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Whole brain white matter changes revealed by multiple diffusion metrics in multiple sclerosis: A TBSS study

Yaou Liu[a,1,2,3], Yunyun Duan[a,1,2], Yong He[b,4], Chunshui Yu[a,5], Jun Wang[b,6], Jing Huang[a,7], Jing Ye[c,8], Paul M. Parizel[d,9], Kuncheng Li[a,*], Ni Shu[b,**]

[a] Department of Radiology, Xuanwu Hospital, Capital Medical University, Beijing 100053, China
[b] State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, Beijing 100875, China
[c] Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing 100053, China
[d] Department of Radiology, Antwerp University Hospital and University of Antwerp, Wilrijkstraat 10, 2650 Edegem, 8 Belgium

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ABSTRACT

Objective: To investigate whole brain white matter changes in multiple sclerosis (MS) by multiple diffusion indices, we examined patients with diffusion tensor imaging and utilized tract-based spatial statistics (TBSS) method to analyze the data.
Methods: Forty-one relapsing-remitting multiple sclerosis (RRMS) patients and 41 age- and gender-matched normal controls were included in this study. Diffusion weighted images were acquired by employing a single-shot echo planar imaging sequence on a 1.5 T MR scanner. Voxel-wise analyses of multiple diffusion metrics, including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) were performed with TBSS.
Results: The MS patients had significantly decreased FA (9.11%), increased MD (8.26%), AD (3.48%) and RD (13.17%) in their white matter skeletons compared with the controls. Through TBSS analyses, we found abnormal diffusion changes in widespread white matter regions in MS patients. Specifically, decreased FA, increased MD and increased RD were involved in whole-brain white matter, while several regions exhibited increased AD. Furthermore, white matter regions with significant correlations between the diffusion metrics and the clinical variables (the EDSS scores, disease durations and white matter lesion loads) in MS patients were identified.
Conclusion: Widespread white matter abnormalities were observed in MS patients revealed by multiple diffusion metrics. The diffusion changes and correlations with clinical variables were mainly attributed to increased RD, implying the predominant role of RD in reflecting the subtle pathological changes in MS.

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system that is usually accompanied by an impairment of motor, sensor, visual and cognitive functions [1,2]. In the past decade, advanced magnetic resonance imaging (MRI) approaches have been extensively used for the assessment of brain structural and functional changes and their association with clinical characteristics in patients with MS [1,3], which are important for our understanding the neuropathological mechanisms of the disease.

Diffusion tensor imaging (DTI) is one of the most promising techniques with regard to MS. It quantifies the amount of nonrandom water diffusion within tissues and provides unique *in vivo* information about the pathological processes that affect water diffusion as a result of brain microstructural damage. Fractional anisotropy (FA) and mean diffusivity (MD) are two important diffusion metrics for the analysis of DTI data: FA reflects the degree of directionality

* Corresponding author. Tel.: +86 13911099059; fax: +86 10 83198376.
** Corresponding author. Tel: +86 15801582918; fax: +86 1058804482.
E-mail addresses: asiaeurope80@gmail.com (Y. Liu), xiaoyun81.love@163.com (Y. Duan), yong.h.he@gmail.com (Y. He), csyuster@gmail.com (C. Yu), jun_wang@bnu.edu.cn (J. Wang), sainthj@126.com (J. Huang), jingye.2007@yahoo.com.cn (J. Ye), paul.parizel@ua.ac.be (P.M. Parizel), kunchengli55@gmail.com (K. Li), nshu55@gmail.com (N. Shu).
[1] Tel.: +86 10 83198515; fax: +86 1083198376.
[2] Dr. Yaou Liu and Dr. Yunyun Duan wish to be regarded as joint first authors.
[3] Dr Yaou Liu was supported by McDonald Fellowship from Multiple Sclerosis International Federation (MSIF) and Neuroradiology Schorlarship from European School of Radiology (ESOR).
[4] Tel.: +86 13552759185; fax: +86 1058804482.
[5] Tel.: +86 10 83198399; fax: +86 1083198376.
[6] Tel.: +86 15910906565; fax: +86 1058804482.
[7] Tel.: +86 10 83198291; fax: +86 1083198376.
[8] Tel.: +86 10 83198378; fax: +86 1083198259.
[9] Tel.: +32 3 821 35 32; fax: + 32 3 821 45 32.

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Table 1
Demographics and clinical characteristics of all participants.

Characteristics	RRMS ($n=41$)	Controls ($n=41$)	P values
Mean age (range) [years]	36.8 (18–58)	34.6 (18–58)	0.34
Gender (M/F)	13/28	13/28	>0.99
Median EDSS (range)	2.0 (1.0–6.0)	–	
Median disease duration (range) [months]	36 (3–204)	–	
Median TWMLL (range) [ml]	7.5 (0.1–45.0)	–	

Abbreviations: RRMS, relapsing-remitting multiple sclerosis; EDSS, expanded disability status scale; TWMLL, total white matter lesion loads.

of cellular structures (i.e., structural integrity) within the fiber tracts by measuring anisotropic water diffusion [4], while MD represents the diffusion in the noncolinear direction or free diffusion [4]. They are believed to provide a general, nonspecific measure of tissue alteration. More recently, the directional diffusivity metrics axial diffusivity (AD), λ_1 and radial diffusivity (RD), λ_{23} of white matter tracts have been hypothesized to more specifically differentiate axonal injury from demyelination in white matter tracts, respectively [5–7].

Previous DTI studies have focused on the FA and/or MD changes of the white matter in MS patients without consideration of the directional diffusivity metrics. Reduced FA and increased MD both within the focal white matter lesions and normal appearing white matter (NAWM) in MS were reported using various analysis methods [8–12]. However, the alterations for other diffusivity metrics (i.e., AD and RD) across the brain remain largely unclear. Until now, only one study has reported the alterations in AD and RD in regions with reduced FA in MS patients and found an increase in RD and a less pronounced increase of AD [10]. However, to date, there is no report of the whole-brain white matter changes in MS patients by analyzing each of the component eigenvalues of the diffusion tensor.

To give a comprehensive view of the change patterns of the white matter in MS patients, we used the newly developed tract-based spatial statistics (TBSS) method [13] and multiple diffusion metrics (FA, MD, AD and RD) to systematically study MS-associated changes in white matter tracts across the whole brain. The TBSS method is a fully automated whole-brain analysis technique that uses voxel-wise statistics on diffusion metrics but simultaneously minimizes the effects of misalignment using a conventional voxel-based analysis method [13]. In recent years, TBSS has been increasingly used to study FA changes in cerebral white matter in MS patients [10,11,14,15]. However, the alterations of the diffusivity metrics (i.e., AD, RD and MD) and their clinical relevance have been seldom studied. Therefore, in our study we used a comprehensive analysis of multiple diffusion metrics for describing the white matter change patterns across the whole brain in MS patients.

2. Materials and methods

2.1. Participants

This study included 41 patients with clinically definite relapsing-remitting multiple sclerosis (RRMS) [16] (28 females; mean age 36.8 ± 10.3 years) and 41 age-, gender-matched normal controls (NC) (28 females; mean age 34.6 ± 10 years) with normal findings on neurological examination and without history of neurological dysfunction. All subjects were assessed clinically by a single neurologist (J.Y, with 16 years of experience in neurology), who was unaware of the MRI results. None of the participating patients had been treated with related medications (e.g., corticosteroids and immunosuppressants) within 3 months before MRI scanning. The main demographic and clinical characteristics of the participants are reported in Table 1. Written informed consent was obtained from each participant and this study was approved by the institutional review board of Xuanwu Hospital.

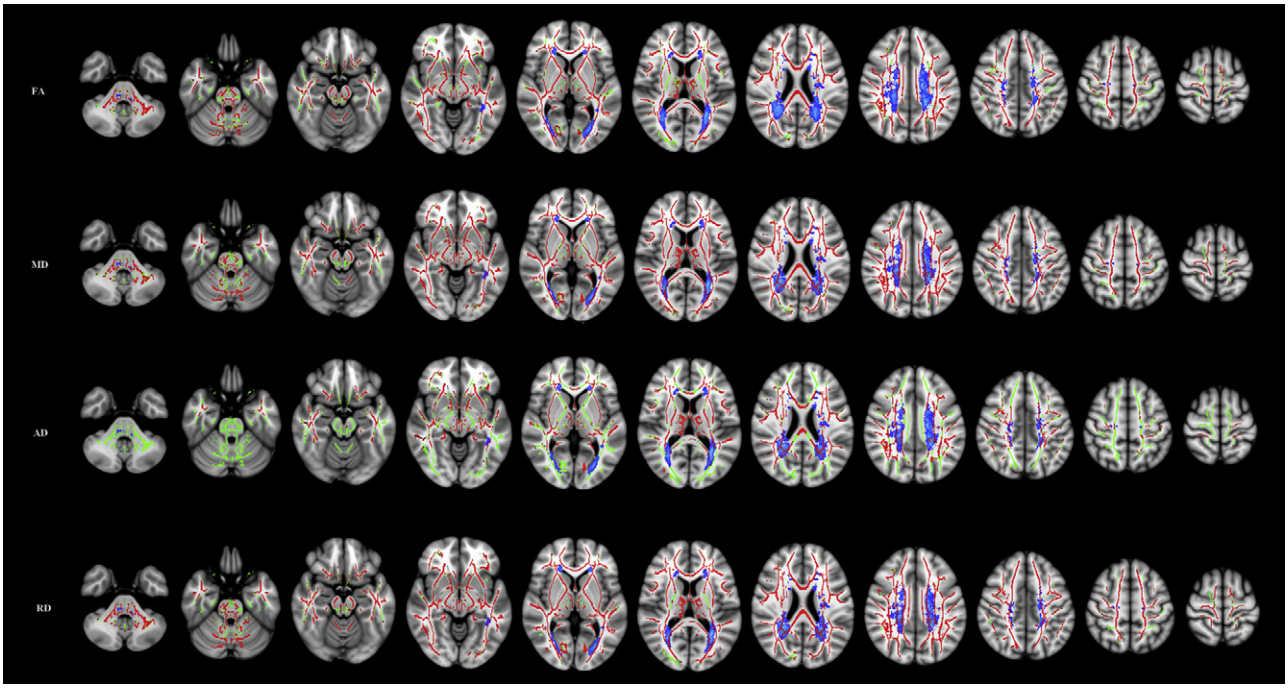

Fig. 1. TBSS results of FA, MD, AD and RD images between controls and MS patients. Green represents mean FA skeleton of all participants; red represents regions with decreased FA (1st row), increased MD (2nd row), increased AD (3rd row) and increased RD (4th row) in MS patients ($P<0.05$, FWE corrected for multiple comparisons); blue represents mean lesion probability distribution thresholded at 10%.

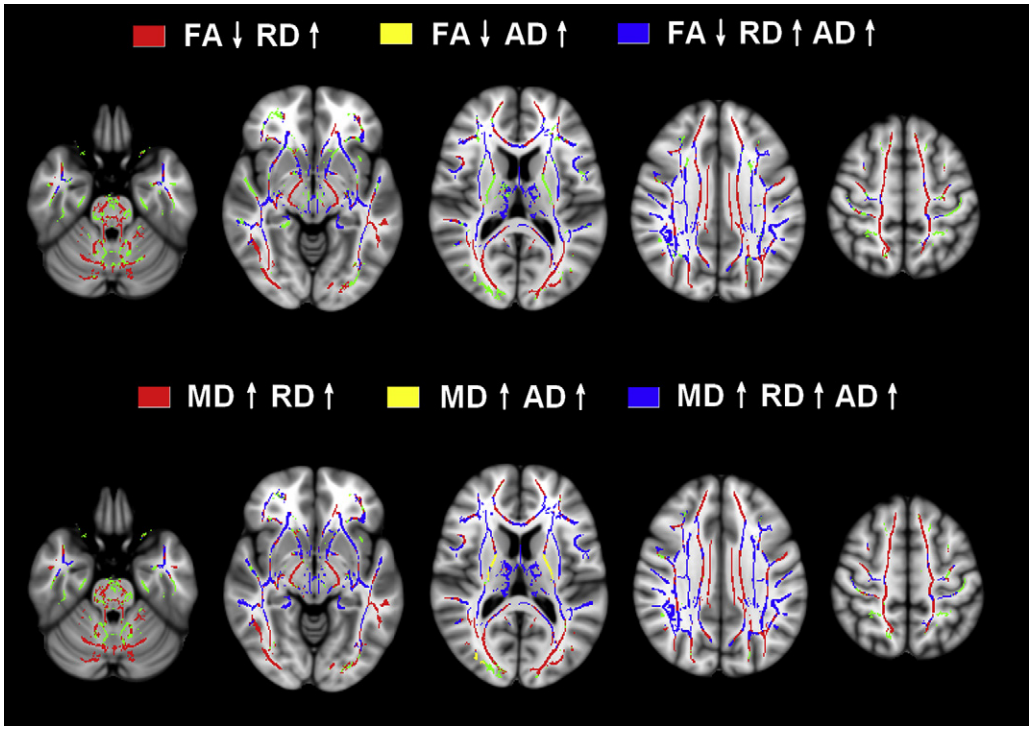

Fig. 2. Axial representations of overlapping white matter areas for decreased FA with increased RD and increased AD (top) and for increased MD with increased RD and increased AD (bottom) in MS patients. It depicts different change patterns of diffusion metrics in white matter skeletons in MS.

2.2. Data acquisition

All participants were imaged with a 1.5 T MR unit (Sonata; Siemens Medical Systems, Erlangen, Germany). The brain was imaged by using the following sequences with an identical field of view (240 mm × 210 mm), number of axial slices (30), slice thickness (4 mm) and inter-slice gap (0.4 mm): (a) T2-weighted turbo spin echo (repetition time (TR)/echo time (TE) = 5500/94 ms; number of excitation (NEX) = 3; echo train length = 11; matrix = 256 × 224), (b) T1-weighted spin echo (TR/TE = 650/6 ms; NEX = 3; matrix = 256 × 224), and (c) spin-echo single-shot echo planar (TR/TE = 5000/100 ms; NEX = 10; matrix = 128 × 112). A total of seven image sets were acquired: six with noncollinear diffusion-weighting gradients and *a*, *b* value of 1000 s/mm^2 and one without diffusion weighting.

2.3. Data preprocessing

First, eddy current distortions and motion artifacts in the DTI dataset were corrected by applying affine alignment of each diffusion-weighted image to the b0 image, using FMRIB's Diffusion Toolbox (FDT) (FSL 4.1.4; www.fmrib.ox.ac.uk/fsl). After this process, the first volume of the diffusion data without gradient applied (i.e., b0 image) was used to generate a binary brain mask with the Brain Extraction Tool. Finally, DTIfit was used to independently fit diffusion tensor to each voxel. The output of DTIfit yielded voxelwise maps of FA, MD, AD and RD for each subject.

2.4. Tract-based spatial statistics (TBSS)

Tract-based spatial statistics of FA images was carried out using TBSS in the FMRIB software library (FSL 4.1.4; www.fmrib.ox.ac.uk/fsl; for detailed description of methods, see Smith et al. [13]).

2.5. Statistical analyses

First, we calculated the mean diffusion measures (FA, MD, AD and RD) in the whole-brain white matter skeleton for each subject, and performed two-sample *t*-tests to compare the mean diffusion measures between the MS and NC groups. Age was considered as a covariate in the analyses.

Second, voxel-wise statistics in TBSS were carried out using a permutation-based inference tool for nonparametric statistical thresholding ("randomize", part of FSL). In this study, voxel-wise group comparisons were performed using non-parametric, two-sample *t*-tests between the MS and NC groups after controlling for the effect of age. The mean FA skeleton was used as a mask (thresholded at a mean FA value of 0.2), and the number of permutations was set to 5000. The significance threshold for between-group differences was set at $p < 0.05$ [Familywise Error Rate (FWE) corrected for multiple comparisons] using the threshold-free cluster enhancement (TFCE) option in the "randomize" permutation-testing tool in FSL. Similarly, group comparisons of MD, AD and RD images were performed, respectively.

Additionally, correlation analyses between each diffusion measure (FA, MD, AD and RD) and EDSS scores, disease durations and white matter lesion volumes of the MS patients were performed in a voxelwise manner with a mask of areas with group differences in each diffusion measures, respectively. We used a linear regression model to perform correlation analyses while treating age as a covariate of no interest, significant correlation was set at $P < 0.05$ (FWE corrected for multiple comparisons).

2.6. Measurement of white matter lesions

An experienced reader (Y.L, with 7 years of experience in neuroradiology) marked hyperintense WM lesions on the T2-weighted images manually, in line with common practice in brain imaging studies. Subsequently, WM lesion volumes were measured with an

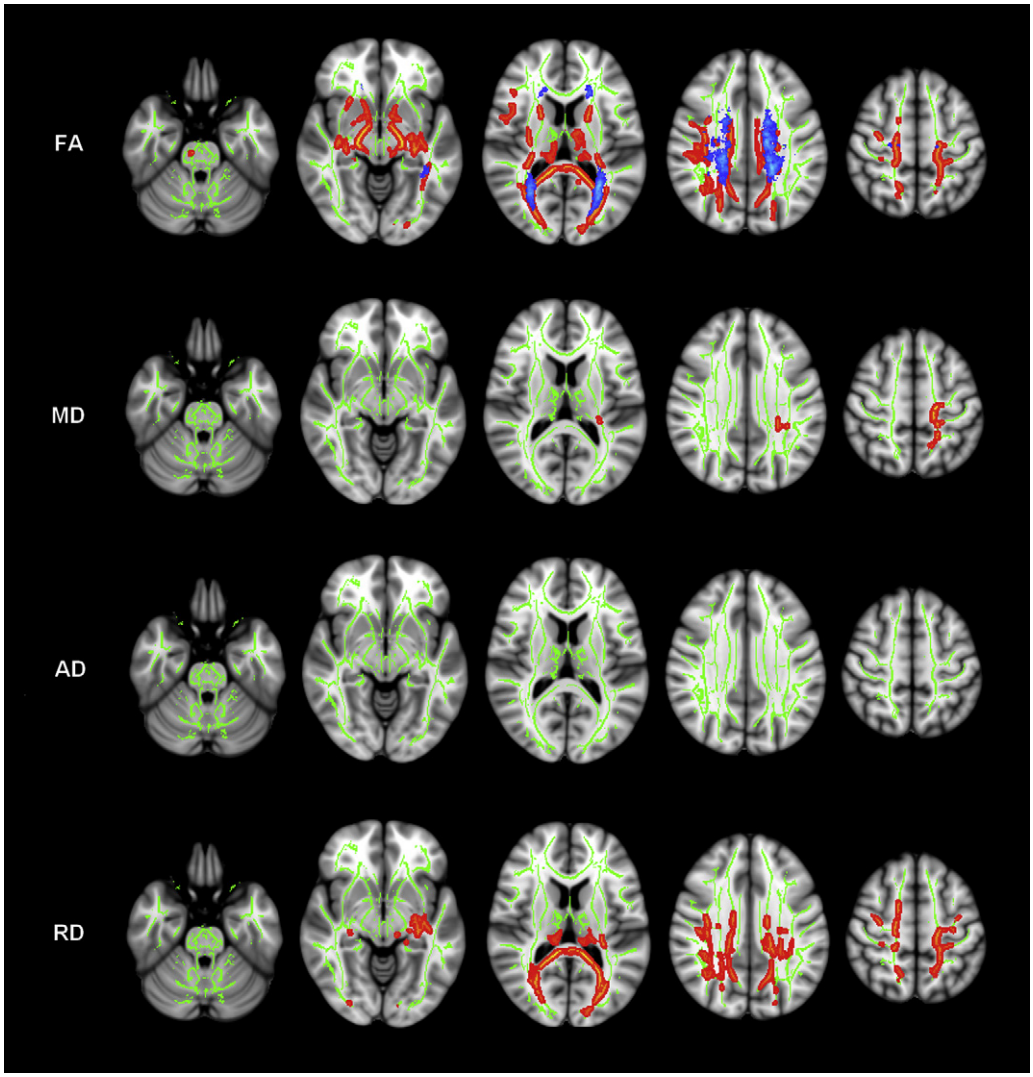

Fig. 3. TBSS results from linear regression analysis showing voxels (in red-yellow, thickened for better visibility) where an increase in EDSS scores was significantly correlated with FA decrease, MD, AD and RD increase (from top to bottom) at $P<0.05$ (FWE corrected for multiple comparisons). Green represents the mean WM skeleton of all the participants.

in-house-developed software. Each individual binary lesion mask was obtained by setting the signal within lesion boundaries to one and the remainder of the brain to zero.

3. Results

3.1. Group differences in multiple diffusion metrics

First, the mean diffusion metrics (FA, MD, AD and RD) in the white matter skeleton were extracted for each subject. Statistic analysis revealed significant group differences in all mean diffusion metrics of the white matter skeletons (for all measures: $P<10^{-8}$). Compared with the normal controls, MS patients had significantly decreased FA [MS (mean ± std): 0.40 ± 0.04; NC (mean ± std): 0.44 ± 0.02; 9.11%], increased MD [MS (mean ± std): $(8.35\pm0.57)\times10^{-4}$; NC (mean ± std): $(7.71\pm0.15)\times10^{-4}$; 8.26%], increased AD [MS (mean ± std): $(1.21\pm0.04)\times10^{-3}$; NC (mean ± std): $(1.17\pm0.02)\times10^{-3}$; 3.48%] and increased RD [MS (mean ± std): $(6.46\pm0.69)\times10^{-4}$; NC (mean ± std): $(5.71\pm0.18)\times10^{-4}$; 13.17%] in the white matter skeletons. The percentage means the extent of diffusion metric changes in patients relative to the mean diffusion metrics of controls.

Second, TBSS analyses revealed widespread white matter regions with abnormal diffusion changes (decreased FA, increased AD, RD and MD) in MS patients (Fig. 1). For AD and RD, different change patterns were found. Specifically, increased RD is involved in whole-brain white matter, while only several regions exhibited increased AD, such as inferior temporal and frontal gyrus, external capsule and the regions around the cerebral ventricles. Some white matter regions have increased RD, but no changes in AD, including cingulum bundles, superior longitudinal fasciculus, optic radiation, internal capsule, cerebral peduncle, brain stem and cerebellum. Some white matter tracts have increased both AD and RD, such as corpus callosum, corticospinal tracts and external capsule (Fig. 2).

3.2. Correlations between diffusion metrics in damaged areas and clinical variables

In MS patients, white matter regions with significant correlations between the diffusion metrics and the EDSS scores were identified, such as increasing EDSS and decreasing FA in the

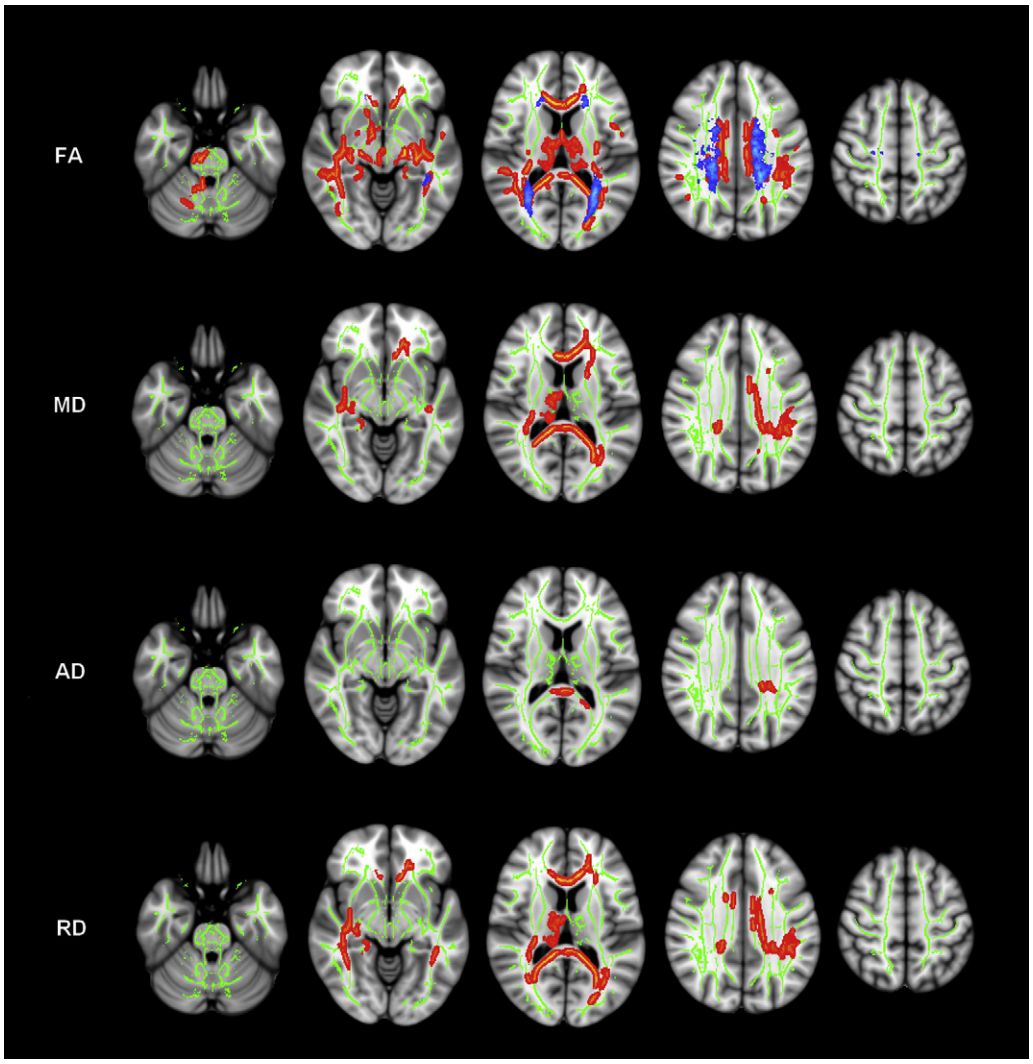

Fig. 4. TBSS results from linear regression analysis showing voxels (in red-yellow, thickened for better visibility) where an increase in disease durations was significantly correlated with FA decrease, MD, AD and RD increase (from top to bottom) at $P < 0.05$ (FWE corrected for multiple comparisons). Green represents the mean WM skeleton of all the participants.

splenium of corpus callosum, left cingulum bundle and bilateral corticospinal tracts (Fig. 3). Regions with significant negative correlations between the FA and the disease durations were identified in the whole corpus callosum and bilateral cingulum bundles (Fig. 4). Additionally, significant negative correlations between the FA and the lesion volumes were identified across the whole-brain white matter (Fig. 5). For diffusivity metrics, similar regions with clinical correlations were identified but to a much less extent.

4. Discussion

In this study, we determined global maps of the white matter changes in RRMS patients by measuring FA, MD, AD and RD across the brain, offering a comprehensive view of the landscape of white matter damage in RRMS patients. Compared with the normal controls, the MS patients exhibited significantly reduced FA and increased AD, RD and MD in widespread white matter regions, which suggests that MS is a disease that affects the brain globally. Increase of RD was not only much larger than increase of AD, but also distribute in more areas. Both the decreased FA and increased MD were mainly caused by increased RD, implying the predominant role of RD in reflecting the pathological changes and improving clinical–radiological correlations in MS. Furthermore, significant correlation between the diffusion measures and the clinical variables, such as EDSS scores, disease durations and white matter lesion loads were found in several white matter tracts. These correlations between white matter DTI metrics and clinical variables may suggest a role for DTI in monitoring disability and progression of the disease.

Through TBSS analysis, we found that MS is involved in diffusion changes of widespread white matter regions, including both focal lesions and NAWM, which is consistent with the findings of previous DTI studies using various analysis methods [1,15,17]. Early DTI studies used histogram analyses [12], region of interest (ROI)-based or conventional voxel-based analysis [8,10,11] to investigate the white matter changes in MS. In this study, we employed a more sophisticated and reproducible voxel-based approach TBSS to overcome the disadvantages of the ROI-based (low reproducibility) or conventional voxel-based methods (alignment and smoothing issues). The TBSS output for group differences overlaid onto the lesion probability maps is involved in nearly all the NAWM besides the lesion regions, which confirm and extend the results of previous TBSS studies of MS [10,11,14,15].

Through correlation analysis between the diffusion metrics in damaged areas and clinical variables in MS patients, we found significant correlations between EDSS scores and FA values in the

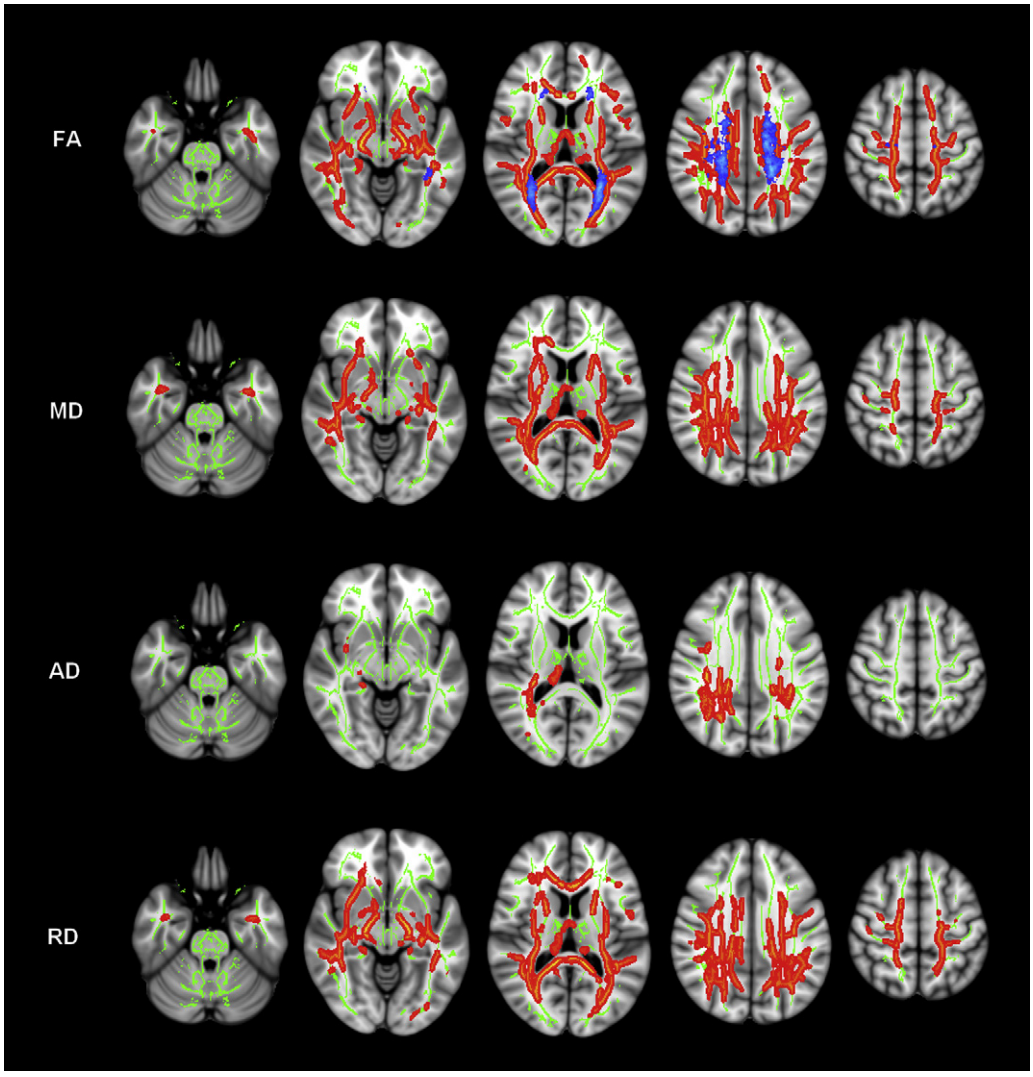

Fig. 5. TBSS results from linear regression analysis showing voxels (in red-yellow, thickened for better visibility) where an increase in whole brain T2 lesion load was significantly correlated with FA decrease, MD, AD and RD increase (from top to bottom) at $P < 0.05$ (FWE corrected for multiple comparisons). Green represents the mean WM skeleton of all the participants.

splenium of corpus callosum, left cingulum tract and bilateral corticospinal tracts. This finding is in line with a recent TBSS study of MS, where a correlation between EDSS scores and decreasing FA values in the corpus callosum and the corticospinal tract was reported [15]. Moreover, we identified a significant correlation between disease duration and FA in the whole corpus callosum, bilateral cingulum bundles, bilateral inferior longitudinal fasciculus and fornix. Compared with a previous study, where a significant correlation between the disease duration and FA values was localized in lesion areas [15], in our study, regions with significant correlations were not only confined to lesion regions, but also extended to NAWM. For the white matter lesion loads, we found a significant inverse correlation between lesion volume and FA values across the whole-brain white matter, suggesting FA is a sensitive measure for detecting direct damage caused by MS lesions but also degeneration in regions distant from focal damage (i.e., NAWM).

In the present study, we employed multiple diffusion indices rather than a single metric (such as FA) across the brain. MS patients had reduced FA and increased MD across the whole white matter, which is consistent with the findings of previous DTI studies in MS patients [10,11,14,15]. Different patterns of AD and RD changes in different fiber tracts may indicate that various WM pathological changes occur with MS. When assessing the contribution of AD and RD in those WM regions showing significantly decreased FA and increased MD, we found these were driven predominantly by increases in RD. The AD was either increased or unchanged in these regions. Changes in AD and RD have been suggested to relate to axon or myelin damage, respectively, in mouse models of MS [5–7]. RD, together with FA and MD, was shown to be a robust predictor of myelin content in postmortem human brain, prior to and after fixation [18]. Moreover, evidence from other studies suggested that increases in RD, but not AD in NAWM may reflect wallerian degeneration secondary to spatially remote lesions in connected white matter tracts [8,19]. The interpretation of AD variations is controversial in pathological conditions, because both increase and decrease have been reported [8,19]. In our study, the pathological increase of AD in MS is consistent with the recent findings from Roosendaal et al. [10]. One possible explanation is that severe decreases in axonal packing density (e.g., from a greater loss of myelin or axons) would lead to a global increase in extracellular water, resulting in larger RD increases and subsequent AD increases. The other plausible factors include fiber re-organization, increase in membrane permeability, destruction of intracellular compartments and glial alterations [8,19]. In the WM

regions showing associations of FA and clinical measures, we found that these were driven predominantly by increases in RD. This finding is in accordance with the reports from Giorgio et al. [15], which further attests to the predominant role of RD in both detecting subtle pathological damages and improving clinical–radiological correlations in MS. Widespread increase of RD may also suggest demyelination as a key factor among various pathological changes in RRMS. Furthermore, RD measured by TBSS technique may have great potential to be a MRI biomarker in monitoring the response to therapy in MS patients.

Several limitations should be addressed. First, the present study used a 4-mm slice thickness for the DTI data and a suboptimal DTI sequence with six diffusion-encoding gradient directions. Based on the results of this study, all of the clusters with diffusion changes in patients were more than 4 mm in length, and this result may minimize the limitations of slice thickness. Nonetheless, the analysis should be performed on the new datasets derived from optimal scanning parameters to further evaluate the reproducibility of our results. Second, the correspondence between axon and myelin damage and tensor diffusivity is still controversial. Thus, we cannot resolve the histopathological implications in AD versus RD changes and the clinical correlations in MS.

5. Conclusions

In this study, we generated a global map of the white matter changes in RRMS patients by analysis of multiple diffusion indices across the brain by means of TBSS method. Compared with normal controls, the MS patients showed significantly reduced FA and increased AD, RD and MD in distributed white matter regions. Although different diffusion change patterns were observed in different white matter tracts, the diffusion changes and clinical correlations were mainly contributed by increase of RD, implying the predominant role of RD in reflecting the subtle pathological changes in MS.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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