Journal of the Neurological Sciences 343 (2014) 138–143

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Myelin paucity of the superior cerebellar peduncle in individuals with Friedreich ataxia: an MRI magnetization transfer imaging study

Louise A. Corben [a,b,e,*,1], Saman R. Kashuk [c,d,1], Hamed Akhlaghi [c], Sharna Jamadar [c,e], Martin B. Delatycki [a,e,f], Joanne Fielding [e], Beth Johnson [c,e], Nellie Georgiou-Karistianis [e], Gary F. Egan [c,e]

[a] Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Parkville 3052, Victoria, Australia
[b] Monash Medical Centre, Monash Health, Clayton, Victoria 3168, Australia
[c] Monash Biomedical Imaging, Monash University, Clayton, Victoria 3800, Australia
[d] College of Engineering and Science, Victoria University, Melbourne 8001, Victoria, Australia
[e] School of Psychological Sciences, Monash University, Clayton, Victoria 3800, Australia
[f] Department of Clinical Genetics, Austin Health, Heidelberg, Victoria 3084, Australia

ARTICLE INFO

Article history:
Received 29 December 2013
Received in revised form 23 May 2014
Accepted 26 May 2014
Available online 2 June 2014

Keywords:
Friedreich ataxia
Axonopathy
Magnetization transfer ratio
Myelin
Frataxin
Oligodendrocytes

ABSTRACT

The dentate nucleus (DN) is the major relay station for neural connection between the cerebellum and cerebrum via the thalamus, and is a significant component of the neuropathological profile of Friedreich ataxia (FRDA). We have previously shown that the size of the superior cerebellar peduncle (SCP), which links the DN to cortical and subcortical structures via the thalamus, is significantly reduced in individuals with FRDA compared to control participants. This study used magnetization transfer imaging (MTI) to examine and contrast the integrity of white matter (WM) in the SCP and the corpus callosum (CC) (control region) in ten individuals with FRDA and ten controls. Individuals with FRDA demonstrated a significant reduction in the magnetization transfer ratio (MTR) in the SCP compared to control participants. However, there was no significant difference between groups in MTR in the CC. When comparing regions within groups, there was a significant reduction in MTR in the SCP compared to CC in participants with FRDA only. We suggest that the reduction in MTR in the SCP may be indicative of lack of myelin secondary to axonal loss and oligodendroglial dysfunction in WM tracts in individuals with FRDA.

1. Introduction

Friedreich ataxia (FRDA), the most common of the hereditary ataxias, is a complex multisystem autosomal recessive condition, characterized by progressive ataxia, spasticity, weakness, absent lower limb reflexes, impaired vibration sense and proprioception, scoliosis, foot deformity and cardiomyopathy [1,2]. In about 93% of cases, FRDA is due to homozygosity for an expansion of a GAA trinucleotide repeat in intron one of *FXN* [3]. The remaining 7% are compound heterozygous for a GAA expansion and a point mutation or deletion in *FXN* [4]. The GAA expansion in intron one of *FXN* leads to reduced levels of the encoded protein frataxin. Whilst the exact role of frataxin is still not fully understood, there is a consensus that frataxin is a mitochondrial protein involved in iron sulfur cluster synthesis and iron chaperone activity [5–7].

The major sites of neuropathology in FRDA include the dorsal root ganglia (DRG), posterior columns, spinocerebellar tracts and corticospinal tracts of the spinal cord, and the dentate nucleus (DN) of the cerebellum [8,9]. The DN is the major relay station for cerebello-thalamic-cortical projections [10,11], via the superior cerebellar peduncle (SCP). Atrophy of the SCP in individuals with FRDA has been reported, and becomes more apparent in those individuals with earlier age of disease onset, greater disease severity and longer disease duration [12–14]. However, the source of the reduction in volume remains unclear [13].

Diffusion tensor imaging (DTI) has been widely used for *in vivo* investigation of microstructural white matter (WM) changes, axonal degeneration and demyelination [15]. A number of studies have mapped the extent of WM changes in individuals with FRDA using DTI [12,16,17]. In particular, these studies have noted WM changes in the brainstem, bilateral SCP, the cerebellar peri-dentate region, the optic chiasm and deep cerebral WM [12,16,17]. Disruption of WM tracts, linking the cerebellum to the cerebrum via the thalamus may compromise cerebellar access to more distal structures critical to both motor and non-motor tasks. Indeed, a recent study reported disrupted cerebello-cerebral connectivity in several distant cortical and subcortical areas including the supplementary motor area, frontal cortices,

* Corresponding author at: Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Parkville 3052, Victoria, Australia. Tel.: +61 3 8341 6228; fax: +61 3 8341 6390.
E-mail address: louise.corben@vcgs.org.au (L.A. Corben).
[1] Denotes equal first authors.

http://dx.doi.org/10.1016/j.jns.2014.05.057
0022-510X/

putamen, pallidum, cingulate cortex and hippocampus in individuals with FRDA [18]. Disruption to cerebello-thalamo-cerebral connectivity may underlie the non-motor symptoms reported in individuals with FRDA and indicates that the neuropathology associated with FRDA has a far wider effect on neural networks than previously thought [19–21].

Magnetization transfer imaging (MTI) is a magnetic resonance imaging (MRI) technique that utilizes an off-resonance magnetization pulse in order to measure variation in the exchange of protons between free water and macromolecules [22]. Neuropathological processes that result in a reduction of macromolecular bound protons lead to a reduction in the magnetization transfer ratio (MTR) value. MTI changes in brain imaging studies, expressed as a reduction in MTR value, are indicative of changes in the degree of axonal myelination and this is thought to be a more specific measure of myelination status than provided by DTI techniques [23]. Most of the published studies examining MTR values in the human brain have examined myelination in people with multiple sclerosis and have established MTR as a sensitive biomarker for clinical trials [24,25]. What remains elusive is an understanding of the pathological processes underlying WM changes in individuals with FRDA. MTI provides an important opportunity to semi-quantitatively examine myelination changes in major white matter tracts *in vivo*.

The aim of this study was to use MTI to examine the integrity of myelination (characterized by the MTR value) in WM regions connecting the cerebellum to cerebral structures. Given the previously documented reduction in size of the SCP, and the structural relevance of this region in terms of cerebro-cerebellar connectivity, we elected to examine the MTR values in the SCP and the CC in individuals with FRDA compared to control participants [13,14]. Consistent with previous findings [13,14], we hypothesized that individuals with FRDA would have reduced MTR in the SCP compared to control participants but that there would be no difference in the MTR value in the CC between groups. In addition, we hypothesized that the degree of MTR reduction in the SCP would correlate with clinical measures of disease severity.

2. Methods

2.1. Participants

Ten right-handed individuals homozygous for a GAA expansion in intron one of *FXN* (6 males) with a mean age of 37.7 years (SD = 11.2) participated in this study. An age- and sex-matched group of ten control participants (6 males) with no known neurological disorders and mean age of 38.2 years (SD = 7.9) also participated. A one-way ANOVA revealed no significant difference in age between the groups (f[1,18 = 0.013, p = 0.91). See Table 1 for clinical and demographic details of participants. Participants provided informed consent prior to inclusion in the study, and Ethics Committee approval was provided by the Monash University Human Research Ethics Committee, in accordance with the declaration of Helsinki.

2.2. Imaging data acquisition

MRI images were acquired using a 3-T Siemens Skyra scanner (Siemens, Erlangen, Germany) at Monash Biomedical Imaging, Victoria, Australia. T2 weighted images were acquired using a 32-channel head coil (TE = 8.4 ms, TR = 734 ms, flip angle = 30°, voxel size = $0.9 \times 0.9 \times 3.3$ mm^3, FOV = 230×172.5 mm^2, matrix size = 256×205, 46 slices). Images were acquired with and without saturation, using a magnetization transfer pulse placed before each slice-selective excitation.

2.3. MR image analysis

Before processing, all DICOM files were initially converted into ANALYZE format using FSL (FSL version 4.1.8, FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) to make a 3-D brain image of each scan. There were two 3-D brain images for each participant, one 3-D image acquired with the saturation pulse (MT image) and one 3-D image acquired without the saturation pulse (MT0 image). The ANALYZE 3-D images were skull-stripped using the brain extraction tool (BET) [26].

To calculate MTR, the MT image of each participant was first linearly registered to the same subject's MT0 image using a 7-degree transformation of the FMRIB's linear image registration tool (FLIRT) [27] to avoid stretching and skewing. The MTR for each voxel was then calculated using the following formula: MTR = 100*(MT0-MT)/MT$_0$ [22] (see Fig. 1).

Our primary region of interest (ROI) was the SCP. We also examined the CC as a control region in order to compare changes in MTR values between individuals with FRDA and controls and also between the ROI within each group. The boundaries of the SCP were defined according to the following visual criteria: the posterior border of the pons defined the inferior border, the CSF within the fourth ventricle defined the medial border, subarachnoid CSF defined the lateral border and the inferior colliculus defined the superior border. A 3-D mask was subsequently created manually for each individual in the central part of the SCP and the mean MTR was calculated for the masked area (see Fig. 2).

We compared the mean MTR values in the SCP and CC between individuals with FRDA and control participants using a two-way repeated measure ANOVA with the factors of group (FRDA, controls) and ROI (SCP, CC). Separate paired t-tests explored the significant group by ROI interaction by comparing MTR values in the SCP and CC for each group. The analysis was conducted using SPSS Statistics V.20 software (SPSS IBM Corporation, Armonk, New York).

Table 1
Mean (M), standard deviation (SD) and range (R) of group characteristics and screening measures for participants with FRDA and controls.

Group characteristics	FRDA			Controls			p
Male	6			6			–
Female	4			4			–
	M	**SD**	**R**	**M**	**SD**	**R**	
Age (y)	36.6	11.2	22–48	38.2	7.9	31–51	n/s
Age at Disease Onset (y)	20.1	7.6	8–34	–	–	–	–
Disease Duration (y)	16.5	7.1	6–25	–	–	–	–
GAA1	533	184	126–837	–	–	–	–
GAA2	950	216	462–1345	–	–	–	–
FARS Score	95	17.4	69–124	–	–	–	–
MTR SCP	49.3	1.9	46.5–52.6	54.9	2.6	51.8–60	$p < 0.001$
MTR CC	55.7	0.9	54.4–57.6	56.5	2.2	52.4–56.7	n/s
ΔMTR	6.4	2.2	3–9	1.6	2.7	−3.2–5.4	$p < 0.001$

GAA1 = *FXN* GAA repeat size of the smaller allele; GAA2 = *FXN* GAA repeat size of the larger allele; FARS = Friedreich ataxia rating scale; dashes indicate where descriptives were not applicable; MTR SCP = magnetization transfer ratio, superior cerebellar peduncle; MTR CC = magnetization transfer ratio, corpus callosum; ΔMTR = difference between MTR in SCP and CC; n/s = not significant.