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Longitudinal White Matter Alteration in Prolonged Disorders of Consciousness due to Traffic Accidents

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Abstract

Objectives: We analyzed longitudinal white matter alterations by diffusion tensor imaging (DTI) in patients with prolonged disorders of consciousness (PDC).

Participants: Seventeen patients with PDC having an average of 328.8 days post-injury and eleven control participants.

Main measures: All participants underwent two DTI studies. Fractional anisotropy (FA) values were analyzed using whole-brain analysis (WBA) of 7 patients with no marked brain deformities; mean FA values of forceps minor (mFAFM) were subsequently measured by regional-brain analysis (RBA). Relationships between WB and regional FA values and recovery from PDC were evaluated.

Results: WBA in the PDC group showed that FA values were significantly lower in multiple white matter regions ($P < 0.05$), particularly in part of the forceps minor, in the second scan than they were in the first scan. RBA showed that mFAFM significantly decreased ($P < 0.01$). Further, WBA revealed that a significant positive correlation was observed between the degree of recovery from PDC and the difference in the number of voxels with FA values > 0.2 ($r = 0.65$, $P < 0.01$) between the first and second scans.

Conclusion: Our results showed microstructural white matter changes in patients with PDC, suggesting recovery from PDC with long-term treatment.

Keywords

Traumatic brain injury, Prolonged consciousness disorder, Diffusion tensor imaging, White matter, Longitudinal alteration, Fractional anisotropy, Vegetative state, Minimal consciousness state, Traffic accident, Tract-based spatial statistics

Introduction

Severe traumatic brain injury (TBI) is one of the most common causes of long-term disability conditions, including coma and persistent vegetative or minimally conscious states [1], which are widely accepted to arise from various forms of structural damage, such as diffuse axonal injury (DAI) [1,2]. Patients with severe prolonged disorders of consciousness (PDC),including vegetative states, typically have unfavorable outcomes although a few patients exhibit slow, subtle, and minor clinical changes [3,4]. For the past 3 years, our institution has provided in patients suffering from PDC due to traffic accident-related severe brain injury not only traditional therapies, such as physical therapy, occupational therapy, and nursing care, but also other specific non-traditional treatments, such as music therapy, aroma massage, and exposure to natural environments (i.e., feeling the sunlight, blowing wind, and seasonal temperature changes). Indeed, a few patients have shown slight positive reactions during inpatient residency at our institution.

There are validated scoring tools to evaluate the severity of consciousness disorders during the acute stages after TBI [5,6], but there are no appropriate scoring tools for evaluating chronic consciousness disorders. In particular, there are no scoring tools with proven utility for evaluating slight improvements from PDC. Therefore, it is difficult to assess the benefits of providing any intervention to patients with PDC. The "Kohnan score", developed at our institution to resolve this issue [5], reportedly displayed unidimensionality and higher intra- and inter-rater reliability [6]. Using this measure, we found that some patients with PDC exhibited improvement. However, there are no valid predictors of long-term positive clinical response in patients with PDC. Long-term practices without specific clinical goals are provided to patients with PDC by many medical personnel. Therefore, it is important to

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Table 1: Characteristics of patients with prolonged consciousness disorders.

No.	Age (y)	Gender	Diagnosis	Days until admission following injury	Days until first scan from injury	Days until second scan from injury
1	75	female	Right acute subdural hematoma, cerebral contusion	407	415	768
2	60	male	Traumatic subarachnoid hemorrhage, pneumocephalus, diffuse axonal injury	264	266	548
3	68	male	Traumatic subarachnoid hemorrhage, diffuse axonal injury, brain stem contusion	296	311	636
4	68	female	Cerebral contusion, traumatic subarachnoid hemorrhage, diffuse axonal injury, brain stem contusion	126	135	416
5	70	male	Right acute subdural hematoma, cerebral contusion, left intracranial hemorrhage	345	348	635
6	78	female	Left subdural hematoma, traumatic subarachnoid hemorrhage, intracerebral hemorrhage	629	629	885
7	86	male	Traumatic subarachnoid hemorrhage	155	158	591
8	44	female	Traumatic subarachnoid hemorrhage, diffuse axonal injury	230	232	396
9	58	male	Traumatic subarachnoid hemorrhage, left intracerebral hemorrhage	147	149	286
10	77	female	Traumatic subarachnoid hemorrhage, cerebral contusion, left intracerebral hemorrhage	226	228	580
11	31	male	Left intraventricular hemorrhage, left intracerebral hemorrhage, diffuse axonal injury	482	490	1173
12	56	male	Cerebral contusion, intraventricular hemorrhage, brain stem contusion	350	360	779
13	68	male	Traumatic subarachnoid hemorrhage, diffuse axonal injury	224	230	663
14	64	male	Traumatic subarachnoid hemorrhage, diffuse axonal injury, cerebral contusion	391	400	849
15	23	female	Right acute subdural hematoma, traumatic subarachnoid hemorrhage, cerebral contusion, diffuse axonal injury	798	807	1200
16	41	male	Hypoxic encephalopathy	264	266	581
17	33	male	Traumatic subarachnoid hemorrhage, cerebral contusion, left cerebral hemorrhage	256	258	540
N1	32	female				
N2	31	male				
N3	31	male				
N4	36	male				
N5	26	male				
N6	60	female				
N7	49	female				
N8	44	male				
N9	43	female				
N10	54	female				
N11	46	female				

develop predictors of long-term change elicited through long-term therapeutic intervention within these patients.

Decreased fractional anisotropy (FA) in some regions [7], measured by magnetic resonance diffusion tensor imaging (MR–DTI) [8], recently proved useful for assessing white matter damage *in vivo* [2,9-20]. Especially, corpus callosum was frequently reported to show significant damage from traumatic brain injury in previous studies [9,11]. Long-term patient outcomes were reportedly associated with the degree of white matter alteration revealed by DTI findings in patients with mild or severe TBI [2,9-20]. Furthermore, several studies reported that longitudinal alteration occurred in patients with severe TBI and that unfavorable outcomes correlated with decreased FA values detected in several brain regions [11,18].

This study analyzed longitudinal alteration in anatomical connections of white matter in patients with PDC due to traffic accidents and evaluated the association of microstructural imaging biomarkers in white matter with clinical markers to determine their potential clinical utility.

Methods

Patients

We retrospectively reviewed 17 patients at our institution with chronic, severe PDC resulting from traffic accident-related injury. All patients with PDC underwent 3.0T MR-DTI studies at admission and after one year of residency. The age range of patients was 23-86 (mean, 58.8 ± 18.3) years, and the median number of days from injury to recruitment for the study, i.e., admission to our institution, median was 264 (IQR: 226 - 391) days. Patient characteristics are presented in table 1. The first MR–DTI scan was performed at admission [266 (230 - 400) days after the initial injury], and the second scan was performed approximately one year after admission [340 (284 - 429) days after the initial injury].

Furthermore, we prospectively recruited 11 healthy normal volunteers (4 males and 7 females) for comparison. The age range of the healthy participants was 26-60 (41.1 ± 10.8) years (Table 2).

Standard protocol approvals, registrations, and patient consents

This study was conducted in compliance with the ethical principles that originated in the Declaration of Helsinki regarding biomedical research on human subjects and informed consent regulations. Approval from the institutional ethics committee was obtained prior to the initiating of the study.

PDC assessment

PDC was assessed using the Kohnan score. The Kohnan score was developed to evaluate the severity of consciousness disorder, with a

Table 2: Clinical characteristics of patients with prolonged consciousness disorders.

No.	GCSE at admission	GCSE at 1YA	KS at admission	KS at 1YA	Difference in KS from admission to	mFAFM at first scan	mFAFM at second scan	Difference in mFAFM from first scan to second scan	mFAWB at first scan	mFAWB at second scan	Difference in mFAWB from first scan to second scan	VsFA0.2 at first scan	VsFA0.2 at second scan	Difference in VsFA0.2 from first scan to second scan
1	2	2	65	63	2	0.39	0.33	0.06	0.22	0.23	-0.01	184507	190416	-5909
2	3	3	34	5	29	0.49	0.46	0.03	0.26	0.25	0.01	227050	208051	18999
3	2	2	67	68	-1	0.45	0.4	0.05	0.26	0.23	0.02	211742	201840	9902
4	2	2	63	56	7	0.48	0.46	0.02	0.24	0.24	0	175971	168775	7196
5	2	2	68	69	-1	0.28	0.22	0.06	0.22	0.29	-0.07	208770	168775	-71813
6	2	2	64	62	2	0.28	0.25	0.03	0.24	0.24	0	202977	212090	-9113
7	2	2	68	64	4	0.38	0.39	-0.01	0.31	0.27	0.04	310168	260886	49282
8	2	3	55	34	21	0.43	0.46	-0.03	0.28	0.25	0.03	237291	199895	37396
9	3	3	29	0	29	0.52	0.47	0.05	0.32	0.24	0.08	314163	214154	100009
10	2	2	63	62	1	0.32	0.3	0.02	0.23	0.23	0	185291	172749	12542
11	2	2	67	64	3	0.42	0.38	0.04	0.21	0.21	0	186705	185048	1657
12	2	2	62	52	10	0.43	0.39	0.04	0.32	0.27	0.05	283215	231824	51391
13	2	2	60	61	-1	0.39	0.37	0.02	0.23	0.23	0	173868	172998	870
14	2	2	65	64	1	0.44	0.42	0.02	0.24	0.23	0.01	214459	201762	12697
15	2	2	67	65	2	0.31	0.3	0.01	0.26	0.23	0.03	199347	167305	32042
16	2	2	68	66	2	0.39	0.38	0.01	0.2	0.19	0.01	164703	141284	23419
17	2	3	55	34	21	0.29	0.31	-0.02	0.28	0.28	-0.01	355768	271046	84722
N1									0.26	0.29	-0.03	275647	316920	-41273
N2									0.28	0.28	0	374602	368973	5629
N3									0.28	0.28	0	364937	366585	-1648
N4									0.28	0.27	0.01	349935	348378	1557
N5									0.27	0.27	0	310792	312301	-1509
N6									0.26	0.26	0	236622	235726	896
N7									0.26	0.26	0	275647	285765	-10118
N8									0.26	0.26	0	321268	328659	-7391
N9									0.27	0.27	0	307768	313357	-5589
N10									0.26	0.29	-0.03	337975	338298	-323
N11									0.28	0.28	0	294943	298536	-3593

GOSE: extended glasgow outocome scale; KS: kohnan score; 1YA: 1 year after admission; mFAFM: mean fractional anisotropy value of forceps minor; mFAWB: mean fractional anisotropy value for the whole brain; VsFA0.2: number of voxels with a fractional anisotropy value more than 0.2 in the whole brain; N: normal participants.

particular focus on the persistent vegetative state. (Appendix) [6]. This score comprises even parameters, with each parameter divided into 5 grades: extreme (10 points), severe (9 points), moderate (7 or 8 points), mild (5 points) and slight (0 points). We additionally assessed general functional recovery using the Extended Glasgow Outcome Scale (GOSE) [21], which ranges from 1 to 8, with higher scores suggesting better functional outcomes. These assessments were performed at admission and at 1 year after admission.

Image acquisition

MRI was performed using a 3.0 T Signa Excite HD scanner (General Electric, Milwaukee, WI, USA). The general scan parameters for MR-DTI were as follows: echo time, 59 ms; repetition time, 9,000 ms; flip angle, 90°; slice thickness, 3 mm with no gap; field of view, 28.8 × 28.8 cm; acquisition matrix, 96 × 96; image matrix, 256 × 256 with a voxel size of 1.125 × 1.125 × 3.0 mm; number of excitations, 1; and bandwidth, 250 kHz. Images were obtained with 15-directional diffusion encoding (b value, 1,000 s/mm^2 in each direction) and without diffusion encoding (b value, 0 s/mm^2). A total of 46 axial section images covering the entire cerebrum were obtained. The inferior MR–DTI slices were positioned at the medulla oblongata during acquisition.

Two MR-DTI scans were performed in healthy, normal participants; the second scan was performed 1 year after the first scan. Scan parameters for MR-DTI in normal participants were different from those in patients with PDC because of an MRI equipment upgrade. The scan parameters were as follows: echo time, 65.8 ms; repetition time, 15,000 ms; flip angle, 90°; slice thickness, 3 mm with no gap; field of view, 28.8 × 28.8 cm; acquisition matrix, 96 × 96; image matrix, 256 × 256 with a voxel size of 1.125 × 1.125 × 3.0 mm; number of excitations, 1; and bandwidth, 1953.12 kHz. Images were obtained with 15-directional diffusion encoding (b value, 1,000 s/mm^2 in each direction) and without diffusion encoding (b value, 0 s/mm^2). A total of 46 axial section images covering the entire cerebrum were obtained. Other settings were the same as in patients with PDC.

We performed a whole-brain (WB) statistical analysis of FA maps with a tract-based spatial statistics (TBSS) technique using the diffusion toolbox implemented in the Oxford Centre for Functional MRI of the Brain(FMRIB) software library (FSL:http://www.fmrib.ox.ac.uk/fsl/). Images underwent eddy current distortion correction [22] and skull-stripping using the Brain Extraction Tool [23]. The FA maps of each patient were aligned into the Montreal Neurological Institute spaces using nonlinear registration algorithms with FNIRT in FSL. The FA values of each patient were subsequently projected onto a mean FA skeleton, which represented centers of white matter tracts common to all patients. The resulting FA skeleton data were used in the following voxel-wise WB statistical analysis. The longitudinal changes in FA values between the first and second scans were evaluated using a permutation-based randomized test and inference using the threshold-free cluster enhancement method implemented in FSL. The statistical threshold for all image analyses was set at $P < 0.05$, with family-wise errors corrected for multiple comparisons of the voxel-wise WB analysis. We also measured the mean FA value in WB (mFAWB) and the number of voxels in which FA values were > 0.2 in WB (VsFA0.2) in the FA skeleton.

After TBSS analysis, we performed a regional brain assessment with a tract-specific analysis (TSA) of FA values using dTV II and Volume-One 1.72 software, developed by Masutani, *et al.* (http://www.volume-one.org/).

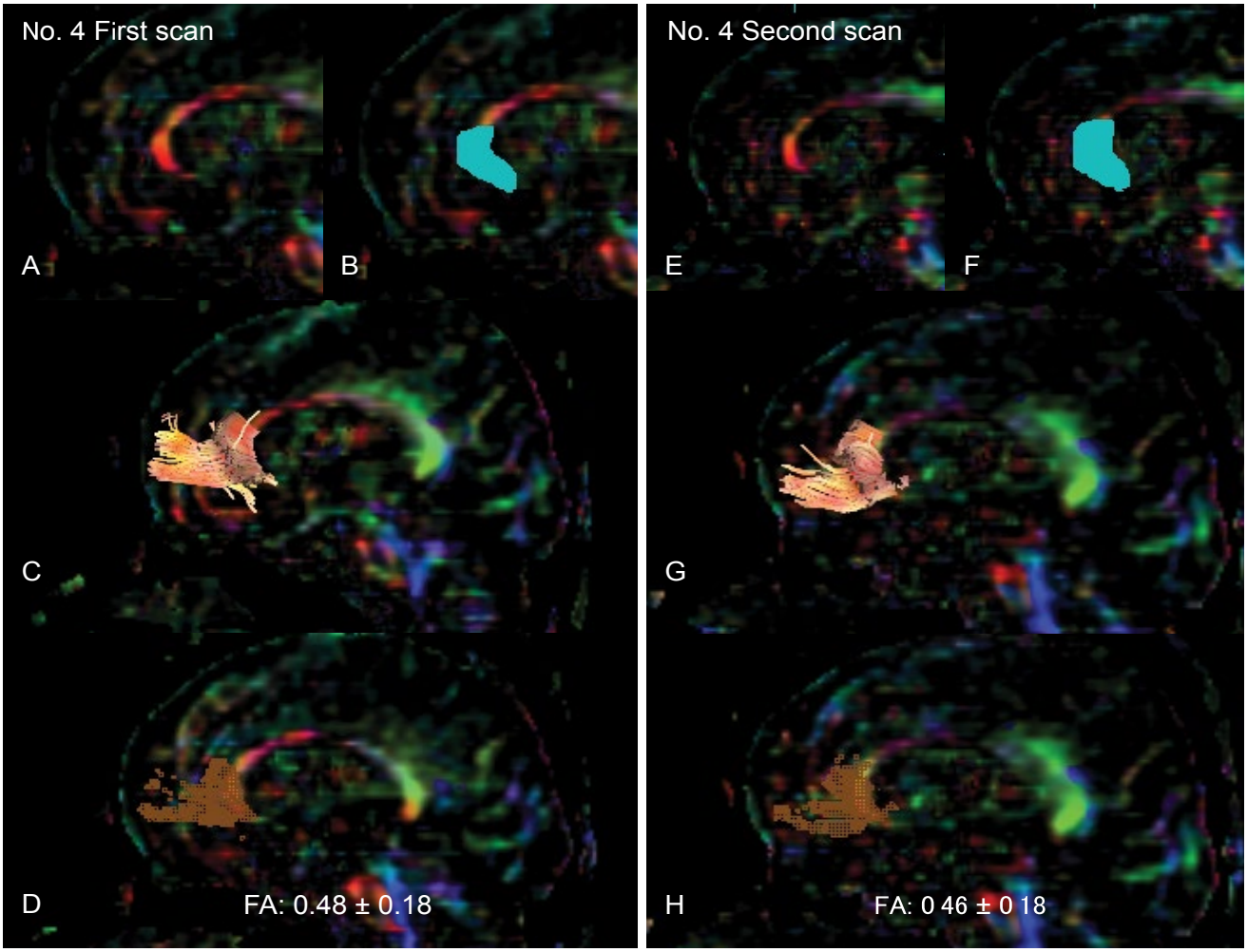

Figure 1: The drawing strategy for tractography and voxelization along the tractography of the forceps minor. Both panels show the tractography of the forceps minor and voxelization along the tract of the forceps minor in the representative subject (patient No. 4).

The left panel shows the tractography in the first scan. The right panel shows the tractography at the second scan. The FA value of the forceps minor decreased from 0.48 ± 0.18 in the first scan to 0.46 ± 0.18 in the second scan.

White matter tractography was assessed in all subjects using the region-of-interest (ROI) method. Three-dimensional anisotropy contrast (3DAC) color-coded maps were used to precisely and objectively position ROIs in white matter tracts (Figure 1A and Figure 1E). The relatively large target ROI, including the entirety of the genu of the corpus callosum, was manually placed on a reconstructed mid-sagittal section of the 3DAC image (Figure 1B and Figure 1F). Drawn tractography was generated using the threshold values of line-tracking termination as FA > 0.18 (Figure 1C and Figure 1G). This tractography was considered to be the forceps minor (FM). The dTV II software provides a track-line voxelization function that extracts the tracking line of the white matter tract to 3D voxels while preserving original tensor parameters. Voxelization along the FM tract was also performed (Figure 1D and Figure 1H). All voxels, including the tracked lines, were evaluated, and the mean FA values in the registered voxels within the FM core (mFAFMs) were measured [7,8]. In all subjects, we also measured FA values regarding mFAWB and VsFA0.2 in the whole brain, including both the white and gray matter regions. Therefore, we were unable to extract only white matter because some patients had too much deformity to perform accurate segmentation.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 24.0 for Mac (IBM SPSS; Chicago, IL, USA). Comparisons over time for Kohnan scores, GOSE, mFAWB, VsFA0.2, and mFAFM of the groups were conducted using either the paired *t*-test or the Wilcoxon rank-sum test based on the results of the Shapiro–Wilk test. The Spearman rank correlation coefficient was used to evaluate the relationships among age, the number of days from injury to admission, degree of recovery from PDC as determined by change in the Kohnan score during the first year after admission, and FA values. The FA values used for comparison were FAFM, mFAWB, and VsFA0.2 in the first scan and the differences in mFAFM, mFAWB, and VsFA0.2 between the first and second scans. We also calculated the partial correlation coefficient between each FA values that showed a significant relationship with the degree of recovery from PCD according to the Spearman rank correlation coefficient and with that from PCD with age and gender as covariates.

Results

In this study, 15 patients had an at-admission GOSE score of 2 (vegetative state), and two patients had an initial GOSE score of 3 (severe disorder). However, the Kohnan scores at admission showed more variability (range, 29-68), ranging from the minimally conscious state (< 39) to the completely vegetative state (> 65). After one year, there was no significant change in GOSE scores ($P = 0.16$, Wilcoxon rank-sum test): 13 patients had a GOSE score of 2, whereas 4 patients had a GOSE score of 3. In contrast, a subset of PDC patients showed a significant improvement as evidenced by the Kohnan score (60.0 ± 11.5 vs. 52.3 ± 21.4, $P < 0.01$; Wilcoxon rank-sum test) (Table 3). The mean difference in the Kohnan scores assessed in the first and second scans was 7.7 ± 10.5.

In patients with PDC, mFAWB and VsFA0.2 values in the first scan were 0.25 ± 0.04 and 225,646.76 ± 56,386.77, respectively, whereas those in the second scan were 0.24 ± 0.03 and 204,747.41 ± 38,384.88, respectively (Table 3). Patients with PDC showed no significant difference over time in mFAWB ($P = 0.12$, paired *t*-test)

Table 3: Characteristics of seven patients with prolonged consciousness disorders evaluated by TBSS.

No.	mFAWB at first scan in WMS	mFAWB at second scan in WMS	VsFA0.2 at first scan in WMS	VsFA0.2 at second scan in WMS
2	0.38	0.36	140253	133175
3	0.35	0.32	139299	135832
4	0.34	0.33	139765	134141
8	0.36	0.35	144283	142292
9	0.42	0.36	145503	141011
12	0.41	0.37	150795	139888
14	0.35	0.34	149989	146279

mFAWB: mean fractional anisotropy value for the whole brain; TBSS: tract-based spatial statistics; VsFA0.2: number of voxels with a fractional anisotropy value of more than 0.2 in the whole brain; WMS: white matter skeleton.

Table 4: Correlation between the degree of recovery from prolonged consciousness disorder and variables for 17 patients.

	Degree of recovery from PCD	
Variable	***r* (Spearman correlation coefficient)**	***P* values**
Age	-0.39	0.13
Day of institution admission from injury	-0.29	0.26
mFAFM at first scanb	0.40	0.11
mFAWB at first scan	0.60	0.01
VsFA0.2 at first scan	0.56	0.02
Difference in mFAFM from first to second scan	−0.24	0.35
Difference in mFAWB from first to second scan	0.43	0.09
Difference in VsFA0.2 from first to second scan	0.67	< 0.01

mFAFM: mean fractional anisotropy value of the forceps minor; mFAWB: mean fractional anisotropy value for the whole brain; VsFA0.2: number of voxels with a fractional anisotropy value greater than 0.2 in the whole brain.

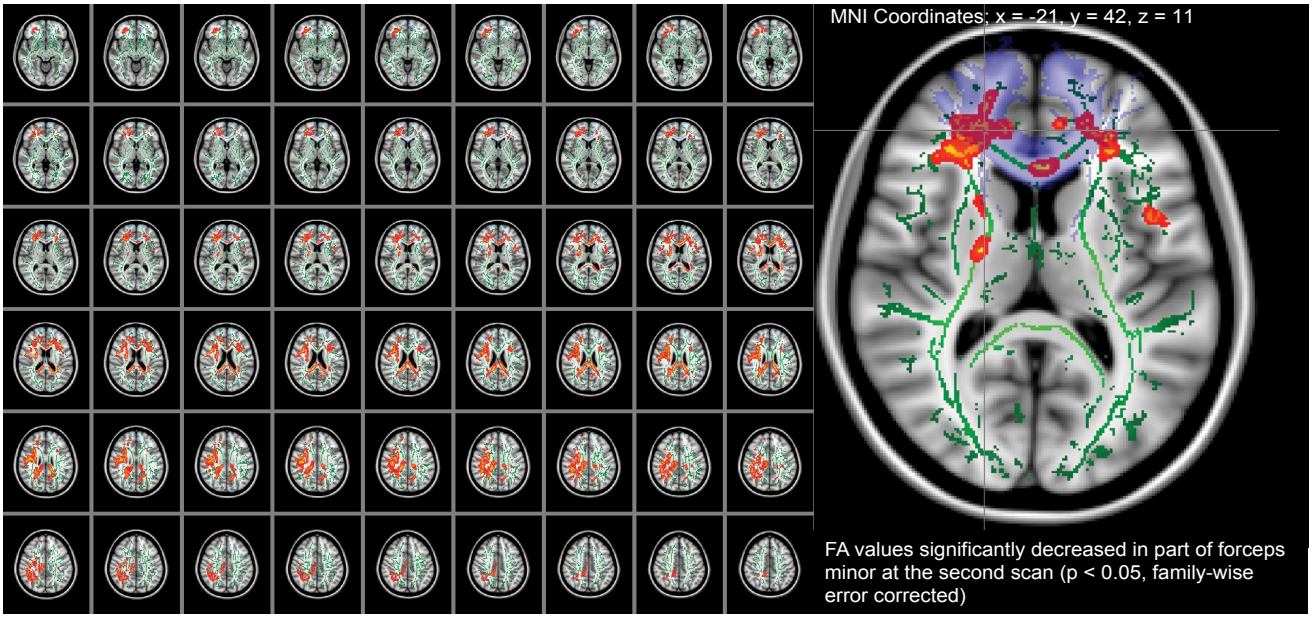

Figure 2: A comparison of longitudinal diffusion tensor imaging after traumatic axonal injury in patients during the chronic phase (at admission) and during the extended chronic phase (1 year after admission). The images (red-yellow) depict instances where diffusion tensor imaging measures during the chronic phase were more than those of the extended chronic phase. The green tracts are representative of the skeleton created by tract-based spatial statistics. The light violet regions are representative of the forceps minor as specified by the Johns Hopkins University white matter tractography atlas provided with the FSL. The FA values significantly decreased in part of the forceps minor in the second scan (panels B) as compared with that in the first scan (0.46 ± 0.18 vs. 0.48 ± 0.18, P < 0.05, family-wise error correction).

value, including gray matter comparison. In contrast, they showed significant difference over time in VsFA0.2 ($P = 0.02$, Wilcoxon rank-sum test) value. There were no significant differences in mFAWB ($P = 0.16$, paired t test) and VsFA0.2 ($P = 0.16$, paired t-test) values between the first and second scans in normal, healthy participants (Table 2).

In some patients exhibiting marked brain atrophy, extended cerebral ventricles, and/or brain deformities, their imaging scans showed considerably missed registrations. Therefore, 10patients with severe brain deformities were excluded. Thus, TBSS included five male and two female patients with a mean age of 59.7± 8.4 years and a mean of 264 (188-323) days from injury to admission (Table 3). TBSS revealed that the FA values in the second scan were significantly lower than those in the first scan in numerous, small white matter regions (Figure 2). In particular, TBSS revealed a significant decrease over time in FA values for parts of the genu of the corpus callosum. Similarly, mFAWB in white matter skeleton (WMS) (0.37 ± 0.032 vs. 0.35 ± 0.018, $P < 0.05$) and VsFA0.2 in WMS (144,269.57 ± 4,794.88 vs. 138,945.43 ± 4,764.62, $P < 0.01$) values were significantly lower in the second scan than those in the first scan (Table 3). In contrast, by TBSS, there were no significant WB changes in the 11 normal participants.

Following TBSS, regional analysis using TSA was performed to determine the FA values of FM for all 17 patients with PDC. The results indicated a significant decrease in mFAFM over time (0.39 ± 0.1 vs. 0.37 ± 0.1, $P < 0.001$, Figure 3).

In the correlation analysis of all 17 patients, no significant correlations were observed between the degree of recovery from PDC and the difference in mFAFM over time (Table 4). However, a significant positive correlation was observed between the degree of recovery from PDC and time-dependent changes in VsFA0.2 ($r = 0.56$, $P = 0.02$) between the first and second scans, and VsFA0.2 ($r = 0.67$, $P < 0.01$), mFAWB ($r =$

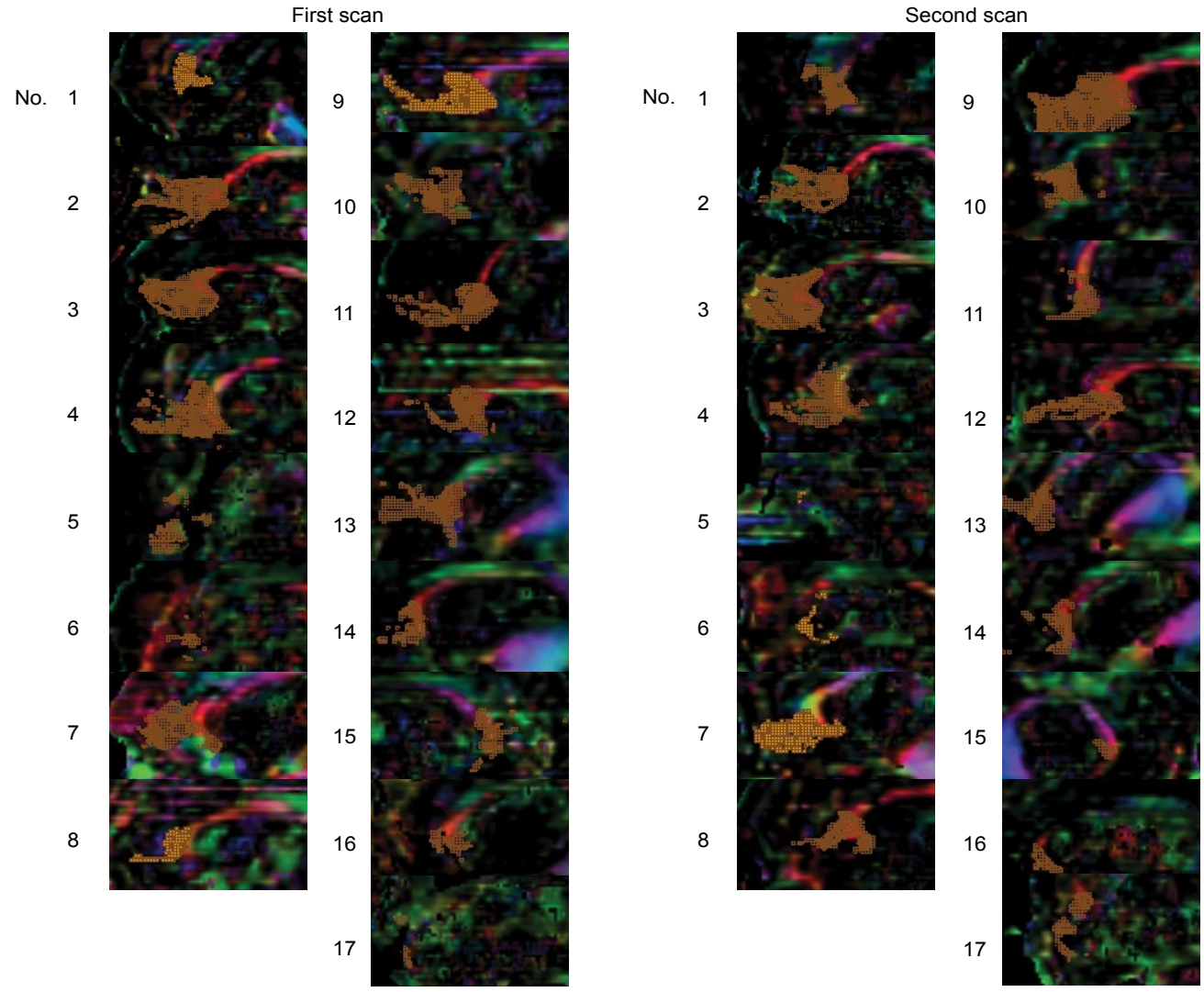

Figure 3: Voxelization along the FM tract was performed on 17 patients. In patient 15, the right-sided view is presented as left-side tractography because drawing the forceps minor was not possible with this patient.

0.60, $P = 0.01$) at first scan (Figure 4). Similarly, a significant positive correlation was observed between the degree of recovery from PDC and time-dependent changes in VsFA0.2 ($r = 0.61$, $P = 0.02$) between the first and second scans and between VsFA0.2 ($r = 0.62$, $P = 0.02$) and mFAWB ($r = 0.60$, $P = 0.02$) at first scan according partial correlation analysis. There was a significant correlation between the change in VsFA0.2 values and the VsFA0.2 value in the first scan ($r = 0.67$, $P < 0.01$).

Discussion

We explored the evolution of damaged white matter by examining MR-DTI changes in patients with PDC obtained at admission to our institution and again at 1year after admission. Seven patients with severe complicated DAI who demonstrated subcortical white matter lesions and had not undergone invasive treatment were evaluated. Results showed decreased FA values in multiple regions of some WMSs. Siminally, mFAWB in WMS and VsFA0.2 in WMS values significantly decreased. In particular, the decreased FA voxels were concentrated in the corpus callosum (i.e., FM) compared with no such significant changes detected in normal participants upon using TBSS. TSA performed on 17 patients with PDC revealed that mFAFM was significantly decreased during the year after admission; however, no significant correlations were observed among the degree of recovery from PDC, the difference in the mFAFM values over time and the mFAFM value in the first scan. In contrast, a significant correlation was observed between the degree of recovery from PDC and the time-dependent changes in VsFA0.2, mFAWB, and VsFA0.2 values in the first scan. Currently, there are no valid predictors of long-term positive clinical response in patients with PDC. Several clinicians provide long-term care without established, specific clinical goals for patients with PDC. Therefore, it is critical to develop predictors of long-term changes elicited through long-term therapeutic intervention in these patients. Our results demonstrated microstructural white matter changes occurring in patients with PDC, suggesting that the assessment of white matter changes facilitates the development of valid long-term outcome predictors.

Although previous studies have evaluated chronic DAI, we used TBSS, a voxel-based refinement of MR-DTI data analysis, in this study. Spatially, diffusion changes observed in the chronic stage occurs across various white matter regions, including the expected areas (i.e., corpus callosum), as revealed by histopathological and radiological studies of DAI [2,9-11,13-20]. This finding is consistent with prior reports of decreased FA values during the chronic phase of DAI [11,14,15,18-20]. Sidaros, *et al.* examined longitudinal microstructural white matter alteration in individuals with severe TBI in the late sub-acute and chronic stages and investigated potential correlations with 1-year clinical outcomes [11]. They found decreased FA values in all the investigated white matter regions, such as the posterior aspect of the corpus callosum (PCC), posterior limb of internal capsule (PLIC), centrum semiovale (CSO), and cerebral peduncle (CP) in the late sub-acute stage, approximately 8 weeks post-injury. Follow-up MR-DTI, which was performed approximately 12 months post-injury, showed FA normalization in PLIC and CSO, primarily in patients with unfavorable outcomes (i.e., GOSE score of 4 [moderate disability] or 5 [low disability]). Similarly, FA values remained depressed in PCC and CP, particularly

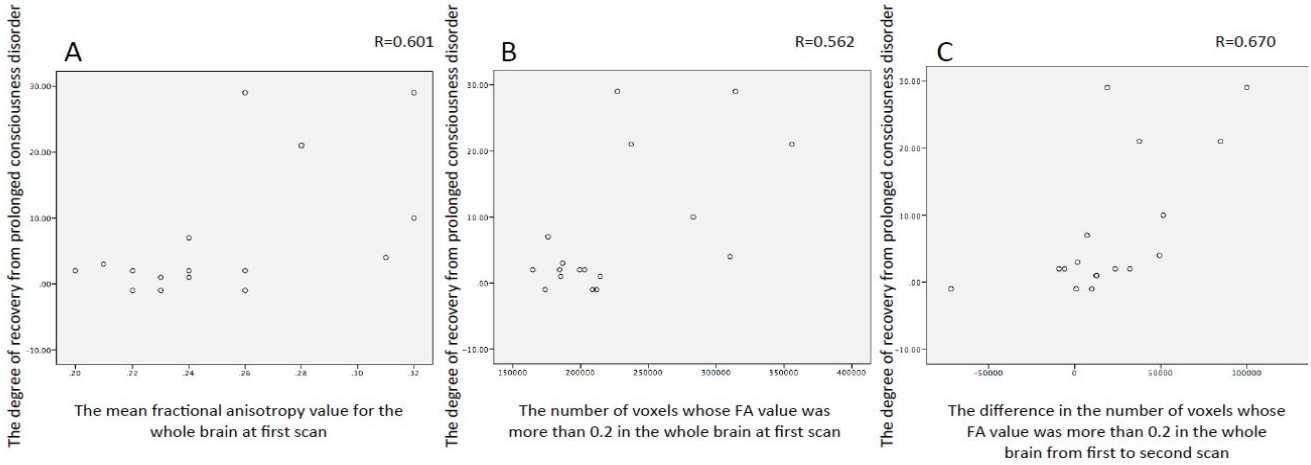

Figure 4: Analysis of positive correlation between the degree of recovery from prolonged consciousness disorder and the mean fractional anisotropy value for the whole brain at first scan (A, r = 0.601, P = 0.011). Analysis of positive correlation between the degree of recovery from prolonged consciousness disorder and the number of voxels whose FA value was more than 0.2 in the whole brain at first scan (B, r = −0.562, P = 0.019). Analysis of positive correlation between the degree of recovery from prolonged consciousness disorder and the difference in the number of voxels whose FA value was more than 0.2 in the whole brain from first to second scan (C, r = 0.670, P = 0.003).

in patients with unfavorable outcomes (i.e., GOSE scores ≤ 3 [severe disability]). Although TBSS showed decreased FA values in multiple WMS regions, there were differences between the subjects in previous studies and our study. Our subjects generally had more severe outcomes; all our patients had GOSE scores ≤ 3, indicating more severe disability than those in previous studies [2,9-15,18-20]. Thus, inter-study differences in results may reflect DAI severity.

Our first scan data were collected 334.2 ± 176.7 days after injury, thus reflecting chronic white matter damage. Furthermore, longitudinal white matter alteration, indicated by the difference in VsFA0.2 values between the first and second scans, was associated with the degree of PDC recovery. In contrast, our results showed no correlation between the degree of PDC recovery and the change in mFAFM, which suggested that patients with PDC have multiple regions with white matter injury. Thus, we speculated that an overall white matter evaluation is a better indicator of recovery than a focal area evaluation in PDC.

These results suggested the existence of progressive microstructural changes in the white matter of patients with chronic PDC due to severe DAI. PDC was shown to cause microstructural alteration in white matter. The time-dependent change in VsFA0.2 values positively correlated with the degree of recovery from PDC, indicating that a time-dependent decrease in FA values could lead to a better outcome in PDC. These results are inconsistent with those of several previous reports, indicating that a time-dependent decrease in FA values was related to poor outcomes as assessed by cognitive function [18] and GOSE [11]. These contradictory findings may reflect the difference in PDC severity between studies; most of our patients had GOSE scores of ≤ 3 and showed subtle and minor clinical changes. Although significant improvements were revealed by the Kohnan scores, the changes were not significant based on GOSE scores, suggesting that improvements in our patients were lower than those reported in previous studies [11,18]. Conversely, these divergent findings might reflect the difference in the investigated phase. Compared to previous studies, we included patients with PDC in the extended chronic phase. Longitudinal FA value change may differ between the sub-acute (> 7.5 months) [2,9,14-15] and extended chronic phases (> 1 year) as well as between the chronic (approximately within 1 year) [11,17] and extended chronic phases (> 1 year). Moreover, there was a significant correlation between the VsFA0.2 value in the first scan and time-dependent change in the VsFA0.2 value. Our results suggested that a faster assessment of white matter damage is a better predictor of long-term outcome. The change in VsFA0.2 value strongly correlated with the initial VsFA0.2 value, which might indicate that an achievement of recovery from PDC correlated with less DAI. In patients with PDC, the degree of improvement was very small, as detected by the absence of change in GOSE scores. In patients with PDC, the induction of a severe inactive state due to brain injury might lead to secondary neurodegeneration that might be detected by MR–DTI. In the patients with severe whole brain damage, whole brain FA values are very low from the very early stage. Therefore, these patients might have small voxels that might contribute to reduced FA values. In contrast, long-term changes in FA values might result from inactivity in only patients with comparatively less brain damage. MR-DTI was able to detect microstructural changes. Based on our findings, we speculate that only patients with decreased FA values in multiple voxels over an extended time might show improvement from PCD. However, the underlying mechanism behind longitudinal alteration in white matter remains unclear. Thus, further longitudinal studies are warranted that combine MR-DTI with volumetric measurements and other neuroimaging modalities, such as magnetoencephalography. The potential of MR-DTI use as a prognostic tool needs further investigation in studies with larger number of subjects.

In conclusion, our results showed microstructural white matter changes occurring over time in patients with PDC and suggested that FA values are useful indices of white matter alteration in these patients. As a noninvasive modality, MR-DTI provides *in vivo* quantitative pathophysiological information. Tracking microstructural changes in white matter over time has the potential to measure neuroplasticity and repair after TBI and may eventually be utilized to monitor therapeutic responses, which requires further study.

Disclosures

Dr. Hiroaki Abe, Dr. Shimoji Keigo, Dr. Takeo Kondo, Dr. Takanori Kochiyama, Dr. Yoshihide Nagamine, Dr. Satoru Fujiwara, Dr. Yutaka Oouchida, and Prof. Shin-Ichi Izumi report no disclosures.

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