White matter alterations in narcolepsy patients with cataplexy: tract-based spatial statistics

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SUMMARY

Functional imaging studies and voxel-based morphometry analysis of brain magnetic resonance imaging showed abnormalities in the hypothalamus–thalamus–orbitofrontal pathway, demonstrating altered hypocretin pathway in narcolepsy. Those distinct morphometric changes account for problems in wake–sleep control, attention and memory. It also raised the necessity to evaluate white matter changes. To investigate brain white matter alterations in drug-naïve narcolepsy patients with cataplexy and to explore relationships between white matter changes and patient clinical characteristics, drug-naïve narcolepsy patients with cataplexy ($n = 22$) and healthy age- and gender-matched controls ($n = 26$) were studied. Fractional anisotropy and mean diffusivity images were obtained from whole-brain diffusion tensor imaging, and tract-based spatial statistics were used to localize white matter abnormalities. Compared with controls, patients showed significant decreases in fractional anisotropy of white matter of the bilateral anterior cingulate, fronto-orbital area, frontal lobe, anterior limb of the internal capsule and corpus callosum, as well as the left anterior and medial thalamus. Patients and controls showed no differences in mean diffusivity. Among patients, mean diffusivity values of white matter in the bilateral superior frontal gyri, bilateral fronto-orbital gyri and right superior parietal gyrus were positively correlated with depressive mood. This tract-based spatial statistics study demonstrated that drug-naïve patients with narcolepsy had reduced fractional anisotropy of white matter in multiple brain areas and significant relationship between increased mean diffusivity of white matter in frontal/cingulate and depression. It suggests the widespread disruption of white matter integrity and prevalent brain degeneration of frontal lobes according to a depressive symptom in narcolepsy.

INTRODUCTION

Narcolepsy is a disabling sleep disorder characterized by excessive daytime sleepiness (EDS) and manifestations of abnormal rapid eye movement (REM) sleep, including cataplexy, sleep paralysis, and hypnagogic or hypnopompic hallucinations (Dauvilliers *et al.*, 2007). Besides sleep-related symptoms, regulation of emotion, cognitive impairments and mood disorder can be seen in narcolepsy (Naumann *et al.*, 2006).

Over the last two decades, numerous neuroimaging studies have investigated the pathophysiology of narcolepsy. Functional imaging studies showed metabolic (Joo *et al.*, 2004) and perfusion (Joo *et al.*, 2005) abnormalities in the hypothalamus–thalamus–orbitofrontal pathway and other brain areas in patients with narcolepsy, demonstrating alterations in the hypocretin pathway. Brain magnetic resonance imaging (MRI) studies in patients demonstrated distinct structural changes of multiple brain areas, accounting for problems in wake–sleep control, attention and memory

(Brenneis *et al*., 2005; Draganski *et al*., 2002; Joo *et al*., 2009, 2011; Kaufmann *et al*., 2002; Overeem *et al*., 2003).

Hypoperfusion in white matter (WM) was noted in the frontal and parietal lobes of patients in perfusion studies (Joo *et al*., 2005), which proposed the necessity of appropriate imaging methods to evaluate WM changes. Diffusion tensor imaging (DTI) is a quantitative MRI technique that measures the random motion of water within the tissue microstructure in the brain (Moseley *et al*., 1991). Fractional anisotropy (FA) of DTI is a scalar parameter of the degree of anisotropy, and is related to the myelination, axon density and packing density fibre bundle (Pierpaoli *et al*., 1996). The mean diffusivity (MD) of DTI is a quantitative measure of the mean motion of water, and is affected by cellular size and neurophil numbers (Basser *et al*., 1994; Pierpaoli *et al*., 1996).

So far, three studies have investigated WM changes in narcolepsy (Menzler *et al*., 2012; Nakamura *et al*., 2013; Scherfler *et al*., 2012). Two studies have followed similar approaches to voxel-based morphometry (VBM) techniques (Nakamura *et al*., 2013; Scherfler *et al*., 2012), originally developed for the investigation of local changes in grey matter (GM) density in T1-weighted images. In this VBM-style DTI analysis, each subject's scalar map of DTI is normalized into a common space, and then voxel-wise statistics were performed to find group differences or correlations of interests (e.g. normal versus patients, or disease severity). There are considerable debates on the technical limitations of VBM-style approaches due to the inaccurate alignment between subjects and the ambiguity in choosing the extent of smoothing (Bookstein, 2001; Davatzikos, 2004; Gitelman *et al*., 2001; Smith *et al*., 2006). A third study by Menzler *et al.* used tract-based spatial statistics (TBSS), which may alleviate some of the limitations of VBM-style approaches, as WM alterations were found in the hypothalamus, midbrain, medulla and bilateral frontal lobe of patients compared with controls (Menzler *et al*., 2012).

Despite the mounting evidences of co-morbid cognitive dysfunction and depression in patients with narcolepsy (Fortuyn *et al*., 2010; Naumann *et al*., 2006), the investigation on the relationships between cognition/mood and the WM are scarce. The aim of this study was to investigate: (1) WM alteration, as reflected by FA or MD using TBSS; and (2) the correlation between WM changes and cognitive function and mood in drug-naïve patients with narcolepsy with cataplexy.

MATERIALS AND METHODS

Participants

Twenty-eight narcolepsy patients were consecutively recruited from the sleep clinic of one university-affiliated hospital. Patients were recruited if they had no history of taking central nervous system stimulants or cataplexy medication. The diagnosis of narcolepsy with cataplexy was made according to the revised International Classification of Sleep Disorders (Westchester, 2005). The presence of cataplexy was determined according to the criteria suggested by Mignot *et al.* (1997). Patients underwent a sleep study consisting of overnight polysomnography (PSG) followed by a multiple sleep latency test (MSLT). The MSLT consisted of five naps scheduled at 2-h intervals starting at about 09:00 hours. Subjects with a mean sleep latency of 8 min or less and two or more sleep-onset REM periods (SOREMPs) were evaluated for human leukocyte antigen (HLA)-DQB1*0602 and DRB1*1501, which are the best genetic predictors of narcolepsy in human (Chabas *et al*., 2003; Doherty *et al*., 1998). Detailed information, including the presence of sleep attacks, hypnagogic hallucinations and sleep paralysis as well as a positive family history of narcolepsy, was obtained from patients and/or their families.

Thirty healthy controls from the local community were recruited through an advertisement. Each candidate control underwent a detailed clinical interview, sleep questionnaire and PSG, and the results were evaluated and interpreted by a sleep specialist (J. E. Y.). If a control had an apnea–hypopnoea index of 5 or greater or evidence of another sleep disorder (e.g. periodic limb movement disorder) on PSG, he or she was excluded from further participation.

Patients and controls were excluded if they exhibited any of the following: (1) mean daily sleep time less than 7 h; (2) abnormal sleep–wake rhythms; (3) other sleep disorders apnea–hypopnoea index $\geq$5 h^{-1} or period limb movement during sleep index more than $\geq$15 h^{-1}); (4) heart or respiratory disease; (5) history of cerebrovascular disease; (6) other neurological or psychiatric diseases; (7) alcohol or illicit drug abuse or current intake of psychoactive medications; or (8) presence of a structural lesion on brain MRI. Four patients were excluded because they had concomitant moderate to severe obstructive sleep apnea syndrome (OSA), and two patients were excluded due to negative HLA typing. Four controls were excluded due to moderate to severe OSA. Therefore, 22 patients with narcolepsy with cataplexy and 26 controls were included in the study. All subjects were right-handed.

All participants gave written informed consent before the study began. The Institutional Review Board at Samsung Medical Center authorized the informed consent form and the study protocol.

Neuropsychological assessments

Participants underwent a battery of neuropsychological tests in six broad domains: working memory; executive functioning; verbal information processing; verbal memory; visual memory; and verbal fluency. The whole neuropsychological test took 2.5 h to perform. Details of the neuropsychological assessments were previously described (Noh *et al*., 2012). To examine emotional state, the Beck Depression Inventory (BDI) was administered on the day of the sleep study. The BDI scores form two subscales: general depressive symptoms and somatic symptoms.

Brain MRI acquisition

Magnetic resonance imaging was performed using a Philips 3.0 Tesla scanner (Achieva, Philips Medical Systems, Best, and the Netherlands) with a 16-channel head coil. All participants underwent diffusion-weighted MRI. In the whole-brain DT-MRI examination, sets of axial diffusion-weighted, single-shot echo-planar images were collected with the following parameters: 128 × 128 acquisition matrix; 1.72 × 1.72 × 2 mm^3 voxels; 70 axial slices; field of view, 22 × 22 cm^2; echo time, 60 ms; repetition time, 7696 ms; flip angle, 90 °; slice gap, 0 mm; and b-factor, 600 s mm^{-2}. Using the baseline image without weighting [0, 0, 0], diffusion-weighted images were acquired from 32 different directions. All axial sections were acquired parallel to the inter-commissural (anterior/posterior commissure) line.

DTI processing

Diffusion tensor imaging processing was performed using the FMRIB Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl). First, motion and eddy-current distortion were corrected through affine registration by taking the B0 volume as a reference using FSL's Diffusion Toolbox. The diffusion tensors were then fit using linear least-squares optimization (Smith *et al*., 2004). FA and MD images were obtained from the eigenvalues of the tensors using DTIFIT. Next, voxel-wise statistical analysis of FA and MD images was performed using TBSS (Smith *et al*., 2006). FA images were aligned into standard space (FMRIB58_FA) using the non-linear registration tool (Andersson, 2007.). A mean FA image was created and thresholded by a FA value of 0.2 to exclude peripheral tracts and GM regions. Each subject's aligned FA images were projected onto the skeleton, and the resulting images were fed into voxel-wise cross-subject statistical analysis. MD images were analysed in the same manner.

Statistical analysis

Statistical analysis of demographic and clinical data was performed using the SPSS 20.0 package (SPSS, Chicago, IL, USA). A Chi-squared test was used to compare gender distributions between groups. Independent-samples *t*-test was used to compare age, PSG parameters and neuropsychological test scores between groups.

To evaluate group differences in MRI parameters and correlations between clinical characteristics and MRI parameters, voxel-wise statistical analysis was performed using the FSL randomize program. Differences in FA and MD between patients and controls were examined using permutation-based statistical analysis with 5000 permutations (Nichols and Holmes, 2002). The Threshold-Free Cluster Enhancement was used to correct for family-wise error (FWE) of multiple comparisons (Nichols and Holmes, 2002). Age was a covariate because it is known to be a major factor influencing FA and MD values. Relationships between diffusion indices and sleep parameters and neuropsychological scores were evaluated using Pearson correlation coefficients. For the analysis of the correlation of DTI measurements and clinical scores [BDI, duration of EDS and cataplexy, Epworth Sleepiness Scale (ESS) and MSLT sleep latency], only the patients with narcolepsy were selected to avoid confounding the clinical state. DTI measurements were regressed against the clinical scores at each skeleton voxel using age as nuisance variable. To explore statistical significance, a permutation-based statistical analysis was performed with 5000 permutations. The FWE-corrected level of significance was set at $P < 0.05$.

It defines as follows:

$$Y \sim b_0 + b_1\text{Age} + b_2\text{BDI} + b_3\text{EDS} + b_4\text{ESS} + b_5\text{MSLT} + b_6\text{Cataplexy} + \varepsilon$$

where Y represents DTI measurements, b_0 the intercept, $b_{1\sim6}$ the regression coefficients and ε is the residual error.

All statistical results were inflated using TBSS fill for visualization purposes.

RESULTS

Clinical characteristics and sleep studies

For patients, the age of onset of EDS was 16.5 ± 5.9 years (range, 6.2–32.5 years) and cataplexy was 21.6 ± 6.0 years (range, 12–37.2 years). The mean duration of EDS was 11.0 ± 6.9 years and cataplexy was 6.3 ± 5.4 years. Twenty-one patients (76%) had hypnagogic and/or hypnapompic hallucinations, and 18 patients (67%) had a history of sleep paralysis. All patients showed positive HLA typing (DR2 and DQB1*0602). Patients had significantly higher ESS scores (15.4 ± 3.1 versus 4.3 ± 1.3; *t*-test, $P < 0.01$) and were significantly more depressed (BDI scores, 13.5 ± 4.3 versus 6.19 ± 2.8, $P < 0.001$) compared with controls. In a subscale analysis of BDI, patients experienced more severe general depressive symptoms (10.6 versus 3.29, $P < 0.001$) than controls, but not the somatic symptoms (2.86 versus 2.9, $P = 0.386$). None of the patients had taken antidepressants or had been previously diagnosed with major depressive disorder. Brain MRI revealed no gross abnormalities in any participant. Further participant demographics and sleep-related parameters are summarized in Table 1.

Neuropsychological tests

The digit span and the Corsi block-tapping tests were used to examine visual attention, and the trail making test (parts A and B) was adopted for measuring attention, speed, mental flexibility and executive control. Compared with controls, patients exhibited significantly lower scores for attention, working memory and verbal information processing (measured by the digit symbol test). Verbal fluency by the Controlled Oral Word Association Test, verbal memory by

Table 1 Sleep characteristics of patients with narcolepsy and controls

	Patients (n = *22*)	*Controls* (n = *26*)	P-*value*
Men : women, no.	10 : 12	15 : 11	0.441
Age, years	26.9 ± 7.9 (19–44)	30.1 ± 11.1 (20–44)	0.212
PSG			
Sleep latency, min	4.1 ± 4.2 (0–21)	12.8 ± 1.5 (10–15)	<0.001*
REM sleep latency, min	49.1 ± 55.6 (0–203)	95.5 ± 14.3 (75–149)	0.015*
Apnea–hypopnoea index, no. of events per h	2.0 ± 2.1 (0–7.3)	3.7 ± 2.0 (0–6.5)	0.546
Apnea index, no. of events per h	15.4 ± 6.3 (5.2–32.1)	15.6 ± 5.2 (5.2–28.0)	0.630
MSLT			
Mean sleep latency, min	2.4 ± 1.9 (0.2–5.6)		
SOREMP, no.	3.8 ± 1.2 (2–5)		
Mean REM latency, min	3.8 ± 2.9 (0.2–10.7)		

Data are presented as mean ± standard deviation (range). Controls did not undergo the MSLT.
MSLT, multiple sleep latency test; PSG, polysomnography; REM, rapid eye movement; SOREMP; sleep-onset REM period.
*$P < 0.05$, independent-samples *t*-tests.

the Korean California Verbal Learning Test, and visual memory evaluated by the Rey Complex Figure Test were significantly reduced in patients. However, executive function measured by the Stroop test did not show significant differences between patients and controls (details are presented in Table 2).

DTI analysis

Compared with controls, patients exhibited significant FA decreases in the bilateral cingulate gyri, corpus callosum genu, WM adjacent to the fronto-orbital area, anterior limb of the internal capsule, as well as WM of the left anterior and medial thalamus. A detailed description of these changes, including their size and *P*-values, was given in Table 3 and Fig. 1.

To investigate the relationships between WM alterations and clinical characteristics, correlations between diffusion indices and BDI scores, duration of EDS and cataplexy, ESS, and MSLT sleep latency were performed after controlling for age and gender. After Bonferroni correction for multiple comparisons, significant positive correlations were found between depressive mood and MD in the bilateral superior frontal gyri, bilateral fronto-orbital gyri and right superior parietal gyrus in narcoleptic patients (Fig. 2; Table 4). There were no significant correlations between FA/MD of other brain regions and other clinical characteristics or neuropsychological test scores.

DISCUSSION

In this study, it was found that drug-naïve patients with narcolepsy with cataplexy showed reduced FA in the bilateral corpus callosum compared with controls. Decreased FA of the corpus callosum has been found in patients with geriatric (Yang *et al.*, 2007) or unipolar depression (Lacerda *et al.*, 2005), suggesting that microstructural lesions of the corpus callosum contribute to functional alterations in the inter-hemispheric system of emotional regulation (Kieseppa *et al.*, 2010). Higher incidence of mood-related symptoms of patients with narcolepsy compared with healthy people (Fortuyn *et al.*, 2010) would be directly linked to the pathophysiology of narcolepsy (Dauvilliers *et al.*, 2009). It is uncertain whether WM alteration precedes the depressive mood or not; however, similar findings were observed in a DTI study of newly diagnosed, naïve patients with depression (Ma *et al.*, 2007). This may suggest a close relationship between mood disturbances and WM changes, and a possibility of the shared pathway of narcolepsy and depression. It was also found that the greater increases in MD of fronto-parietal gyri were associated with more severe depressive symptoms. Although patients showed statistically higher total BDI score than controls, the scores per se of patients did not satisfy the criteria of clinically significant depression that required a score above 20. In BDI subscales, patients showed the higher general depressive symptom (10.6 ± 3.8 versus 3.29 ± 2.1, $P < 0.001$) than controls, while somatic symptoms showed no difference (2.86 ± 1.0 versus 2.9 ± 2.0, $P = 0.386$). Previously, one study reported 56.9% of patients with narcolepsy were thought to be depressed (BDI ≥ 10) and only 15.1% scored as moderate or severe depression (BDI ≥ 20; Daniels *et al.*, 2001), and the other reported that among 55.1% of narcoleptics with depressive symptoms only 5.6% showed severe depression (Dauvilliers *et al.*, 2009). This may explain relatively lower scores of self-reported mood questionnaire compared with actual symptoms in patients. Several reports were also found showing that central nervous system stimulants (dextroamphetamine, methylphenidate or modafinil) improved the mood and vigilance and some aspects of cognition, although those studies observed short-term effects from 90 min to 6 weeks (Becker *et al.*, 2004; Zwicker *et al.*, 1995). Preclinical and clinical studies presented that hypocretin and their receptors are involved in the physiopathology of depression, although the precise role of hypocretin in depression is undecided (Nollet and Leman, 2013). Taken together, DTI changes of

Table 2 Neuropsychological test scores for patients with narcolepsy and controls

	*Patients (*n = *22)*	*Controls (*n = *26)*	P-*value*
Working memory composite score	−0.17 ± 0.42	0.36 ± 0.36	<0.001
Digit span, forward	7.6 ± 2.0	9.5 ± 2.0	
Digit span, backward	5.4 ± 1.5	8.6 ± 2.1	
Corsi block, forward	7.8 ± 1.0039	10.7 ± 1.8	
Corsi block, backward	7.5 ± 1.6	9.9 ± 0.9	
Trail making test A, time to completion	57.4 ± 52.4	34.8 ± 10.4	
Trail making test B, time to completion	125.9 ± 94.2	82.0 ± 30.1	
Executive functioning composite score	−0.05 ± 0.87	0.05 ± 0.49	0.6
Stroop test, correct responses	112 ± 1.0	106.0 ± 14.9	
Stroop test, correct response time	112.7 ± 11.8	108.4 ± 12	
Verbal information processing composite score	−0.47 ± 1.03	0.4 ± 0.8	<0.001
Digit symbol test	49.5 ± 12.5	65.2 ± 12.1	
Verbal memory composite score	−0.53 ± 1.05	0.48 ± 0.64	<0.001
Korean california verbal test			
Total	45.4 ± 12.2	57.2 ± 7.4	
Short-delay free recall	9.4 ± 2.8	12.5 ± 3.0	
Long-delay free recall	9.7 ± 3.3	12.7 ± 2.7	
Recognition	14.4 ± 1.3	15.1 ± 1.3	
Verbal fluency composite score	−0.22 ± 0.83	0.20 ± 0.71	<0.0001
Controlled oral word association test			
Phonetic word fluency	27.9 ± 11.9	32.1 ± 12.7	
Semantic word fluency	31.6 ± 8.2	36.2 ± 7.7	
Animal	15.2 ± 4.81	20.8 ± 5.1	
Supermarket	16.3 ± 5.2	15.4 ± 4.8	
Visual memory composite score	−0.41 ± 0.77	0.37 ± 0.62	<0.001
Rey complex figure test			
Copy	30.9 ± 6.82	34.6 ± 2.5	
Immediate recall	13.8 ± 7.7	21.6 ± 7.3	
Delayed recall	13.7 ± 7.8	20.8 ± 7.1	
Recognition	19.5 ± 1.9	20.8 ± 1.9	
BDI	13.5 ± 4.3	6.19 ± 2.8	<0.001
General depressive symptoms	10.6 ± 3.8	3.29 ± 2.1	<0.001
Somatic symptoms	2.86 ± 1.0	2.9 ± 2.0	0.386

BDI, beck depression inventory.

corpus callosum and correlation with fronto-parietal regions in patients suggest that the WM substrates of anterior parts of brains may be related to the mood disturbances in patients with narcolepsy.

It was found that patients with narcolepsy showed reduced FA in WM of the anterior limb of the internal capsule, anterior/medial thalamus and connected prefrontal regions, suggesting a disorganization of fronto-thalamic-striatal circuitry in narcolepsy. Thalamo-cortical fibres in the anterior limb of the internal capsule connect medial and anterior thalamic nuclei to the frontal lobes. A consensus is that medial dorsal nuclei of the thalamus, prefrontal cortex and ventral striatum form an interconnected neural circuit that subserves the certain working memory functions (Block *et al.*, 2007; Floresco *et al.*, 1999). In line with previous literatures (Moraes *et al.*, 2012; Naumann *et al.*, 2006), patients showed lower scores in tests of working memory. Furthermore, a former functional MRI study revealed that the ventrolateral prefrontal cortex was a key structure underlying episodic memory, with the left side involved in verbal information processing and the right side involved in visual information processing (Dickerson and Eichenbaum, 2010). Therefore, the decreased FA of bilateral frontal lobe WM in patients could explain the observation of visual and verbal memory impairments in narcolepsy. Cognitive performance of narcolepsy may be greatly influenced by varying degrees of daytime sleepiness (Fulda and Schulz, 2001). The severity of sleepiness and effort to fight against sleepiness during the tests may explain most of the variability between cognitive results in drug-naïve patients with narcolepsy. Total time for DTI scanning is about 7 min in this hospital. To reduce the risks of drowsiness in patients, MRI studies were performed after more than 8 h of nocturnal sound sleep and a brief nap (<15 min) immediately before scanning in all subjects. Cognitive function test was performed similarly to MRI scanning. Brief napping is believed to enhance alertness in narcolepsy. Nevertheless, some patients have experienced drowsiness or fall asleep during the scans or cognitive tests, which could have affected the results. It may be a major research limitation in drug-naïve patients with narcolepsy. In the meantime, it is notable that EDS or MSLT sleep parameters did not show significant relationships with DTI parameters in this study.

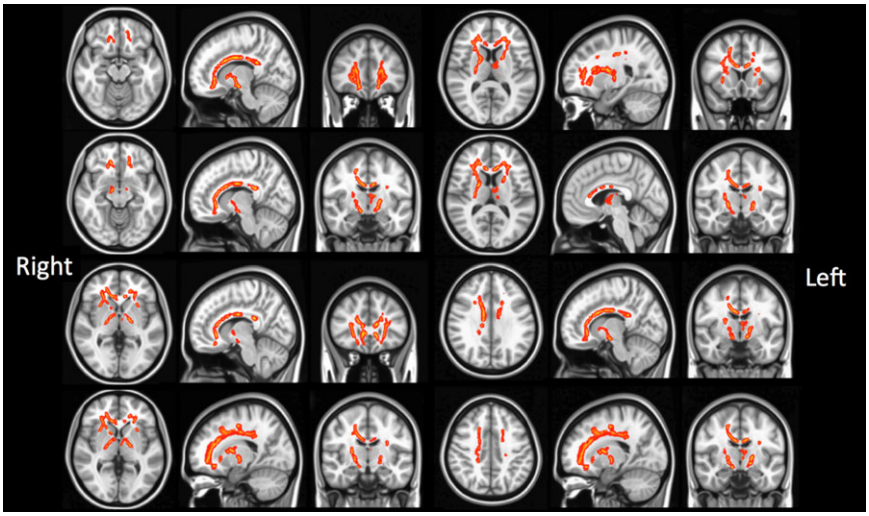

Figure 1. Significant fractional anisotrophy (FA) decreases in patients with narcolepsy compared with controls ($P < 0.05$, threshold-free cluster enhancement (TFCE). The underlay image is the standard Montreal Neurological Institute template.

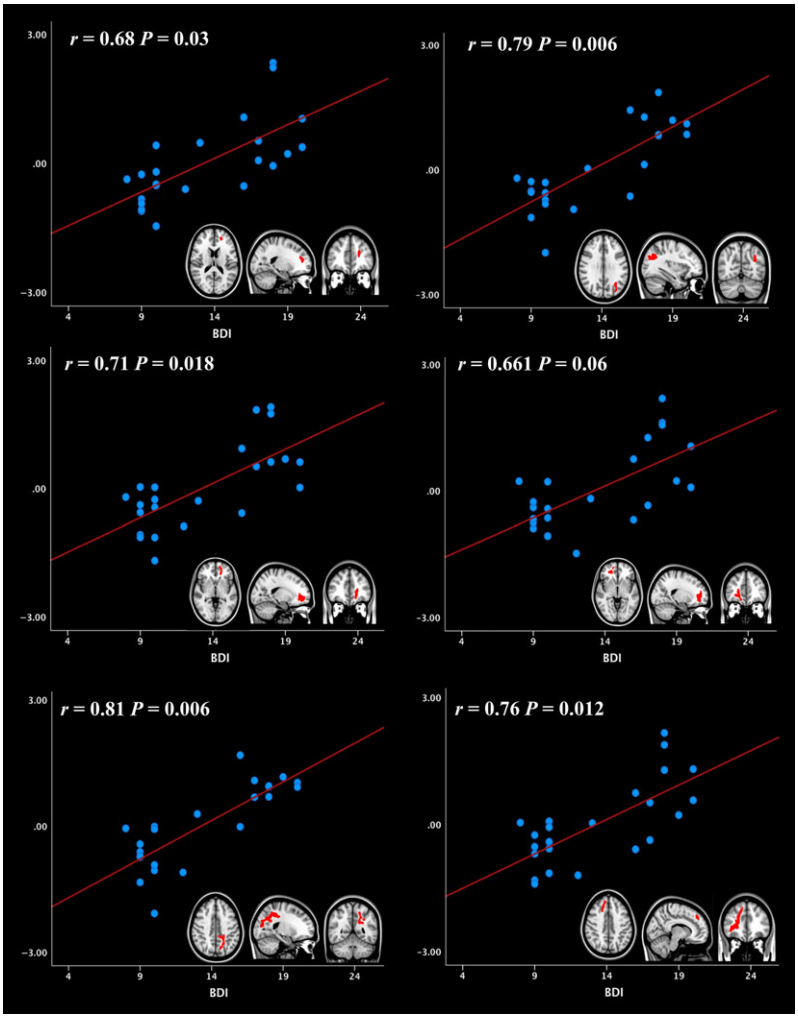

Figure 2. Partial correlations between average values of mean diffusivity (MD) and total Beck Depression Inventory (BDI) scores in patients with narcolepsy. The average values of MD were calculated by averaging across all voxels in the significant regions. The average values of MD and total BDI scores were adjusted for age.

There have been three DTI studies of patients with narcolepsy (Menzler *et al.*, 2012; Nakamura *et al.*, 2013; Scherfler *et al.*, 2012). Two of them reported changes in hypothalamus WM (Menzler *et al.*, 2012; Nakamura *et al.*, 2013), whereas the third and the current studies found no evidence of structural changes in the hypothalamus (Menzler *et al.*, 2012; Nakamura *et al.*, 2013; Scherfler *et al.*, 2012). Besides the hypothalamus, the brain areas with significant FA values varied among studies. Nakamura *et al.* reported increased diffusivity for the left amygdala in patients, and related the findings with cataplexy (Nakamura *et al.*, 2013). Scherfler *et al.* found the changes in FA in the superior

Table 3 Brain regions with significant differences in FA values between patients with narcolepsy and controls

Brain region	*Cluster size (mm^3)*	*Maximum* P-*value*	*MNI coordinates* x	y	z
Significant FA decreases					
WM of fronto-orbital gyrus, right	1183	0.014	16	37	−13
WM of fronto-orbital gyrus, left	933	0.028	−15	37	−11
Cingulate, right	55	0.031	13	−6	31
Cingulate, left	165	0.041	−13	−6	32
Corpus callosum genu	600	0.018	−12	30	−1
Thalamus, anteromedial, left	41	0.039	−6	−13	12
Anterior limb of internal capsule, right	348	0.028	16	7	8
Anterior limb of internal capsule, left	134	0.045	−19	12	8
Posterior limb of internal capsule, left	121	0.047	22	−16	0
WM of frontal lobe, right	165	0.041	19	−12	40
WM of frontal lobe, left	55	0.031	−18	3	40

Maximum *P*-values <0.05 after Bonferroni correction.
FA, fractional anisotropy; MNI, Montreal Neurological Institute; WM, white matter.

Table 4 Brain regions with positive correlations between MD values and total BDI scores in patients with narcolepsy

Brain region	*Cluster size (mm^3)*	*Maximum* P-*value*	*MNI coordinates* x	y	z
MD positively correlated with total BDI score					
WM of fronto-orbital gyrus, right	512	0.048	15	46	−12
WM of fronto-orbital gyrus, left	373	0.046	−15	46	−12
WM of superior frontal gyrus, right	891	0.040	34	−10	52
WM of superior frontal gyrus, left	1092	0.046	17	26	40
WM of superior parietal gyrus, right	702	0.040	16	−54	56

Maximum *P*-values <0.05 after Bonferroni correction.
BDI, Beck Depression Inventory; MD, mean diffusivity; MNI, Montreal Neurological Institute; WM, white matter.

temporal gyrus, which suggested the involvement with hallucinations (Scherfler *et al*., 2012). Menzler *et al.* showed decreased FA in the caudate nucleus and pre-postcentral gyri, which support the hypothesis of striatal dysfunction and stimulants effects on the motor area in narcolepsy (Menzler *et al*., 2012). Actually those brain areas with significant FA values in patients were mostly overlapped in previous structural imaging studies exploring GM of narcolepsy (Brenneis *et al*., 2005; Draganski *et al*., 2002; Joo *et al*., 2009, 2011; Kaufmann *et al*., 2002; Overeem *et al*., 2003). It is not clear why such variances exist in studies, it might be attributed to differences in methods of calculating, the effect of central nervous system stimulants, disease duration or the age of patients (Nakamura *et al*., 2013).

In this study, group differences of MD between patients and controls were not found. MD is defined as the average of the three eigenvalues of the diffusion tensor, measuring the magnitude of diffusion. FA is defined as a coefficient of variation of the eigenvalues, capturing the directionality of diffusion. The change of FA is not always accompanied by the change of MD, which was revealed in previous DTI studies in narcolepsy (Menzler *et al*., 2012; Nakamura *et al*., 2013; Scherfler *et al*., 2012), as well as in multiple sclerosis (Filippi *et al*., 2001) and Alzheimer's disease (Naggara *et al*., 2006). MD and FA provide complementary and partially independent information regarding pathology.

Between two kinds of DTI technologies, DTI-VBM approaches have several potential problems, such as the misalignment of fine structures and inconsistencies in the amount of smoothing, which can substantially affect study results. To overcome these limitations, DTI-TBSS emerged, which improves the sensitivity, objectivity and interpretability of analysis (Smith *et al*., 2006). It should be noted that GM exploration by DTI-VBM would substantiate the FA findings, as WM changes are frequently linked to alterations in the connected GM. Previously, VBM analyses of GM (not DTI analyses) were performed in drug-naïve patients with narcolepsy with 1.5 T MRI (Joo *et al*., 2009). It was observed that many brain regions with decreased GM concentration were overlapped with areas showing FA changes in the current study. The DTI-TBSS technique adopted in the present study did not provide GM exploration. It is supposed that inconsistent findings among DTI studies using different techniques might compromise the reliability of DTI results.

The previous study with TBSS by Menzler *et al.* had enrolled a small sample size (10 patients and 12 controls) and low power to detect significant differences between groups (Menzler *et al.*, 2012). In the current study, a larger sample size was employed (22 patients and 26 controls), and WM alterations were identified in hypocretin projection sites of the inferior frontal and cingulate cortex. This would be responsible for the attention and memory impairments of patients with narcolepsy. This favours the hypothesis of poor structural integrity within the frontal attention network of patients with narcolepsy with cataplexy, and raises the possibility that mood disturbances in patients may be a direct consequence of the pathophysiology of narcolepsy.

This study has several limitations. Because all patients were drug-naïve, vigilance levels were not always good while performing MRI scanning and neuropsychological tests. Central nervous system stimulants, modafinil awaken patients during the studies and lead to an improvement of executive function in patients (Becker *et al.*, 2004). However, it may demonstrate the medication effect rather than disease per se. The number of patients ($n = 22$) in this study may be insufficient to reveal the DTI changes, which were different from previous studies. Further study needs to clarify the inconsistent results with more numbers of patients.

In conclusion, this TBSS study demonstrated that drug-naïve patients with narcolepsy had reduced FA of WM in multiple brain areas, and a significant relationship between increased MD of WM in frontal/cingulate and depression. This suggests the widespread disruption of WM integrity and prevalent brain degeneration of frontal lobes according to depressive symptoms in narcolepsy.

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AUTHOR CONTRIBUTIONS

E. Y. J. and S. B. H. were involved in acquisition of data. E. Y. J., Y. K. P. & O.-H. K. were responsible for conception and design of the study, conducted the statistical analysis and drafted the manuscript. J. H. K., J. M. K. and S. T. K. gave critical revision of the manuscript for important neuroimaging analyses. E. Y. J. has full access to all the data in the study, and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

No conflicts of interest declared.

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