Title page:

Title: Cognitive deficits in Friedreich ataxia correlate with micro-structural changes in dentatorubral tract

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Abstract:

Background: Atrophy of the dentate nucleus is one of the major neuropathological changes in Friedreich ataxia (FRDA). Neuroimaging studies demonstrated white matter (WM) degeneration in FRDA. In this study, we used advanced tractography techniques to quantitatively measure WM changes in the dentato-thalamic and dentato-rubral tracts, and correlated these changes with cognitive profiles of FRDA. We also analyzed diffusivity changes of the thalamo-cortical tract to assess whether neurological degeneration of WM extends beyond the primary site of involvement in FRDA.

Methods: Twelve genetically proven individuals with FRDA and fourteen controls were recruited. 60 directions diffusion tensor images were acquired. The WM bundles from the dentate nucleus were estimated using a constrained spherical deconvolution method and the diffusivity characteristics measured. The Simon task was used to assess cognitive profile of FRDA.

Results: The dentato-rubral, dentato-thalamic and thalamo-cortical tracts manifested significantly lower fractional anisotropy, higher mean diffusivity and increased radial diffusivity in FRDA compared with controls. There was no difference in axial diffusivity between the two groups. The mean and radial diffusivity of the dentato-rubral tract was positively correlated with choice reaction time, congruent reaction time, incongruent reaction time and Simon effect reaction time and negatively with the larger GAA repeat.

Conclusions: Significant changes in diffusivity characteristics were observed in the dentato-thalamic and thalamo-cortical tracts, suggesting extensive WM degeneration and affected WM structures in FRDA. Correlation of WM changes in the dentato-rubral tract with the cognitive assessment suggested that this tract is an important contributor to cognitive disturbances in FRDA.

Key words: Friedreich's ataxia, dentato-rubral tract, dentato-thalamic tract, dentate nucleus, diffusion MRI

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Conflict of Interest

Dr. Hamed Akhlaghi reports no disclosures.

Mr. Johnson Yu reports no disclosures.

Dr Louise Corben reports no disclosures.

Associate Professor Nellie Georgiou-Karistianis reports no disclosures.

Emeritus Professor John L. Bradshaw reports no disclosures.

Professor Elsdon Storey has received honoraria (payable to his institution) from Pfizer for lecturing on non-drug related issues at an education course. He is a co-investigator on an NIH trial for which Merck is providing active drug (aspirin and placebo).

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Introduction:

Friedreich's ataxia (FRDA), the most common hereditary ataxia, manifests with sensory and cerebellar ataxia, areflexia and sensory deficits, commencing late in the first decade or early in the second decade of life [1]. The underlying cause in 98% of affected individuals is homozygosity for an expansion of a GAA trinucleotide repeat in intron 1 of the *FXN* gene on chromosome 9, which results in decreased production of the iron homeostasis protein, frataxin, with subsequent mitochondrial iron accumulation and cell death [2]. Neuronal loss and white matter degeneration have been observed in peripheral sensory fibers, dorsal root ganglia and the spinal cord in several pathological studies in individuals with FRDA [3, 4]. From a gross anatomical point of view, atrophy of the cerebellum, brain stem and medulla has been reported in several imaging studies [5-7]. Previous functional and PET studies [8-11] have confirmed dysregulation and dysfunction of brain regions other than the cerebellum, such as the thalamus and basal ganglia, suggesting widespread functional or even structural changes in the brain in FRDA.

Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) technique that allows investigation of white matter degeneration and demyelination *in vivo* [12]. Diffusion-weighted imaging (DWI) is based on the measurement of Brownian motion of water molecules, reflecting the diffusion properties of water in each pixel of an image [12]. This technique allows visualization of the location and anisotropy (degree of inequality of diffusion in different directions) of the brain's white matter. The source of anisotropy in the brain is thought to be primarily a consequence of parallel orientation of myelinated axons, as evidenced by higher anisotropy in white matter (WM) than grey matter (GM), however, the axonal membranes may play a role as well [13]. In order to investigate axonal microstructure, the three dimensional diffusion profile needs to be fully characterized at each voxel in an image, the trajectories of axonal fibers must be estimated, and the density and orthogonal diffusivity values of the estimated white matter tracts computed [12, 14].

Atrophy of the dentate nucleus is a well-reported pathological finding in FRDA [15, 16]. Recent DTI studies on individuals with FRDA [17-19], have shown cerebellar as well as cerebral white matter degeneration. Della Nave et al. (14) observed a nearly symmetric decrease of fractional anisotropy (FA) in many WM tracts of the brainstem and cerebellum including the inferior and superior cerebellar peduncles, the corticospinal tract at the level of the medulla (pyramis) and in WM tracts of the right cerebellar hemisphere in individuals with FRDA. In the cerebral hemispheres, a decrease of the FA was observed only in the right occipitofrontal fasciculus and the inferior longitudinal fasciculus. They also reported mean diffusivity (MD) increases in FRDA patients compared

to controls in the deep cerebellar WM, the superior cerebellar peduncles, the posterior limbs of the internal capsule and retrolenticular area, bilaterally and the left central sulcus.

The dentato-rubral (from the dentate nucleus to the contra-lateral red nucleus) and the dentato-thalamic (from the dentate nucleus to the contralateral ventro-lateral thalamic nucleus) axonal bundles are two major tracts originating from the dentate nucleus and exiting the cerebellum via the superior cerebellar peduncle. To date there have been no reports examining the diffusivity characteristics of these two major neuronal bundles in FRDA. In this study, we examined the two major white matter bundles originating from the dentate nucleus using diffusion MR tractography, and calculated the diffusivity characteristics of these tracts in individuals with FRDA and in controls. In addition, we examined thalamo-cortical neuronal fibers located post-synaptic to the thalamic ventro-lateral nucleus in order to determine whether secondary white matter degeneration was present in FRDA. We hypothesised that the dentato-rubral tract is involved in the cognitive disturbances in individuals with FRDA and therefore we assessed the association of white matter degenerative changes of this tract with cognitive behaviour in individuals with FRDA. To further justify and validate our hypothesis, we also assessed the correlation of the diffusivity changes of the dentato-thalamic and thalamo-cortical tracts, which are mainly involved in motor coordination and performance, with cognitive behaviour in individuals with FRDA.

Methods:

Subject recruitment

Twelve right handed individuals with FRDA were recruited from Monash Medical Centre Friedrich Ataxia Clinic in Victoria, Australia. Fourteen age matched controls with no neurological disease were also recruited. This study is a part of the research project which involved volumetric and functional studies as well. The exclusion criteria for participants including structural analysis have been mentioned in the previous published article [20, 21]. All individuals with FRDA were homozygous for a GAA repeat in intron 1 of the *FXN* gene [22]. The GAA length was determined by PCR or Southern blot assay in the one laboratory to ensure consistency of results. Each individual's Friedreich Ataxia Rating Scale (FARS) score [23] was determined. All participants were required to complete the Beck Depression Inventory (BDI) [24] to screen for symptoms of depression, as depression can negatively influence speed of information processing, executive function, attention and memory [25, 26]. There was no significant difference in BDI scores between individuals with FRDA and controls (see Table 1 and Table 2).

All participants gave written consent to participate in the study, in accordance with the Declaration of Helsinki. The study was approved by the Royal Children's Hospital (HREC 26147 C) and Melbourne Health (National Neuroscience Foundation, HREC 2007.027) Human Research Ethics Committees.

MR imaging data acquisition and processing

All participants were scanned in a Siemens Tim Trio 3T scanner (SiemensAG, Erlangen, Germany) located at the Royal Children's Hospital, Melbourne, Australia. An eight channel standard head coil was used and each subject's head was held stationary by several foam pads to reduce involuntary head motion during the scanning session. The study was divided into two 45 minute scanning sessions with a 30 minute break. The MR session consisted of acquisition of a whole brain T1-weighted image, a whole brain T2-weighted image, two functional studies and one DTI study. During the first scanning session, a T1-weighted scan and one functional study were performed. In the second session, a T2-weighted, DTI and second functional study were carried out. The volumetric and functional studies are reported elsewhere [21, 27].

An anatomical T1-weighted image was acquired in axial orientation for each participant. Each T1-weighted image contained 512 contiguous 0.5 mm thickness slices with $0.875 \times 1.0 \text{ mm}^2$ in-plane resolution (field of view $224 \times 256 \text{ mm}^2$, acquisition matrix 256×256 , TR [time repetition] / TE [time echo] / inversion time / FLIP angle = 11.8ms / 2.4 ms / 900ms / 9°). An anatomical T2-weighted image was acquired in axial orientation

containing 28 slices with 4.8 mm slice thickness and $0.68 \times 0.68 \text{ mm}^2$ in-plane resolution (field of view $187 \times 220 \text{ mm}^2$, acquisition matrix 272×320 , TR / TE / FLIP angle = 5000 ms / 102 ms / 140°). DTI whole brain images were acquired using a double spin echo diffusion-weighted EPI sequence. The diffusion sensitizing gradient encoding was applied in 60 directions, with a b value of 2000 s/mm^2 . Ten DTI images were acquired with no gradient sensitizing (b value = 0 s/mm^2). DTI images consisted of 64 slices with 2.3 mm slice thickness and $2 \times 2 \text{ mm}^2$ in-plane resolution (field of view $128 \times 128 \text{ mm}^2$, acquisition matrix 256×256 , repetition time / echo time / FLIP angle = 7720 ms / 88 ms / 90°).

The diffusion images were corrected for head motion and eddy current distortion [28]. A mask of the brain was created for each participant's DTI image using the Brain Extraction Tool (BET) algorithm [29]. A diffusion tensor model was fitted for each participant to measure main eigenvalues and eigenvectors in order to calculate fractional anisotropy (FA) and mean diffusivity (MD) maps.

Tractography from the dentate nucleus (dentato-rubral and dentato-thalamo-cortical tracts)

To generate images of efferent tracts from the dentate nucleus, several way-point regions of interest (ROI) were required to constrain the tractography. A way-point is a reference point or area through which all fibers represented in the final tractography result must pass; any fibers not passing through the way point are discarded. For the dentato-rubral tract, the dentate nucleus was set as the origin of the tract, passing through the ipsilateral superior cerebellar peduncle as the way-point to reach the contra-lateral red nucleus as the termination of the tract. For the dentato-thalamo-cortical tract, the dentate nucleus was again set as the origin while the ipsilateral superior cerebellar peduncle and contralateral thalamic ventro-lateral nucleus were set as the way-points to reach the contra-lateral cortical motor area as the termination region of the tract.

The ROI mask of the dentate nucleus and the red nucleus were drawn manually on the T2-weighted image of each participant. The ROI mask of the superior cerebellar peduncle was drawn manually on the T1-weighted image of each participant [21]. The group results from a finger tapping functional study [20] were used to generate the ROI masks of the thalamic and motor cortex regions. All ROI masks in T1-weighted, T2-weighted and fMRI space were linearly registered to each participant's DTI space using a rigid body transformation algorithm (FLIRT, Oxford University, UK) [30].

To generate the efferent tracts from the dentate nucleus, the constrained spherical deconvolution method (Tournier et al., 2007) implemented in the MRtrix software package (www.brain.org.au/software) was used. The dentato-thalamo-cortical tract was then divided into the dentato-thalamic and thalamo-cortical tracts. The

dentato-rubral and dentato-thalamic tracts were considered as the primary output tracts of the dentate nucleus, and the thalamo-cortical tract was considered as the main post-synaptic tract of the dentate nucleus for further analysis.

For each tract, the FA, MD, axial diffusivity (AD) and radial diffusivity (RD) were calculated. Because there were no significant laterality differences in the diffusivity values, the mean values of the left and right tracts were used for further analysis.

Behavioural assessment

One day prior to the MR scanning session, each participant's baseline motor performance was measured using simple reaction time (SRT) and choice reaction time (CRT) tasks. Each participant was asked to sit directly in front of a laptop computer, 70 cm from the screen, with two button boxes located on non-slip mats on either side of the laptop. The stimuli for the SRT and CRT consisted of left ($\leftarrow \bullet \rightarrow$) and right ($\rightarrow \bullet \leftarrow$) pointing arrows. The stimulus appeared for 2500 ms or until a response was made. Three inter-stimulus intervals of varying durations, short (750ms), intermediate (1500ms) or long (3000ms), were pseudorandomly distributed throughout each stimulus block. During the SRT trials, participants were instructed to press a button with the right index finger only regardless of the arrow direction. This procedure was repeated for the left index finger for each participant. For the CRT trials, participants were instructed to press the right button box with the right index finger for stimuli that pointed to the right, and to press the left button box with the left index finger for stimuli that pointed to the left. For the SRT trial, there were 2 blocks of 36 arrow stimuli (72 stimuli in total, compromising one run). Equal numbers of left and right pointing arrows were presented in a pseudorandom order, and the order of the stimuli were changed for each run. The SRT run (consisted of two blocks) was performed two times with the right hand and two times with the left hand, resulting in 144 stimuli presented for each hand. The same stimulus presentation was used for the CRT with the participants completing two runs, resulting in 144 CRT stimuli in total.

The Simon task consisted of both congruent and incongruent stimuli. Congruent stimuli had either a rightward-pointing arrow located on the far right ($\rightarrow \bullet \rightarrow$) or a leftward pointing arrow located on the far left ($\leftarrow \bullet \leftarrow$). Incongruent stimuli comprised either a rightward pointing arrow located on the far left ($\rightarrow \bullet \leftarrow$) or a leftward pointing arrow located on the far right ($\leftarrow \bullet \rightarrow$). Participants were instructed to respond using a button box placed on either side of their body as quickly and accurately as possible, using the hand on the same side as the arrow pointed, regardless of the side of the screen on which it appeared. The stimulus duration was 2500 ms

with an inter-stimulus interval of 500 ms. Stimuli were presented in four consecutive experiments. Each experiment started with an 18s rest epoch, followed by 10 consecutive stimulus blocks of 12 stimuli each lasting a total of 36s, interspersed with 18s rest epochs, and ended with a final 18s rest epoch. Each experiment period was 9 minutes and 18 seconds. Each block of 12 stimulus presentations included two incongruent stimuli presented in a pseudorandom order as either the 4th, 5th or 6th stimulus during the 12 stimuli in each block. Thus, each experiment contained 100 (83%) congruent and 20 (17%) incongruent stimuli. Of the incongruent stimuli 10 were left pointing and 10 were right pointing. To avoid anticipation, the number of left incongruent stimuli preceded by a left congruent stimulus was the same as the number of left incongruent stimuli preceded by a right congruent stimulus, and vice versa for right incongruent stimuli. The Simon effect is a function of the higher cognitive load associated with the incongruent spatial stimuli resulting in greater reaction time. The Simon reaction time is the difference between the mean incongruent reaction time and mean congruent reaction time. Participants were instructed to respond to the arrow stimuli as quickly and accurately as possible. The reaction time in all tasks was defined as the period between the presentation of the stimulus on the screen and the time the participant pressed the response button box. All reaction times and errors during the SRT, CRT and Simon tasks were recorded using the E-prime software tool (Version 1.2; <http://www.pstnet.com/eprime.cfm>) and were analysed to calculate the mean and standard deviations of the reaction times and errors. The reaction times during the SRT, CRT and Simon tasks were correlated with each participant's micro-structural measurements in order to assess how behavioural performance in individuals with FRDA may be associated with characteristics of the WM tracts emanating from the dentate nucleus [31].

Statistical analysis

All statistical analyses were undertaken using SPSS version 15 (SPSS Inc., Chicago, IL). Multivariate analysis corrected for multiple comparisons (Bonferroni correction) was used in this study with p -value < 0.05 . To correct for age related brain changes, the age of the participants was set as a covariate in multivariate analyses. For the behavioural data a two-way ANOVA with the factors of Group (FRDA, Controls) and Response Type (SRT, CRT) was conducted to ascertain if there was a significant difference in response speed between the groups in either the SRT or the CRT task. A further two-way ANOVA with the factors of Group (FRDA, Controls) and Congruency (Incongruent, Congruent) was conducted to ascertain incongruency effects between groups.

Data from the individuals with FRDA were further analyzed for correlations between diffusivity, clinical and behavioural parameters, using Pearson correlation coefficients. The clinical parameters were age at disease onset (age in years at which clinical symptoms of FRDA were first noticed by the individual or parents); disease duration (age when tested minus age at disease onset); large and small GAA repeat size; and the FARS score. The researchers were not blind to the subjects or the results of cognitive tests.

Results:

Behavioural and volumetric data analysis

Despite being inherently slower in movement time, individuals with FRDA were not differentially impaired in their response to the CRT versus the SRT task compared with control participants. However, individuals with FRDA were differentially disadvantaged in response time to incongruent stimuli compared with congruent stimuli, indicating a disparate Simon effect between the two groups. There was a significant negative correlation between age at disease onset and the incongruency effect ($r = -0.588$, $p < 0.05$), however there were no other significant correlations between clinical and movement parameters (see Corben et al. [31] for description of results).

The mean brain volume of controls was $1490 \pm 110 \text{ cm}^3$ (95% CI: 1423.5 to 1556.5 cm^3) and was $1420 \pm 150 \text{ cm}^3$ (95% CI: 1319.2 to 1520.8 cm^3) in the individuals with FRDA. The mean cerebellar volume of controls was $146 \pm 11 \text{ cm}^3$ (95% CI: 139.3 to 152.6 cm^3) and was $131 \pm 15 \text{ cm}^3$ (95% CI: 122 to 140.1 cm^3) in the individuals with FRDA. There brain and cerebellar volume was not significantly differ between two groups ($p = n/s$)

Tractography from the dentate nucleus

In individuals with FRDA, axial diffusivity ($\lambda_{\parallel}$) was not significantly different from controls. The dentato-rubral, dentato-thalamic and thalamo-cortical tracts manifested significantly lower FA and higher MD and increased radial diffusivity ($\lambda_{\perp}$) in individuals with FRDA compared with controls ($p < 0.05$) (see Table 3, Figure 2).

The mean diffusivity and radial diffusivity of the dentato-rubral tract were positively correlated with choice reaction time ($r = 0.696$, $r = 0.715$ [$p < 0.05$]), congruent reaction time ($r = 0.622$ [$p < 0.05$], $r = 0.610$ [$p < 0.001$]), incongruent reaction time ($r = 0.736$, $r = 0.740$ [$p < 0.001$]), and Simon effect reaction time ($r = 0.732$, $r = 0.746$ [$p < 0.001$]) (see Figure 3 and Figure 5) and negatively with the larger *FXN* GAA repeat ($r = -0.622$, $r = -0.592$ [$p < 0.05$]) respectively (see Figure 4). No significant correlation was observed between other diffusion findings and behavioural or disease profile parameters.

Discussion

In this study, white matter micro-structural changes were observed in dentato-thalamo-cortical and dentato-rubral tracts in individuals with FRDA, demonstrating that extensive white matter structures are affected in the disease. We also observed that the white matter tract projecting from the motor nucleus of the thalamus to the motor cortex illustrated similar diffusivity characteristics and white matter micro-structural changes as in the dentato-thalamic and dentato-rubral white matter tracts. These findings illustrate the potential role of DWI and tractography for *in vivo* investigation and characterization of brain white matter atrophy in individuals with FRDA.

The dentato-rubral and dentato-thalamic tracts, the two main efferent tracts of the dentate nucleus, showed decreased FA and increased MD in individuals with FRDA compared with controls. We measured diffusivity characteristics in these tracts similar to those reported in previous DTI studies [18, 19] namely demonstrated decreased FA and increased MD in the superior cerebellar peduncles. Our further analysis of the diffusivity changes in these tracts demonstrated that the main underlying abnormality was increased radial diffusivity, whereas axial diffusivity did not significantly differ in these two tracts between individuals with FRDA and controls. In the recent study performed by Della Nave and colleagues [17], increased axial and radial diffusivity was observed at the decussation of the superior cerebellar peduncles.

There may be several explanations for the increased radial diffusivity in individuals with FRDA.

Conventionally, increased radial diffusivity is considered a biomarker of demyelination [32]. In FRDA, there is no confirmed pathological report of central demyelination and this disease is categorised as one of axonal degeneration [15, 33]. Several pathological studies on peripheral sensory nerves, especially the sural nerve, confirmed loss of large myelinated fibers, frank myelin destruction and hypomyelination while the total number of axons per unit cross-sectional area was not reduced [4]. Fiber shrinkage and associated secondary paranodal demyelination is hypothesised for the prolonged visual evoked potential latency [34]. One possible explanation of increased MD in the dentato-rubral and dentato-thalamic tracts could be partial demyelination occurring centrally. Alternatively, increased extra-axonal space due to loss of large myelinated fibers can also result in increased MD, by allowing free diffusion of water molecules perpendicular to axons. Axonal membranes are also another source of anisotropy [13], and hence axonal degeneration in FRDA can further contribute to increased radial diffusivity in these tracts. The heterogeneity of white matter degeneration in individuals with FRDA warrants further immunohistochemical analysis of post-mortem brain samples or possibly magnetization transfer ratio (MTR) imaging studies to investigate the underlying neuropathology of increased MD in

individuals with FRDA. Interestingly, we did not observe an increase in axial diffusivity of the white matter tracts in individuals with FRDA as recently reported by Della Nave and colleagues [17].

Fibres originating from the dentate nucleus synapse in the ventro-lateral nucleus of the thalamus or the red nucleus. We measured in individuals with FRDA that the thalamo-cortical tract, which can be considered as a post-synaptic tract of the dentato-thalamic tract, manifested similar diffusion characteristics as those observed in both the dentato-rubral and dentato-thalamic tracts; that is, decreased FA and increased MD and radial diffusivity. The axial diffusivity of this tract did not significantly differ in individuals with FRDA compared with controls.

Pathological findings in the cerebrum are very limited in FRDA and are mainly restricted to the visual pathway and to the loss of Betz cells in the grey matter of the motor cortex [18]. Reduction in the number of functional fibers and nerve fiber depletion of the visual pathway, gliosis of the optic nerve and tract, cell depletion in the lateral geniculate nucleus and loss of myelin in the optic tract and radiation, have been reported [34].

Degeneration of the lateral corticospinal tracts at the level of the cervical spinal cord has been described [35]. Progressive degeneration of the corticospinal tract at the medulla has been established in several post mortem studies, suggestive of a “dying-back” phenomenon in FRDA [36, 37]. In Della Nave’s study [18], only an area of increased MD underlying the left central sulcus was reported. One explanation may be that micro-structural changes in the dentato-thalamic tract can trans-synaptically influence the thalamo-cortical tract and induce degenerative changes. In a post-mortem pathological study on individuals with FRDA, trans-synaptic neuronal atrophy of the dentate nucleus was observed [15]. Furthermore, in familial cortical cerebellar atrophy, depletion of Purkinje cells causes trans-synaptic degeneration in the dentate nucleus [15]. A recent DTI study on individuals with FRDA, reported increased radial diffusivity in the inferior fronto-occipital fasciculus and the corticospinal tracts bilaterally while axial diffusivity did not change in these structures [17].

The dentato-rubral and dentato-thalamo-motor cortical tracts, as defined here, are mainly involved in motor coordination and movement. We failed to identify any correlation between behavioural disease parameters and diffusion characteristics of the dentato-thalamic and thalamo-cortical tracts in individuals with FRDA. However, mean diffusivity and radial diffusivity of the dentato-rubral tract showed significant positive correlation with choice reaction time, congruent reaction time, incongruent reaction time and Simon task reaction time, and negative correlation with the larger GAA repeat in individuals with FRDA. While the functional role of the red nucleus has not been fully elucidated, recent functional connectivity studies have confirmed involvement of the red nucleus in cognitive circuits related to executive control and detection of unexpected movement [38, 39].

Lack of a correlation between diffusivity characteristics of the dentatorubral tracts and simple reaction time, coupled with a strong positive correlation with the more challenging motor tasks (which require higher cognitive demands) could reflect engagement of the red nucleus in cognition. Defective connection between the dentate nucleus and the red nucleus suggested by DWI and tractography could therefore contribute to non-motor disease manifestations in individuals with FRDA. Further electrophysiological or functional studies are required to investigate the role of the red nucleus in individuals with FRDA. The significance of the larger GAA allele in FRDA has been controversial, however Keoppen et al. [40] argued the role of the larger GAA in clinical FRDA phenotype.

Fiber tracking is a technique to infer connectivity of the human brain and investigate micro-structural changes within specific tracts. Accurate segmentation and generation of a desired tract is a key methodological requirement. The dentato-rubral and dentato-thalamic tracts decussate at the midbrain to reach the contra-lateral red nucleus and thalamus respectively. Therefore, the constrained spherical deconvolution method was utilized to resolve crossing fibers of these two tracts in the midbrain [41]. In addition, we used a multi-modal MRI technique to define the waypoint region of interest in order to appropriately constrain the dentato-thalamo-cortical tracts. Changes of diffusion characteristics in the white matter tracts highlight the greater sensitivity of DTI in the assessment of the white matter degeneration in neurological diseases as compared with conventional MRI techniques such as T1 and T2 weighted imaging. The small sample size of this study limits the power of the study. A larger cohort and longitudinal study is required to confirm the findings of the study and investigate the micro-structural white matter changes over time as a potential biomarker of the disease severity.

In summary, we used tractography to investigate white matter micro-structural changes in individuals with FRDA. As expected from the underlying neuropathology of FRDA, we observed decreased FA and increased MD in the dentato-thalamic and dentato-rubral tracts. We also observed that the thalamo-cortical tract, being the post-synaptic projection of the dentato-thalamic tract, revealed white matter degeneration probably as a secondary post-synaptic degenerative response to pathological involvement of the dentato-thalamic tract.

Importantly, white matter changes in these areas were similar to changes observed in the primary regions of pathology in FRDA. We also demonstrated that mean diffusivity and axial diffusivity of the dentato-rubral tract correlated with the choice reaction time, congruent reaction time, incongruent reaction time and Simon task, suggesting cognitive deficits in individuals with FRDA secondarily to the involvement of the dentato-rubral tract. Our findings confirm that the neuropathological extent in FRDA is not confined to the cerebellum, and that other sub-cortical regions are affected.

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Table 1: Demographic and clinical characteristics of individuals with FRDA and controls.

	FRDA	Controls	P value
	Mean (SD)	Mean (SD)	
Number (males, females)	13 (6,7)	14 (5,9)	–
Age, years	35.8 ± 9.7	33.7 ± 7.7	n/s
Disease duration, years	15.7 ± 5.8	–	–
Age of onset, years	20.1 ± 6.5	–	–
Larger GAA repeat, mean	1000 ± 105	–	–
Smaller GAA repeat, mean	608 ± 243	–	–
FARS score, mean (range)	86 ± 15 (63-113)	–	–
BDI Score, mean	7 ± 7.9	3.7 ± 4.7	n/s
Simple reaction time	487 ± 81mSec	300 ± 29mSec	<0.001
Choice reaction time	559 ± 148mSec	327 ± 38mSec	<0.001
Congruent reaction time	673 ± 86mSec	448 ± 57mSec	<0.001
Incongruent reaction time	948 ± 203mSec	610 ± 132mSec	<0.001
Simon effect reaction time	275 ± 134mSec	162 ± 89mSec	<0.001

Table 2: List of medications of individuals with FRDA

Participant	Medications
1	Thyroxin
2	Multivitamin
3	Zoloft 200mg, Vit D, Salazopyrin 500mg, Glucovance, Aspirin 100mg
4	Vit E, Coenzyme Q10
5	Baclofen 2.5mg, Caltrate 600mg, Ramipril 2.5, Glicazide 30mg, Lantus, Novorapid, Oroxine 150mcg, Amiodarone 100mg, Endep 10 mg, Lipitor 10mg
6	None
7	None
8	None
9	None
10	Idebenone 4000mg
11	Coenzyme Q10
12	Vit E 500mg, Coenzyme Q10 400 mcg, Magnesium 520mg, Ditropan 5 mg, Fish oil 4000mg, Lysine 500mg

Table 3: FA, MD, $\lambda_{\parallel}$ and $\lambda_{\perp}$ of dentato-rubral, dentate-thalamic and thalamo-cortical tracts (P-value was corrected for multiple comparison <0.004).

		FRDA	Control	Mean diff	<i>P</i> -value
		mean (SD)	mean (SD)	(95% CI)	
Dentato-rubral tract	FA	0.24 (0.01)	0.36 (0.02)	-0.12 (-0.14, -0.10)	<0.001
	MD $\times 10^{-3} \text{mm}^2/\text{s}$	0.88 (0.05)	0.73 (0.06)	0.14 (0.09, 0.19)	<0.001
	AD $\times 10^{-3} \text{mm}^2/\text{s}$	1.08 (0.06)	1.01 (0.08)	0.06 (0.01, 0.12)	0.03
	RD $\times 10^{-3} \text{mm}^2/\text{s}$	0.77 (0.05)	0.59 (0.06)	0.18 (0.13, 0.23)	<0.001
Dentato-thalamic tract	FA	0.26 (0.06)	0.40 (0.03)	-0.13 (-0.17, -0.09)	<0.001
	MD $\times 10^{-3} \text{mm}^2/\text{s}$	0.81 (0.08)	0.68 (0.05)	0.12 (0.07, 0.18)	<0.001
	AD $\times 10^{-3} \text{mm}^2/\text{s}$	1.02 (0.06)	0.98 (0.06)	0.03 (-0.02, 0.08)	0.20
	RD $\times 10^{-3} \text{mm}^2/\text{s}$	0.70 (0.09)	0.53 (0.05)	0.17 (0.11, 0.23)	<0.001
Thalamo-cortical tract	FA	0.40 (0.02)	0.46 (0.02)	-0.06 (-0.08, -0.04)	<0.001
	MD $\times 10^{-3} \text{mm}^2/\text{s}$	0.61 (0.02)	0.55 (0.01)	0.05 (0.04, 0.07)	<0.001
	AD $\times 10^{-3} \text{mm}^2/\text{s}$	0.89 (0.02)	0.86 (0.03)	0.02 (0.01, 0.05)	0.03
	RD $\times 10^{-3} \text{mm}^2/\text{s}$	0.46 (0.02)	0.39 (0.01)	0.07 (0.05, 0.09)	<0.001

AD: axial diffusivity, RD: radial diffusivity

Figure 1: Left side: regions used to generate tractography in each participant's DTI. All regions were transformed into the DTI space using the FLIRT algorithm, functional mask of motor activation during simple finger tapping (A), functional mask of thalamic activation during simple finger tapping (B), manually drawn mask of the red nucleus using a T2-weighted image (C), mask of the superior cerebellar peduncle in a T1-weighted image (D) and manually drawn mask of the dentate nucleus using a T2-weighted image (E). Right side: the dentato-rubral tract (F) and dentato-thalamo-cortical tract (G).

Figure 2: Diffusivity characteristics of the dentato-rubral, dentato-thalamic and thalamo-cortical tracts. (a) fractional anisotropy, (b) mean diffusivity, (c) axial diffusivity and (d) radial diffusivity. * indicates significant difference between individuals with FRDA and controls ($P < 0.05$).

Figure 3: Correlation graphs between mean diffusivity of the dentato-rubral tract and choice reaction time (A), congruent reaction time (B), incongruent reaction time (C) and Simon effect reaction time (D) in individuals with FRDA.

* $p < 0.05$

** $p < 0.001$

Figure 4: Correlation graphs between larger GAA repeat and mean diffusivity (A) and radial diffusivity (B) of the dentato-rubral tract in individuals with FRDA.

* $p < 0.05$

Figure 5: Correlation graphs between radial diffusivity of the dentato-rubral tract and choice reaction time (A), congruent reaction time (B), incongruent reaction time (C) and Simon effect reaction time (D) in individuals with FRDA.

****** $p < 0.001$