 1) quality of life; 2) mental state; 3) results of neuropsychological testing (NPT); and 4) disorders of speech and cognition. However, it is difficult to perform such a comprehensive diagnosis in routine clinical practice. As accurate diagnosis of MHE is essential for improving prognosis of patients with cirrhosis, objective and repeatable examination methods for diagnosis of MHE need to be established. Recently, metabolic disturbance in the brain of patients with MHE was identified using 3.0 Tesla magnetic

resonance imaging (MRI) (6). This article reported increase in glutamine and reduction in myo-inositol levels in the brain in patients with MHE. Since myo-inositol controls increase in osmotic pressure due to increased glutamine via ammonia metabolism in the brain, these changes were considered as the result of a buffering response for maintaining intracellular osmotic pressure. These findings and findings in previous reports suggest that astrocytes in MHE might become edematous. Thus, diffusion MRI may be used to detect increase in the water component of the brain in patients with MHE.

Diffusion tensor imaging (DTI) is an advanced MR technique that is widely used to quantify the diffusivity and/or anisotropy of water diffusion. DTI parameters include mean diffusivity (MD), which measures the average diffusivity of water, and fractional anisotropy (FA), which measures the degree of directionality of diffusion. Increased MD and/or decreased FA have been reported in patients with MHE (7-9). Recently, diffusion kurtosis imaging (DKI), which can detect minute histological changes of complex brain structures by quantifying the degree of non-Gaussian water diffusion, has been proposed (10). Previous studies suggested that DKI was more sensitive to microstructural changes in various neurological disorders when compared with DTI (11-13). Indeed, usefulness of mean kurtosis (MK), which is one of the parameters calculated from DKI data, has been reported in several diseases, such as Parkinson’s disease, multiple sclerosis, or

migraine (14-16). Although previous studies have established objective findings that can aid in diagnosis of MHE using MRI (7, 9, 17, 18), these studies were based on the comparison of patients with MHE and healthy controls. DKI revealed microstructural changes in patients with cirrhosis compared with healthy controls (19). This result suggested that cirrhosis itself affects DKI. Taken together, microstructural change detected by DKI in previous study may only show change due to cirrhosis. Therefore, whether DKI/DTI identifies abnormalities in the brain microstructure of MHE patients compared with those of patients with cirrhosis without MHE remains unclear. In the present study, we compared DKI/DTI findings of MHE among patients with cirrhosis.

Subjects and methods

Subjects

For this study, 32 subjects who underwent MRI were prospectively registered after providing written informed consent from March 2013 to September 2017 (Figure 1). Inclusion criteria were as follows: 1) diagnosis of liver cirrhosis on the basis of clinical presentation, routine laboratory data, and imaging; and 2) age 18–80 years. Exclusion criteria included a history of mental disorder and overt HE of West Haven Criteria grade 1 or greater. All protocols reported in this paper were approved by the Institutional Review Board of Iwate Medical University, and were performed in accordance with the requirements of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013). Informed consent was obtained from all participants.

Diagnosis of MHE

For diagnosing MHE, NPT was performed using a specific application on a mobile tablet (20). NPT comprises four tests: the number connection tests A and B, the digit symbol test, and the block design test. When patients exhibited abnormalities for two or more tests in NPT, the patients were diagnosed as having MHE (MHE group); those that did not have a diagnosis of MHE were classified into the non-MHE group.

MR image acquisition

MRI was performed using a 3.0 Tesla scanner (Discovery MR750, GE healthcare, Milwaukee, WI, USA) with an 8-channel head coil. DKI/DTI source images were obtained using a single-shot spin-echo echo-planar imaging technique with the following scanning parameters: repetition time/echo time 4000/110 ms; motion-probing gradients, 20 directions with a duration of 31.0 ms and a separation of 39.8 ms; *b* values 0, 1000, and 2500 s/mm^2; field of view 22 cm; matrix size 128 × 128; reconstructed matrix size 256 × 256; slice thickness 3.0 or 4.0 mm without gaps; and acquisition time 10 min 12 s, which were optimised by a previous report (21) and were used in previous reports (11, 16, 22). Conventional MR images, including axial T1- and T2-weighted images, were also obtained.

Analysis of imaging data

One of the authors (K.I.), who was blinded to the clinical presentation of the subjects, performed all image analyses. Diffusion metric maps, such as mean kurtosis (MK), fractional anisotropy (FA), and mean diffusivity (MD), were calculated using in-house software and used in previous studies (11, 16, 21, 22). In this study, FA and MD values were calculated using only DTI data from *b*-values of 0 and 1000 s/mm^2.

To identify changes in the white matter (WM) of the patients with MHE, we carried out voxel-wise statistical analysis of the MK, FA, and MD maps for screening the

whole brain using tract-based spatial statistics (TBSS) implemented in FSL 5.0.9 (FMRIB, Oxford. http://www.fmrib.ox.ac.uk/fsl/) (23). After skull stripping, all subjects' FA maps were aligned into FMRIB58-FA standard space using FMRIB's Non-linear Image Registration Tool. The mean FA image of each subject was created, and thinned and thresholded at FA > 0.20 to generate a mean FA skeleton that represents the canters of major WM tracts being common to all subjects. Further, the mean FA skeleton was masked to extract only the cerebral WM using the Harvard-Oxford Subcortical Structural Atlas implemented in FSL. The FA map of each subject was projected onto this skeleton to obtain skeleton FA maps. MK and MD maps were also projected onto the mean FA skeleton by applying the same transform as that for the FA map. Voxel-wise comparison of MK, FA, and MD values was performed between patients in the MHE and non-MHE groups. The threshold for statistical significance was $p < 0.05$, using threshold-free cluster enhancement (TFCE) with the family-wise effort (FWE) correction for multiple comparison corrections (corrected $p < 0.05$, 5,000 permutations). Moreover, we calculated the ratio of the areas of significant changes in MK, FA, and MD to the mean FA skeleton area. To perform region-of-interest (ROI) analysis in the cerebral WM skeleton, the ROI mask drawn on the mean FA skeleton were transformed into each native space following the back projection procedure in TBSS. Mean MK, FA, and MD values

of the WM were automatically measured by using the coregistered ROI.

To perform ROI analysis in the basal ganglia, the Johns Hopkins University (JHU) Eve atlas was warped to the native space of each subject by using the inverse transformation matrices that were obtained by the registration of the FA and b0 maps into the corresponding maps of the JHU-Eve atlas using methods similar to those described in a previous study (11). Mean MK, FA, and MD values of the caudate nucleus (CN), putamen (Put), globus pallidus (GP), and thalamus (TH) were then automatically measured by using coregistered ROIs.

Child–Pugh score and model of end stage liver disease (MELD)

Child–Pugh scores were calculated using data on prothrombin time international ratio (PT-INR), serum albumin levels (Alb), total bilirubin (T-Bil) and presence of encephalopathy and ascites. Child–Pugh classifications were defined as class A (5–6), class B (7–9), and class C (10–15). The MELD score was calculated using the following formula, based on the results of a hematological examination: MELD = 9.57 loge [Cre (mg/dL)] + 3.78 loge [T-Bil (mg/dL)] + 11.20 loge [PT-INR] + 6.43.

Laboratory data

Plasma PT-INR and serum levels of Alb, alanine aminotransferase, ammonia (NH3), aspartate aminotransferase, and creatinine, as well as platelet count and T-Bil, were

determined by an autoanalyzer (JCA-BM2250, JEOL, Tokyo, Japan).

Statistical analysis

Statistical analyses were performed using the SPSS 17.0 software program (SPSS Inc., Chicago, IL, USA). Continuous variables are presented as mean±standard deviation. Either the Mann-Whitney *U* test or Fisher's exact test was used for comparison of MK, FA, and MD values obtained by ROI analysis as well as demographics between patients with the MHE and non-MHE groups. Diagnostic performance for detection of MHE using DKI/DTI was assessed by using the receiver operating characteristics (ROC) method. The cut-off values of MK, FA, and MD for MHE were estimated by using the area under the ROC (AUROC) and was calculated using the maximum point of the Youden index (sensitivity+specificity-1). For all statistical analysis, a level of significance of $p < 0.05$ was used.

Results

Characteristics of subjects in the MHE and non-MHE groups

Of the 32 subjects evaluated, 10 were classified in the MHE group and 22 in the non-MHE group (Figure 1). After classification of the subjects based on MHE diagnosis, we excluded 5 subjects due to detection of unexpected lesions in the brain: 1 from the MHE group and 4 from the non-MHE group. Further, we excluded 4 subjects from the non-MHE group due to outliers in age-matching between the groups. After exclusion of subjects, we analyzed 9 MHE subjects and 14 non-MHE subjects. Detailed characteristics of the 23 subjects are summarized in Table 1. With regard to the etiology of liver cirrhosis, alcohol consumption, HCV, and non-alcoholic steatohepatitis were the etiological factors in 4, 3, and 2 patients in the MHE group, and 7, 3, and 4 patients in the non-MHE group, respectively. When laboratory data were compared between the two groups, NH3 and MELD score in the MHE group showed significant differences compared with those in the non-MHE group (111 μg/dL vs 53 μg/dL and 8.8 vs 5.2, respectively).

Mean kurtosis using DKI and fractional anisotropy using DTI decreased in the white matter in subjects with MHE

Figure 2 shows the results of the voxel-wise group analysis using TBSS. Compared with patients with non-MHE, MK and FA values of the WM in the patients

with MHE were significantly decreased in widespread WM, whereas MD values were significantly increased around only the left frontal and right occipital regions. Compared with FA and MD, MK detected the broadest area with significant changes in the patients with MHE (MK, 54.8%; FA, 34.3%; MD, 4.0%, respectively) (Figure 2). Regarding ROI analysis, MK and FA values of the WM were significantly lower and MD values were significantly higher in the MHE group (MK, 0.91; FA, 0.43; MD, 0.83, respectively) when compared with the non-MHE group (0.95, 0.45, and 0.80, respectively) (p=0.005, 0.024, and 0.039, respectively) (Figure 3).

Mean kurtosis using DKI and fractional anisotropy using DTI detected decreasing change at basal ganglia in the MHE subjects

Figure 4 shows representative DKI/DTI images of the MHE and non-MHE subjects. MK values in the Put and GP were apparently decreased in the patient with MHE, while FA values were slightly decreased. There were no substantia differences in MD between the groups.

Regarding ROI analysis in the basal ganglia, MK values of the CN, Put, GP, and TH were significantly lower in the MHE group (0.57, 0.70, 0.83, and 0.73, respectively) when compared with the non-MHE group (0.60, 0.77, 0.90, and 0.77, respectively) (p=0.036, 0.002, 0.005, and 0.023, respectively) (Figure 3 (a)). FA values of the CN, Put,

and GP were significantly lower in the MHE group (0.15, 0.19, and 0.29, respectively) when compared with the non-MHE group (0.16, 0.21, and 0.32, respectively) (p=0.012, 0.002, and 0.0001, respectively) (Figure 3 (b)). In contrast, MD values in the basal ganglia showed no apparent differences between the groups (Figure 3 (c)).

Mean kurtosis on the putamen was a useful finding to distinguish patients with MHE among subjects with liver cirrhosis

ROC analyses performed for MK, FA, and MD values of the CN, Put, GP, TH, and WM that showed significant differences between the groups (Table 2). Among the metrics, MK values of the Put achieved AUROC of 0.90, and sensitivity, specificity, positive predictive value, and negative predictive value of more than 80% (0.89, 0.86, 0.80, and 0.92, respectively) between the MHE and non-MHE groups. The AUROC value of GP in FA was the highest at 0.91, while PPV of GP in FA was lower compared with that of Put in MK (0.69 vs. 0.80).

Discussion

In this study, we compared DKI/DTI findings between patients with cirrhosis diagnosed with MHE and those without MHE to identify abnormalities in the brain microstructure that are diagnostic for MHE. Significant findings of the present study are as follows: 1) MRI-derived parameters of water component in WM and basal ganglia showed changes in relation to the development of MHE, 2) these changes were clearly detected by MK, and 3) Put on MK imaging was a diagnostic finding of MHE. According to these results, MHE can be diagnosed objectively and repeatedly by evaluating MK using MRI.

Accurate diagnosis of MHE is important because MHE is considered to precede overt HE and thus, is associated with reduction of both quality of life and activity of daily life (24). Since several papers have reported effectiveness of several agents for treatment of HE(25, 26), appropriate timing for diagnosis of MHE needs to be performed. Diagnostic methods for MHE include specific neuropsychometric and/or neurophysiological examinations such as NPT. However, these tests are time-consuming for outpatients. For ensuring that appropriate intervention is provided by accurate diagnosis of MHE, repeatable and objective diagnostic methods are required. According to our results, subtle changes in basal ganglia and WM can be detected by evaluating MK

and are useful for the accurate diagnosis of MHE. Further, these findings are objective and the examinations using DKI/DTI are repeatable.

The present study was designed for performing a comparison among patients with cirrhosis; however, previous studies that have shown advantages of using MD for diagnosis of MHE compared healthy controls and subjects with MHE (7, 9, 18). Importantly, it has been reported that MR features of the brain microstructure in patients with cirrhosis are different from those in healthy controls (19). In contrast, as the patients in both the non-MHE as well as the MHE group had liver cirrhosis in this study, our findings in the MHE subjects were likely associated with MHE rather than with liver cirrhosis. Thus, we propose that these findings are useful for the diagnosis of MHE. Recently, blood flow in putamen evaluated by functional MRI was changed on MHE patients (27). We considered that this result supported our findings that edematous change in putamen was occurred in MHE patients.

It is hypothesized that the pathophysiology of HE involves swelling of the astrocytes due to imbalance in amino acid metabolism. Indeed, we have previously shown increase in glutamine levels detected by MR spectroscopy in patients with MHE (6). Since the grey matter of the brain contains the cell bodies of the neurons or many kinds of glial cells (28), change in the DTI signal in the gray matter due to the presence of

edematous cells has been described in patients with MHE (9, 17). However, as previous studies compared patients with MHE and healthy controls, the findings in those studies might have varied if a comparison was performed among patients with liver cirrhosis or portal hypertension. In the results of this study, DKI clearly revealed a change in signal at a broad area of the WM. This result indicated that the WM had already been affected by MHE.

The PPVs of Put in MK and GP in FA were 0.80 and 0.69, respectively. PPV is the probability that subjects with a positive test actually have the disease. A high PPV increases the probability that the subject has the disease. Because we focused on establishing objective and reproducible methods for diagnosis of MHE, we concluded that Put in MK with high AUROC and highest PPV was superior in MHE diagnostic performance in this study.

We notice following limitation in this study. Owing to the cross-sectional design of the present study, repeatability of the observations may be low. Although previous studies have reported that findings detected by MRI in subjects with MHE are reversible (7), whether the findings we reveal in this study are reversible remains unknown. To further understand the pathophysiology of MHE, larger and serial-observation studies on MRI of patients with MHE are warranted.

The present study revealed that brain microstructural changes in MHE can be detected by evaluating MR images. Evaluation of MK can be useful to objectively detect subtle changes in the putamen in MHE patients, and these findings are diagnostic for MHE in patients with cirrhosis.

Reference

1. Wijdicks EF. Hepatic Encephalopathy. N Engl J Med **375**: 1660-1670, 2016.
2. Fichet J, Mercier E, Genee O, et al. Prognosis and 1-year mortality of intensive care unit patients with severe hepatic encephalopathy. J Crit Care **24**: 364-370, 2009.
3. Romero-Gomez M, Grande L, Camacho I, Benitez S, Irles JA, Castro M. Altered response to oral glutamine challenge as prognostic factor for overt episodes in patients with minimal hepatic encephalopathy. J Hepatol **37**: 781-787, 2002.
4. Stewart CA, Smith GE. Minimal hepatic encephalopathy. Nat Clin Pract Gastroenterol Hepatol **4**: 677-685, 2007.
5. Wein C, Koch H, Popp B, Oehler G, Schauder P. Minimal hepatic encephalopathy impairs fitness to drive. Hepatology **39**: 739-745, 2004.
6. Kooka Y, Sawara K, Endo R, Kato A, Suzuki K, Takikawa Y. Brain metabolism in minimal hepatic encephalopathy assessed by 3.0-Tesla magnetic resonance spectroscopy. Hepatol Res **46**: 269-276, 2016.
7. Kale RA, Gupta RK, Saraswat VA, et al. Demonstration of interstitial cerebral edema with diffusion tensor MR imaging in type C hepatic encephalopathy. Hepatology **43**: 698-706, 2006.
8. Srivastava A, Chaturvedi S, Gupta RK, et al. Minimal hepatic encephalopathy in children with chronic liver disease: Prevalence, pathogenesis and magnetic resonance-based diagnosis. J Hepatol **66**: 528-536, 2017.
9. Qi R, Zhang LJ, Zhong J, et al. Grey and white matter abnormalities in minimal hepatic encephalopathy: a study combining voxel-based morphometry and tract-based spatial statistics. Eur Radiol **23**: 3370-3378, 2013.
10. Jensen JH, Helpern JA, Ramani A, Lu H, Kaczynski K. Diffusional kurtosis imaging: the quantification of non-gaussian water diffusion by means of magnetic resonance imaging. Magn Reson Med **53**: 1432-1440, 2005.
11. Ito K, Ohtsuka C, Yoshioka K, et al. Differential diagnosis of parkinsonism by a combined use of diffusion kurtosis imaging and quantitative susceptibility mapping. Neuroradiology **59**: 759-769, 2017.
12. Umesh Rudrapatna S, Wieloch T, Beirup K, et al. Can diffusion kurtosis imaging improve the sensitivity and specificity of detecting microstructural alterations in brain tissue chronically after experimental stroke? Comparisons with diffusion tensor imaging and histology. Neuroimage **97**: 363-373, 2014.
13. Marrale M, Collura G, Brai M, et al. Physics, Techniques and Review of Neuroradiological Applications of Diffusion Kurtosis Imaging (DKI). Clin

Neuroradiol **26**: 391-403, 2016.

14. Wang JJ, Lin WY, Lu CS, et al. Parkinson disease: diagnostic utility of diffusion kurtosis imaging. Radiology **261**: 210-217, 2011.
15. Bester M, Jensen JH, Babb JS, et al. Non-Gaussian diffusion MRI of gray matter is associated with cognitive impairment in multiple sclerosis. Mult Scler **21**: 935-944, 2015.
16. Ito K, Kudo M, Sasaki M, et al. Detection of changes in the periaqueductal gray matter of patients with episodic migraine using quantitative diffusion kurtosis imaging: preliminary findings. Neuroradiology **58**: 115-120, 2016.
17. Kumar R, Gupta RK, Elderkin-Thompson V, et al. Voxel-based diffusion tensor magnetic resonance imaging evaluation of low-grade hepatic encephalopathy. J Magn Reson Imaging **27**: 1061-1068, 2008.
18. Qi R, Xu Q, Zhang LJ, et al. Structural and functional abnormalities of default mode network in minimal hepatic encephalopathy: a study combining DTI and fMRI. PLoS One **7**: e41376, 2012.
19. Chen HJ, Liu PF, Chen QF, Shi HB. Brain Microstructural Abnormalities in Patients With Cirrhosis Without Overt Hepatic Encephalopathy: A Voxel-Based Diffusion Kurtosis Imaging Study. AJR Am J Roentgenol **209**: 1128-1135, 2017.
20. Kawaguchi T, Konishi M, Kato A, et al. Updating the neuropsychological test system in Japan for the elderly and in a modern touch screen tablet society by resetting the cut-off values. Hepatol Res **47**: 1335-1339, 2017.
21. Yokosawa S, Sasaki M, Bito Y, et al. Optimization of Scan Parameters to Reduce Acquisition Time for Diffusion Kurtosis Imaging at 1.5T. Magn Reson Med Sci **15**: 41-48, 2016.
22. Ito K, Sasaki M, Ohtsuka C, et al. Differentiation among parkinsonisms using quantitative diffusion kurtosis imaging. Neuroreport **26**: 267-272, 2015.
23. Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage **31**: 1487-1505, 2006.
24. Hanai T, Shiraki M, Watanabe S, et al. Sarcopenia predicts minimal hepatic encephalopathy in patients with liver cirrhosis. Hepatol Res **47**: 1359-1367, 2017.
25. Shiraki M, Shimizu M, Moriwaki H, Okita K, Koike K. Carnitine dynamics and their effects on hyperammonemia in cirrhotic Japanese patients. Hepatol Res **47**: 321-327, 2017.
26. Suzuki K, Endo R, Takikawa Y, et al. Efficacy and safety of rifaximin in Japanese patients with hepatic encephalopathy: A phase II/III, multicenter, randomized, evaluator-blinded, active-controlled trial and a phase III, multicenter, open trial.

Hepatol Res **48**: 411-423, 2018.

27. Li Y, Liu H, Yang J, Tian X, Yang H, Geng Z. Combining arterial-spin labeling with functional magnetic resonance imaging measurement for characterizing patients with minimal hepatic encephalopathy. Hepatol Res **47**: 862-871, 2017.

28. Fields RD, Stevens-Graham B. Neuroscience - New insights into neuron-glia communication. Science **298**: 556-562, 2002 (in English).

Figure Legends

Figure 1. Flow chart of the study design.

Figure 2. Voxel-wise statistical analysis of the mean kurtosis (MK), fractional anisotropy (FA), and mean diffusivity (MD) using tract-based spatial statistics.

a, b, and c: Each panel shows the region with a significant change detected by each method (a: MK, b: FA, or c: MD, respectively). Clusters that survive FWE correction of $p<0.05$ with TFCE are presented as a colored area showing a significant change in white matter. To present differences in the MK, FA, and MD in the area with a significant change, a colored region indicating a skeletal lesion in each MK, FA, and MD is demonstrated.

Figure 3. Mean kurtosis (MK), fractional anisotropy (FA), and mean diffusivity (MD) of the basal ganglia and white matter (WM) were compared between the two groups.

a, b, and c: The caudate nucleus (CN), putamen (Put), globus pallidus (GP), thalamus (TH), and WM were evaluated using ROI analysis to assess MK (a), FA (b), and MD (c). The vertical axis indicates the intensity of each imaging. Closed circle and open circle indicate the minimal hepatic encephalopathy (MHE) group and non-MHE group,

respectively. The horizontal axis indicates the region selected for the analysis. Significant difference between the two groups was analyzed using the Mann-Whitney *U* test. Single asterisk (*) and double asterisks (**) indicate $p<0.05$ and $p<0.01$, respectively.

Figure 4. Diffusion kurtosis and tensor maps of the representative minimal hepatic encephalopathy (MHE) patient and non-MHE patient and regions-of-interest (ROI) of the basal ganglia structures.

The MHE patient was a 63-year-old woman and non-MHE was a 58-year-old man. The atlas-based ROIs of the right caudate nucleus (blue), putamen (green), globus pallidus (yellow), and thalamus (red) are shown on the Johns Hopkins University Eve T1-weighted images. Mean kurtosis (MK) is substantially decreased in the putamen and globus pallidus of the patient with MHE, while fractional anisotropy (FA) is slightly decreased. No apparent changes in mean diffusivity (MD) were observed in the patient with MHE when compared with the non-MHE patient.