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Research Report

# $^{1}$H MR spectroscopy in Friedreich's ataxia and ataxia with oculomotor apraxia type 2

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ABSTRACT

*Background and aim:* Friedreich's ataxia (FRDA) and ataxia with oculomotor apraxia type 2 (AOA2) are the two most frequent forms of autosomal recessive cerebellar ataxias. However, brain metabolism in these disorders is poorly characterized and biomarkers of the disease progression are lacking. We aimed at assessing the neurochemical profile of the pons, the cerebellar hemisphere and the vermis in patients with FRDA and AOA2 to identify potential biomarkers of these diseases. *Methods:* Short-echo, single-voxel proton ($^{1}$H) magnetic resonance spectroscopy data were acquired from 8 volunteers with FRDA, 9 volunteers with AOA2, and 38 control volunteers at 4T. Disease severity was assessed by the Friedreich's Ataxia Rating Scale (FARS). *Results:* Neuronal loss/dysfunction was indicated in the cerebellar vermis and hemispheres in both diseases by lower total N-acetylaspartate levels than controls. The putative gliosis marker *myo*-inositol was higher than controls in the vermis and pons in AOA2 and in the vermis in FRDA. Total creatine, another potential gliosis marker, was higher in the cerebellar hemispheres in FRDA relative to controls. Higher glutamine in FRDA and lower glutamate in AOA2 than controls were observed in the vermis, indicating different mechanisms possibly leading to altered glutamatergic neurotransmission. In AOA2, total N-acetylaspartate levels in the cerebellum strongly correlated with the FARS score ($p<0.01$). *Conclusion:* Distinct neurochemical patterns were observed in the two patient populations, warranting further studies with larger patient populations to determine if the alterations in metabolite levels observed here may be utilized to monitor disease progression and treatment.

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*Abbreviations:* FRDA, Friedreich's ataxia; AOA2, ataxia with oculomotor apraxia type 2; ARCA, autosomal recessive cerebellar ataxia; MRS, magnetic resonance spectroscopy; FARS, Friedreich's Ataxia Rating Scale; NAA, N-acetylaspartate; NAAG, N-acetylaspartylglutamate; tNAA, NAA+NAAG; Glu, glutamate; Gln, glutamine; tCr, creatine+phosphocreatine; tCho, choline-containing compounds; *myo*-Ins, *myo*-inositol; GSH, glutathione

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## 1. Introduction

Autosomal recessive cerebellar ataxias (ARCAs) are a genetically and clinically heterogeneous group of neurodegenerative diseases. The most common ARCA in the Indo-European population is Friedreich's ataxia (FRDA) (Anheim et al., 2009a; Campuzano et al., 1996), and recent epidemiological studies indicate that ataxia with oculomotor apraxia type 2 (AOA2) is the second most frequent ARCA identified so far (Anheim et al., 2009a; Le Ber et al., 2004). Clinically, AOA2 bears some resemblance to FRDA (age of onset, cerebellar signs, and peripheral neuropathy), leading to misdiagnosis in AOA2; however, these diseases belong to different categories of ataxias based on the function of the affected protein. Friedreich's ataxia is considered a progressive, metabolic ataxia, whereas AOA2 belongs to the group of ataxias that result from defects in the DNA repair machinery (Palau and Espinos, 2006).

In FRDA, the gene coding for frataxin is affected (Campuzano et al., 1996). Although its exact function is still unknown, frataxin is involved in iron homeostasis (Calabrese et al., 2005; Lodi et al., 2006; Pandolfo and Pastore, 2009). In patients, a GAA repeat expansion results in decreased protein transcription (Campuzano et al., 1996), which leads to excessive reactive oxygen species production, altered mitochondrial respiratory chain function and iron deposition in cardiac, muscular, spinal and cerebral tissue (Bradley et al., 2000; Calabrese et al., 2005; Koeppen et al., 2007). In particular, the increase in reactive oxygen and nitrogen species and the altered energy balance due to mitochondrial dysfunction are thought to lead to neuronal death (Calabrese et al., 2005; Lodi et al., 2006; Voncken et al., 2004). In the central nervous system, FRDA is characterized by the degeneration of large sensory neurons in the dorsal root ganglia, followed by degeneration of sensory posterior columns, spinocerebellar tracts, corticospinal motor tracts and atrophy of the large sensory fibers in peripheral nerves. In the brain, the dentate nucleus is largely affected and exhibits grumose degeneration and neuronal loss (Koeppen et al., 2007). Imaging studies reveal mild vermian atrophy in advanced stages of the disease (Ormerod et al., 1994). Olivopontocerebellar structures seem unaffected (Anheim et al., 2009a; Mascalchi et al., 2002; Ormerod et al., 1994), although alterations of the pons, medulla and inferomedial portions of the cerebellar hemispheres have been observed in some cases (Della Nave et al., 2004, 2008; Guerrini et al., 2009; Oppenheimer, 1979; Pagani et al., 2010).

AOA2 was recently shown to result from mutations in the senataxin gene (Asaka et al., 2006; Duquette et al., 2005; Moreira et al., 2004), likely causing a loss of function of the senataxin protein. Senataxin is thought to be involved in double strand break DNA repair and response to oxidative stress but also in transcription regulation via exon splicing (Airoldi et al., 2009; Suraweera et al., 2007; Suraweera et al., 2009). Patients with AOA2 have elevated α-fetoprotein (AFP) levels, a tumor marker (Anheim et al., 2009b; Le Ber et al., 2005), but without increased occurrence of cancer as observed in ataxia-telangiectasia. Oculomotor apraxia is not a systematic finding and appears to be present in about 50% of patients (Anheim et al., 2009b). Peripheral neuropathy has also been described in this disease (Asaka et al., 2006; Criscuolo et al., 2006; Gazulla et al., 2009; Le Ber et al., 2004, 2005; Tazir et al., 2009). In the brain, imaging studies revealed extensive vermian atrophy (Anheim et al., 2009a; Bernard et al., 2008; Le Ber et al., 2004; Nicolaou et al., 2008; Schols et al., 2008) which is stable shortly after the onset of the disease (Anheim et al., 2009a). Criscuolo et al. reported extensive Purkinje cell loss and mild fibrous gliosis in the cerebellar cortex, more severely in the vermis compared to the hemispheres (Criscuolo et al., 2006). The same authors reported a slight reduction in the size of the brain stem and spinal cord, and a lower number of neurons in the dentate nuclei.

Little is known about the metabolic status of the brain in both FRDA and AOA2. High field $^{1}$H magnetic resonance spectroscopy ($^{1}$H MRS) is a powerful tool for the non-invasive characterization of biochemical alterations in the brain because it enables the measurement of neurochemical profiles of 10–15 metabolites in localized brain regions in humans (Öz et al., 2010a; Tkáč et al., 2009). Few MRS studies of FRDA have been published to date (Franca et al., 2009; Guerrini et al., 2009; Mascalchi et al., 2002; Viau et al., 2005), reporting metabolite ratios (NAA/creatine, choline/creatine, *myo*-inositol/creatine, and glutamate + glutamine/creatine), while AOA2 has not been studied by MRS so far. Neurochemical levels measured by MRS in ARCAs can provide insights into the disease processes and could potentially provide non-invasive biomarkers of disease progression. Our goal was therefore to measure the neurochemical profiles of the pons, cerebellar hemisphere (encompassing the dentate nucleus), and vermis in patients with FRDA or AOA2 in order to identify disease biomarkers. We used single-voxel, short-echo time MRS at 4T to measure metabolite levels in the brain and investigated their correlation with scores on a validated ataxia rating scale (Fahey et al., 2007; Subramony et al., 2005). In addition, cerebrospinal fluid (CSF) was collected from a subset of the volunteers and $F_2$-isoprostanes were measured as an independent marker of oxidative stress (Greco et al., 2000).

## 2. Results

### 2.1. *Patient demographics and CSF data*

Table 1 shows the patient demographics. The FARS score ranged from 34 to 109 in patients with FRDA, and from 44 to 75 in patients with AOA2. Mean FARS scores of the two patient groups were comparable and significantly higher than controls as expected. $F_2$-isoprostane levels in CSF were comparable between patients and controls. In both groups of patients, CSF glucose concentration was higher than their respective control groups, while lactate levels were comparable between the groups. In controls, lactate concentration correlated with age ($r^2 = 0.8$, $p = 0.00001$).

### 2.2. *Atrophy*

Fig. 1 illustrates the sagittal and coronal $T_2$-weighted images acquired in a control, a patient with FRDA and a patient with AOA2. In patients with FRDA, the spinal cord was visibly atrophied. The cerebellar cortical structures appeared intact on MRI, but mild atrophy was indicated by the CSF content in the vermis voxel (Table 1). The pons was spared. In patients with