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# Diffusion tensor imaging and tract-based spatial statistics analysis in Friedreich's ataxia patients

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ABSTRACT

*Introduction:* Friedreich's ataxia (FRDA) is the most common hereditary ataxia and thinning of the cervical spinal cord is a consistent observation in Magnetic resonance imaging (MRI), although neuropathological examination in FRDA reveals neuronal loss in gray matter (GM) nuclei and degeneration of white matter (WM) tracts in the spinal cord, brainstem and cerebellum. Using diffusion-tensor (DTI) imaging and tract-based spatial statistics (TBSS) we tested the hypothesis that WM damage in FRDA is more extensive than previously described and probably involves normal-appearing WM.
*Methods:* This transversal study included 21 genetically confirmed FRDA patients and seventeen healthy controls that underwent structural MRI of the brain on a 1.5 T scanner. We quantify the severity of ataxia using SARA scale. DTI was performed and diffusion data were analyzed using FMRIB's Diffusion Toolbox in FSL 4.1 in order to identify Fractional anisotropy (FA) decreases in specific brain regions and also the mean, radial and axial diffusivities (MD, RD, AD).
*Results:* The greatest decreases in FA were in the left superior cerebellar peduncle, left posterior thalamic radiation, major forceps, left inferior fronto-occipital fasciculus and corpus callosum and had a significance level of $p < 0.01$. No significant correlation between FA, AD, MD and RD values and the clinical findings, SARA scores and genetic expansion was found.
*Conclusion:* DTI and TBSS techniques clearly demonstrate the extensive cerebral and cerebellar involvement in FRDA, partially explaining the clinical phenotype of the disease. Further studies are needed with larger samples to correlate clinical, genetic findings and ataxia scores.


## 1. Introduction

Friedreich's ataxia (FRDA) is the most common hereditary ataxia and in most patients is caused by a homozygous expansion of a guanine–adenine–adenine (GAA) trinucleotide repeat in intron 1 of the frataxin (X25) gene on chromosome 9q13 [1]. This mutation, which results in a transcriptional defect and deficiency of frataxin, a small mitochondrial protein, is the accepted cause of the entire complex clinical and pathological phenotype of FRDA. Although the function of frataxin is still partly debatable, there is general agreement that it is involved in cellular iron homeostasis and that a deficiency of this protein results in multiple enzyme deficits, mitochondrial dysfunction and oxidative damage [2]. The prevalence of the condition has been estimated in a variety of Western European populations at between 1:20,000 and 1:125,000. Since Nikolaus Friedreich's first description in 1863 of the ataxic syndrome that now bears his name, more detailed descriptions of the phenotype and more comprehensive neuroimaging findings have been reported [3]. FRDA is characterized by progressive gait and

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limb ataxia, poor balance and coordination, leg weakness, sensory loss, areflexia, impaired walking, dysmetria, dysarthria, dysphagia, eye movement abnormalities, scoliosis, foot deformities, cardiomyopathy and diabetes. The non-motor signs on FRDA patients are related to cognition, attention and memory deficits, impairment on visuoconstructive and visuoperceptual capacity, verbal fluency and mental reaction times [4]. Magnetic resonance imaging (MRI) was used in the diagnosis of FRDA before gene testing was available [4], and thinning of the cervical spinal cord was a consistent observation. Since Friedreich's seminal paper in 1876, many generations of medical students have learnt that FRDA is a primary disease of the spinal cord. Now, however, this is known not to be the case. While the clinical features of FRDA have never left any doubt about the existence of a cerebellar component, it was only in 1957 that the condition was confirmed to be associated with lesion of the dentate nucleus (DN) [4]. In addition, neuropathological examination in FRDA reveals neuronal loss in gray matter (GM) nuclei and degeneration of white matter (WM) tracts in the spinal cord, brainstem and cerebellum [3]. More recent MRI studies in FRDA patients revealed not only a vermis and hemispheric cerebellar atrophy but also peridentate white matter atrophy. MRI advanced techniques used to analyze cerebral and cerebellar alterations in FRDA patients also detected important findings in superior and inferior cerebellar peduncles, corticospinal tracts, inferior fronto occipital fasciculus and inferior longitudinal fasciculus [6,8,10].

Diffusion tensor imaging (DTI) is an advanced MRI image acquisition technique that can be used to investigate the fiber architecture of cerebral WM. Tract-based spatial statistics (TBSS) enables unbiased whole-brain quantitative analysis of fractional anisotropy (FA) of water diffusion in cerebral WM tracts in vivo [5].

## 2. Objective

To use diffusion-tensor (DTI) imaging and tract-based spatial statistics (TBSS) to test the hypothesis that WM damage in Friedreich's ataxia is more extensive than previously described and probably involves normal-appearing WM.

## 3. Methods

This transversal study was conducted between 2010 and 2013 and included 21 genetically confirmed FRDA patients who were regularly followed at the Ataxia Outpatient Clinic in the Movement Disorders Unit, Hospital de Clínicas, Federal University of Paraná. All the patients signed an informed consent form that had been approved by the Ethics Committee of The Federal University of Paraná. A molecular diagnosis was established by analysis of PCR amplification of the GAA expansion and the patient's inclusion criteria was the presence of more than 100 GAA repeats in both alleles of intron 1 of the Frataxin (X25) gene. All patients were evaluated by at least one neurologist, who filled up an ataxia protocol based on clinical data, family history, neurological examination and Scale for the assessment and rating of ataxia (SARA) score. Seventeen healthy individuals (8 women and 9 men; mean age 28 ± 11 years), without any personal or family neurological history of FRDA were included in our analysis as controls. In addition to being evaluated according to the clinical protocol, all the patients and 17 controls underwent structural MRI of the brain on a 1.5 T scanner (Magnetom, Avanto, Siemens, Germany). The MRI protocol included axial FLAIR and a sagittal T1-MPRAGE sequence. In addition a DTI acquisition was performed based on an echoplanar imaging sequence with the use of diffusion gradients along 30 noncolinear directions (TR/TE = 11,100/103, voxelsize = 2.2 $mm^3$, 1 b value = 0 and 30 b = 900 $s/mm^2$, readout bandwidth = 1640Px/Hz). All MRI images were reviewed by an experienced radiologist. For voxelwise diffusion modeling, diffusion data were analyzed using FMRIB's Diffusion Toolbox in FSL 4.1. After performing eddy current correction, brain extraction, a diffusion tensor model was fitted at each voxel and diffusion parameters: fractional anisotropy (FA), axial (AD), mean (MD) and radial diffusivities (RD) were calculated for all the subjects. Voxelwise statistical analysis of the FA data was carried out using TBSS, part of FSL. All the subjects' FA data were aligned into a common space by nonlinear registration using the FNIRT tool, which uses a b-spline representation of the registration warp field. The FMRIB58_FA standard-space template (voxelsize 1 mm [3]) was used as the target space in TBSS (http://www.fmrib.ox.ac.uk/fsl/data/FMRIB58_FA.html). Next, the mean FA image was created and thinned to produce a mean FA skeleton representing the centers of all tracts common to the group. Aligned FA data for each subject were then projected onto this skeleton, and the resulting data were fed into voxelwise cross-subject statistics for all voxels with FA ≥ 0.30 to exclude peripheral tracts with significant inter-subject variability and also to avoid partial volume effect with gray matter. The voxelwise analysis was done using permutation-based inference (5000 permutations) corrected for multiple comparisons (family wise error control) with a threshold-free cluster enhancement (TFCE) and a significance level of $p < 0.05$. Corrected TFCE p-value images were computed to enable identification of differences in the FA areas between FRDA patients and healthy control subjects. WM tracts were then identified using the John Hopkins University WM tractography atlas and the International Consortium for Brain Mapping DTI-81 WM labels atlas, both of which are available within FSL. After identifying FA decreases in specific brain regions (MD, RD and AD) were fed into the same TBSS analysis by using the same registration to standard space that was calculated for the FA. Voxelwise analysis was done by using permutation-based inference (5000 permutations) corrected for multiple comparisons (controlling the family wise error), and TFCE. The patients and controls data were compared using a parametric statistical test (T-test).

To correlate the FA, AD, MD and RD values with the clinical findings, SARA scores and genetic expansion and because of the small sample size, we computed Spearman's rho.

## 4. Results

Eight of the FRDA patients were male and thirteen female; mean age of onset was 13.8 years; mean disease duration was 27.7 years; and all patients presented with gait ataxia at the time of examination. All the patient data are shown in Table 1.

The significance maps, corrected for multiple comparisons and threshold free cluster enhancement, are shown in Figs. 1–3. They are color-coded (from red to yellow) with a significance level of $p < 0.05$, in which yellow corresponds to a higher significant result than red, showing areas where FA was significant reduced ($p < 0.05$). The maps are overlaid on the skeleton (green), the area where statistical analysis was done. TBSS showed multiple areas with a significant decrease in FA in the patients with FRDA, mainly in the superior cerebellar peduncles (Fig. 1), fornix, posterior thalamic radiation, forceps, inferior fronto-occipital fasciculus and inferior longitudinal fasciculus (striatum) – (Fig. 2), corpus callosum, corona radiata and corticospinal tracts (Fig. 3). The greatest decreases in FA were in the left superior cerebellar peduncle, left posterior thalamic radiation, major forceps, left inferior fronto-occipital fasciculus and corpus callosum and had a significance level of $p < 0.01$.

MD and RD values were significantly increased in the superior cerebellar peduncles and striatum. RD was also increased in the

**Table 1**
Patient's clinical signs and symptoms.

| Item | Number of patients (%) |
|---|---|
| Total number of patients | 21 |
| Males, n (%) | 8 (38.1%) |
| Consanguinity, n (%) | 3 (14.3%) |
| Family history, n (%) | 9 (42.9%) |
| Allele 1 (>500 repeats), n (%) | 15 (71.4%) |
| Allele 2 (>900 repeats), n (%) | 9 (42.9%) |
| Mean age of onset ± (SD) | 13.8 years (+/−8.7) |
| Mean disease duration ± (SD) | 27.7 years (+/−13.9) |
| Mean SARA score ± (SD) | 21.2 (+/−7.9) |
| Dysarthria, n (%) | 20 (95.2%) |
| Dysphagia, n (%) | 9 (42.9%) |
| Nystagmus, n (%) | 4 (19%) |
| Impaired smooth pursuit eye movements, n (%) | 5 (23.8%) |
| Action tremor, n (%) | 9 (42.9%) |
| Pyramidal signs, n (%) | 10 (47.6%) |
| Gait ataxia, n (%) | 21 (100%) |
| Limb ataxia, n (%) | 19 (90.4%) |
| Sensory loss, n (%) | 20 (95.2%) |
| Areflexias, n (%) | 21 (100%) |
| Scoliosis, n (%) | 10 (47.6%) |
| Foot deformity, n (%) | 14 (66.6%) |
| Diabetes, n (%) | 1 (4.8%) |

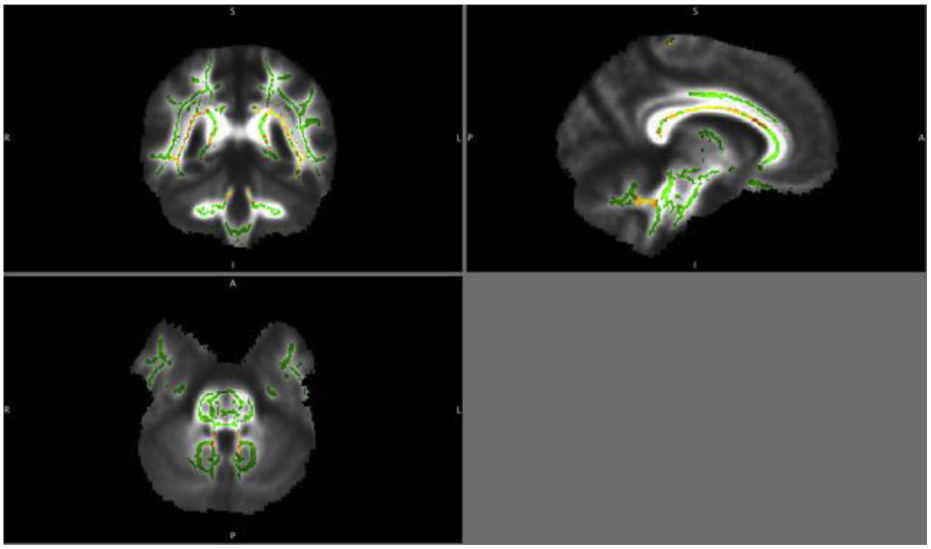

**Fig. 1.** Decrease in FA of superior cerebellar peduncles. The significance maps, corrected for multiple comparisons and threshold free cluster enhancement, are shown in Figs. 1–3. They are color-coded (from red to yellow) with a significance level of $p < 0.05$, in which yellow corresponds to a higher significant result than red, showing areas where FA was significant reduced ($p < 0.05$). The maps are overlaid on the skeleton (green), the area where statistical analysis was done. Fig. 1: Decrease in FA of left and right superior cerebellar peduncles and left and right inferior cerebellar peduncles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

inferior peduncles. AD was significantly decreased in the corticospinal tracts (Table 2 – Supplementary material).

No significant correlation was found between FA, AD, MD and RD values, clinical findings, SARA scores and genetic expansion.

## 5. Discussion

The combination of molecular genetic diagnosis and TBSS allows in vivo assessment of the structural changes associated with inherited ataxias [6]. It was with this in mind that this study, the first in Brazil to use MRI images, DTI and TBSS techniques to map white matter abnormalities in patients with FRDA, was conducted. The results show that FRDA neuroimaging abnormalities are found not only in the spinal cord or a few cerebellar regions such as the DN, as reported previously [7], but also in other WM tracts, providing an explanation for the heterogeneity and variability of FRDA signs and symptoms.

In this study, TBSS showed multiple areas with a significant decrease in FA in patients with FRDA, mainly in the superior cerebellar peduncles, fornix, posterior thalamic radiation, forceps, inferior fronto-occipital fasciculus and inferior longitudinal fasciculus (striatum), corpus callosum, corona radiata and corticospinal tracts. The most important FA decreases were found in the left superior cerebellar peduncle, left posterior thalamic radiation,

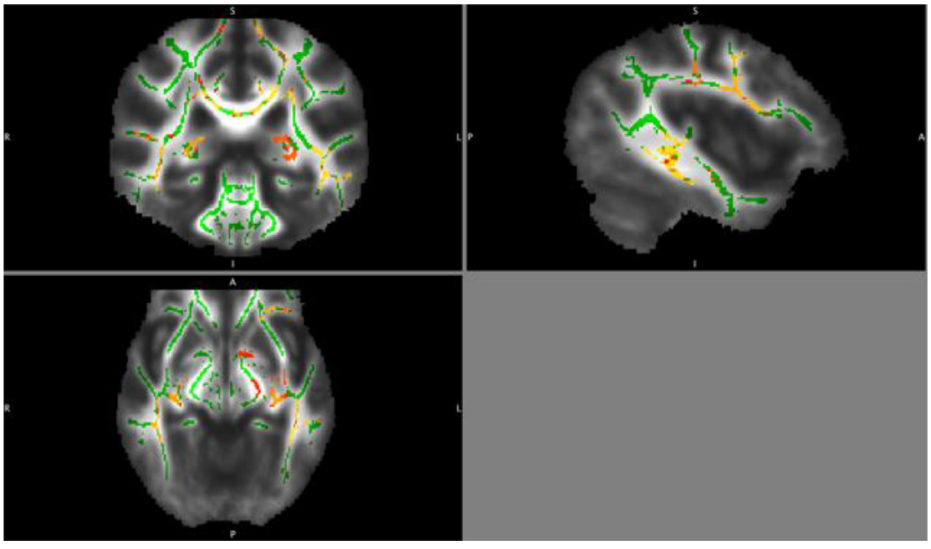

**Fig. 2.** Decreased FA in the inferior fronto-occipital fasciculus and inferior longitudinal fasciculus (striatum). The significance maps, corrected for multiple comparisons and threshold free cluster enhancement, are shown in Figs. 1–3. They are color-coded (from red to yellow) with a significance level of $p < 0.05$, in which yellow corresponds to a higher significant result than red, showing areas where FA was significant reduced ($p < 0.05$). The maps are overlaid on the skeleton (green), the area where statistical analysis was done. Fig. 2: left superior longitudinal fasciculus, corpus callosum, left and right superior corona radiata, left anterior thalamic radiation, internal capsule, left and right posterior thalamic radiation (including optic radiation), left external capsule, left and right retrolenticular part of internal capsule, inferior longitudinal fasciculus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

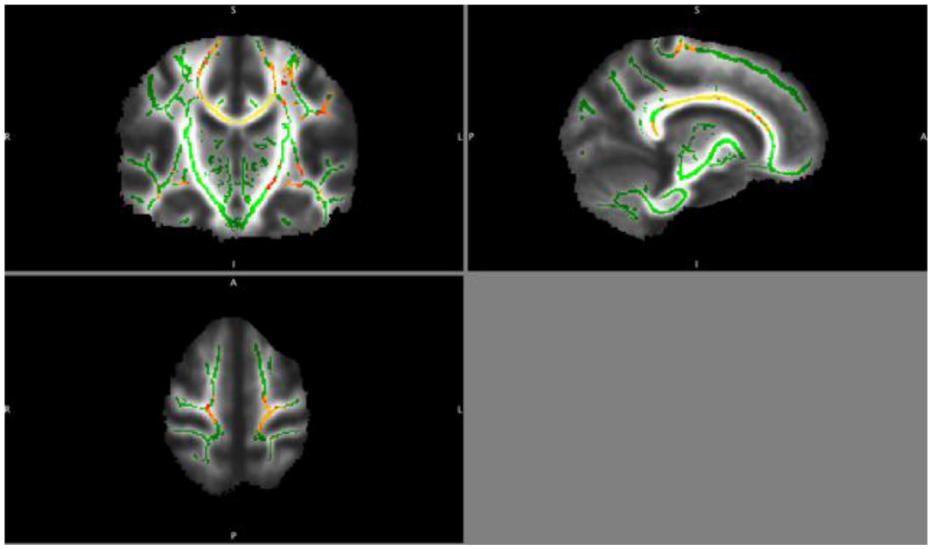

**Fig. 3.** Decreased FA in the corticospinal tracts. The significance maps, corrected for multiple comparisons and threshold free cluster enhancement, are shown in Figs. 1–3. They are color-coded (from red to yellow) with a significance level of $p < 0.05$, in which yellow corresponds to a higher significant result than red, showing areas where FA was significant reduced ($p < 0.05$). The maps are overlaid on the skeleton (green), the area where statistical analysis was done. Fig. 3: decreased FA in left and right corticospinal tracts. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

major forceps, left inferior fronto-occipital fasciculus and corpus callosum and had a significance level of $p < 0.01$. Della Nave et al. found decreased FA in the inferior and superior cerebellar peduncles, corticospinal tract, WM tracts in the right cerebellar hemisphere, right fronto-occipital fasciculus and inferior longitudinal fasciculus [8].

The Dentate Nucleus (previously described as being affected on FRDA) is directly linked to the superior cerebellar peduncle and also to structures like thalamus and corticospinal tracts [4]. In this study, all this regions seemed to be affected in FRDA patients suggesting that the cerebral-cerebellar circuit is completely involved in the pathology of the disease.

In our series the superior cerebellar peduncles and striatum showed decreased FA and increased MD at the same time. In 2008, Della Nave et al. described similar abnormalities in the superior cerebellar peduncles in a series of fourteen FRDA patients [8]. They postulated that this area was probably the most affected as it showed severe degeneration in FRDA patients compared with healthy controls.

The inferior fronto-occipital fasciculus is a ventral tract that connects the inferior and medial occipital lobe to the orbitofrontal cortex. It is probably involved in reading, attention and visual processing. The inferior longitudinal fasciculus is also a ventral tract and connects the occipital and temporal lobes. It is involved in object and face perception, reading, visual memory and language. We believe that the abnormalities found in our series, in particular in the inferior fronto-occipital fasciculus and inferior longitudinal fasciculus, play an extremely important role in the pathophysiology of the disease, demonstrating the extensive cerebral and cerebellar involvement in FRDA, and are probably associated with the non-motor signs in FRDA patients.

AD was significantly decreased in the corticospinal tracts. Our findings failed to confirm the results of the 2010 study by Della Nave et al., which described decreased FA and an increase in both AD and RD in the corticospinal tracts [6].

In an attempt to correlate the FA, AD, MD and RD values with the clinical findings, SARA score and genetic expansion, we carried out a descriptive statistical analysis and calculated Spearman's rho but failed to find any significant differences between patients and controls. Von Hohenberg et al. [9] studied nine FRDA patients and found a correlation between RD in inferior cerebellar peduncles and FARS score and GAA repeats, as well as a positive correlation between RD in the superior cerebellar peduncles and SARA score [9]. Della Nave et al. [8] described a correlation between the severity of the clinical deficit assessed with the IACRS score and decreased FA in the left superior cerebellar peduncle [8]. Although at 21 patients ours is the largest series of FRDA patients in a DTI and TBSS analysis, even this number is too small for a more detailed statistical analysis.

The discrepancies between our findings and reports in the literature probably exist because of the different MRI techniques and ataxia scales used (SARA, FARS and IACRS). Another limitation of our study and of an earlier study [10] is that no neurocognitive tests were applied, as a result of which we were unable to consistently investigate non-motor signs in FRDA and their correlation with our findings.

## 6. Conclusion

Recent functional MRI studies clearly demonstrate the extensive spinal cord, cerebral and cerebellar involvement in FRDA, partially explaining the clinical phenotype of FRDA.

DTI and TBSS techniques can improve our knowledge of the pathophysiology of FRDA and allow a more accurate analysis of cerebral and cerebellar involvement in the disease. Further studies are needed with larger samples to correlate clinical and genetic findings and ataxia scores.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2015.02.021.

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