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TITLE OF THE THESIS:

Early onset hereditary neurodegenerative ataxias: a description of the pathologic modification pattern using advanced neuroimaging techniques and a neuropsychological study for the definition of paraclinical indicators in monitoring the disease progression and verification of treatment efficacy.

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SUMMARY OF THE THESIS

Early onset Hereditary ataxias represent a group of genetically and clinically heterogeneous conditions. The most important clinical presentation symptoms are gait and limb ataxia, dysarthria and eye movement impairment, in addition to other non-neurological involvement. Friedreich's ataxia (FRDA) is the most common autosomal recessive ataxia in terms of frequency and as a form with early onset.

Friedreich's ataxia (FRDA) is a progressive hereditary neurodegenerative condition caused by an autosomal recessively inherited GAA repeat in the FXN gene.

We performed a multidisciplinary overview of FRDA integrating it with an extensive cognitive and neuropsychological assessment. In addition, we used clinical measures and advanced tractography combined to functional MRI (fMRI) to explore white matter (WM) connectivity and motor dysfunction in a cohort of FRDA patients. This study is intended to provide a multidisciplinary overview of the clinical condition integrating it with a comprehensive MRI protocol on FRDA patients compared to controls. We have designed an ongoing longitudinal study in order to be able to describe the disease progression and to search for any potential biomarkers.

METHODS: Twenty one patients with a molecularly confirmed diagnosis of FRDA were recruited. The patients were aged >12 years of age and had an early onset and molecularly defined diagnosis of FRDA. All participants gave their written informed consent. All patients underwent a full clinical (neurological and ataxia scoring scales) and neuropsychological assessment (WISC III, WAIS R), specific tests for the attentive, executive and memory functions and MMPI A for the

personality assessment. Seventeen FRDA patients and 13 healthy controls underwent a neuroimaging study protocol on a 3T MRI scanner that included advanced neuroimaging DTI and fMRI. After the pre-processing, a nonlinear monoexponential model was used to calculate fractional anisotropy (FA), mean, radial and axial diffusivity (MD, RD, AD) maps. Non-parametric voxel-based permutations were performed on the WM maps regions of interest (ROI), considering age and sex via a general linear model (GLM) with critical threshold 0.05 while correcting for multiple tests. An fMRI sequence was acquired during a simple block design finger-tapping task. After a standard pipeline pre-process, intra- and intergroup GLM analysis were conducted, considering age and sex variables and also $p < 0.001$ threshold.

RESULTS: The cohort presents with an early age at onset (AAO) (10.6 ± 4.6, range 4-20). The F:M ration was 16:5. The age at the visit was .9 ±10.3 years (range 12-50) and disease duration was 16.3 ± 8.8 years (range 3-32). FRDA cohort presented as homozygous for the GAA repeat expansion in 96%. The mean GAA repeat expansion in the short allele was 653.7 ± 221 (range 170-946) that correlated negatively with AAO. In most cases the onset was with ataxia, gait clumsiness, and scoliosis, but few with asymptomatic cardiomyopathy and pes cavus. Vibratory sense was impaired in all the patients, with milder deficits in the other senses. Dysarthria was present in all patients. Muscle strength and tone were impaired in almost all the patients. One of them presented with a spastic ataxia with retained DTR. The pyramidal signs were present in 57%. Nystagmus was present in 61.9%. Half of the patients were wheelchair bound. Few patients developed diabetes mellitus. Cardiac involvement was registered in 76.2%, mostly presented as ventricular, septal or apical hypertrophy, but few with arrhythmias and valve prolapsed. The pulmonary system was involved in 28.6% of the patients

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(restricted pulmonary involvement, bronchial asthma and a positive history of ab ingestis pneumonia). Dysphagia was present in 80.9%. in addition, Helicobacter pylori positive gastritis and bowel disturbances were reported. The sensory component afferences was involved as three patients complained hearing loss (14.3%) and one of them visual field reduction (4.8%). Interestingly, our cohort presented with a wide of systemic conditions. Half of the patients had normal to superior IQ tot, followed by borderline presentation and few with mental retardation. The motor impairment (dysmetria, slowness) mostly affected the IQ tot scores. And finally only 10.5 % of the cohort presented with IQ tot values that were allocated within the mental retardation range. The neuropsychological profile assessment of the FRDA patients evidenced impairment in attentive functions at around 47.4% of the cohort, the executive function as phonemic (26.3%) and semantic fluency 21.5, and planning and spatial working memory (57.9%). The personality of the FRDA cohort included mostly concern with bodily symptoms, worries and anxiety, depressive symptoms. Few patients complained hypomania, bizarre behaviours and ideas, awareness of family problems, and very few did confirm hypochondria, low self esteem, anger management difficulties and also aggressive behaviour tendency. Here we report our experience of a cohort of FRDA patients after an extensive clinical and neuropsychological assessment.

The cohort included for the MRI study presented with the following clinical features: mean age at onset 10.65 ± 5.08 (range 4-20 years); F/M: 13/4; mean GAA expansion in the smaller repeat was 651,07 ± 234.39 (n=16) and one patients with a single base pair deletion and 170 GAA repeat. Mean age at assessment was 27.82 ± 10.51years (12-51), mean disease duration was 17.17 ± 8.43 (4-33). The mean age of the control group was 23 ± 4.83 years; F/M= 5/8. From both the voxel-based and ROI-based analysis altered FA and MD parameters were

consistently found in the following four Central Nervous System areas: cerebellar WM (superior, median and inferior peduncles), long sensory-motor pathways (corticospinal and lemnisceal systems, cerebral peduncles), major commissural fibres (splenium and tapetum of the corpus callosum), the thalamic and the optic radiations. The fMRI data were analyzed from 13 patients (mean age 30.05 ± 11.76 years) and 8 controls (mean age 24.5 ± 3.85 years). The finger-tapping task demonstrated intragroup activation of the controlateral motor cortex and the ipsilateral cerebellar cortex both in patients and healthy controls. Intergroup analysis demonstrated a consistent and significantly higher cerebellar cortex activation, in controls compared to the FRDA patients, in particular in the lobules V and VI.

Discussion: Here we present our experience of 21 FRDA affected patients. We show that a comprehensive MRI protocol consistently discriminates FRDA patients from controls. DTI changes in selected areas and BOLD signal in the ipsilateral cerebellar cortex in response to a simple motor task show strong intergroup discriminating power and may prove to be useful paraclinical disease markers. A longitudinal study is undergoing to explore the sensitivity of these indicators to disease progression.

Our results support the evidence that DTI and fMRI techniques may provide reliable quantitative biomarkers that could be used in longitudinal studies for prognostic and therapeutic clinical trials.

Further work is needed to identify which is the best MRI technique that is more sensitive to detect the most efficient biomarker of FRDA at different stages of disease. Probably, even a composition of MRI techniques might provide an appropriate array of measures suitable to complement the clinical assessment.

Acknowledgements

CHAPTER 1: INTRODUCTION TO HEREDODEGENERATIVE ATAXIAS

Early onset Hereditary ataxias represent a group of genetically and clinically heterogeneous conditions. The most important clinical presentation symptoms are gait and limb ataxia, dysarthria and eye movement impairment, in addition to other non-neurological involvement. The mode of inheritance may be autosomal dominant (AD), autosomal recessive (AR), X-linked or diaginic inheritance. Friedreich's ataxia (FRDA) is the most common autosomal recessive ataxia in terms of frequency and as a form with early onset, is followed by Ataxia-Telenagectasia (A-T), ataxia with Oculomotor Apraxia type 1 (AOA1) and type 2 (AOA2) (Ruano et al., 2014). FRDA is the object of this study.

History of Friedreich's Ataxia

FRDA was initially described by Nicholaus Friedreich. He described a cohort of 8 patients, members of 3 families. He published three papers in a cohort of 14 years (1863a, 1863b, 1863c, 1876, 1877). Later on, Brousse proposed to name this condition after Nicholaus Friedreich (Brousse, 1882). In 1890 Ladame presented a review on a very large cohort of patients (n=165) pointing out the difficulty of diagnosis (Ladame, 1890). There had been several controversies when diagnosing and reporting FRDA. The atypical forms of FRDA had been described since 1897, when Hodge described 3 siblings with increased DTR (Hodge, 1897), while Sherman (1934) described a spastic component in patients presenting with FRDA. Wilson firmly reported the diagnosis of FRDA with retained DTR (Wilson, 1940). But it was Bell and Carmichael (1939) that had introduced the idea that a loss of DTR was an early sign in FRDA. In 1981, Harding described a large cohort

of 115 patients (Harding, 1981) presenting an extensive elaboration of clinical presentation and course of FRDA patients and two years later (1983) presenting a refined classification and diagnostic criteria.

Epidemiologic studies

The global prevalence of AR ataxias ranges from 0.0-7.2: 100.000, with an average of 3.3: 100.000 (1.8-4.9: 100.000) and highlighting FRDA as the most frequent form (Ruano et al., 2014).

The geographical distribution of FRDA patients is situated mostly in Caucasians areas, rare in sub-Saharan African and very rare in the far East. There is the hypothesis of a founding mutation origin in Western Europe that appears to be to approximately 682 ± 203 generations ago corresponding to the Paleolythic period (Vankan, 2013).

The prevalence values of FRDA are variable, they range in Caucasian population 1:20.000 to 1:50.000. The highest prevalence is reported in Spain (1:21.000); secondly in North Ireland (1:23.000) and the third highest frequency is in France (1:43.000). In East Europe, the prevalence values are extremely small, and they record as follows: Russia 1:330.000; the Scandinavia records are in the range of the minimun FRDA prevalence with Sweden 1:420.000 and Finland 1: 750.000 (Vankan, 2013). The Italian prevalence was estimated in 3 major epidemiological publications that were conducted in North Italy with 1:83.000 (Leone et al., 1990),

South Italy 1:90.000 (Filla et al., 1981) and for the whole Italian patients 1:90.909 (Romeo et al., 1983) with an incidence of around 1:25.449 new cases per year. The carrier frequency is reported to be 60-1:100.

The observed FRDA distribution in Europe co-localizes with the a chromosomal marker gradient related to R1b that is believed to be present within West Europe. The chromosomal gradient is apparently due to the paleolithic migrations out of Franco-Cantabrian Ice age refugees and Neolithic migration entering West Europe with the advance of agriculture (Vankan 2013).

Genetics

In 1996 there was a publication by Campuzano et al (1996) reporting a linkage of FXN gene to the FRDA. The FXN gene was reported in the critical region for the FRDA locus on chromosome 9q13-q21.1. The gene contains 5 exons (1, 2, 3, 4, 5a) and two splicing form (exons 5b and 6), and also 4 introns. It codifies for a protein called frataxin, expressed in 2 isomers according to whether either exon 5a (210 amino acids) or exon 5b (170 amino acids) are transcribed. This protein apparently has a high level of expression in the heart, an intermediate level of expression in the liver, skeletal muscle and pancreas, and very low level in the spinal cord, cerebellum and cerebral cortex. Campuzano et al. reported, after a screening of 184 FRDA patients, three point mutations from three families of French, Spanish and Southern Italian origin (1996). They found that in 79 molecularly defined FRDA patients, including 5 with point mutations, there were GAA repeat expansion that appeared to be disease correalated. About 98% of FRDA had GAA repeat expansions. The sizes of GAA repeat were between 200 and 900, mostly containing 700-800 repeats. In the following year, 1997 the latter research group (Campuzano et al., 1997) published the functional work on FXN gene by reporting the reduction of FXN protein levels in FRDA patients and also localizing the protein within the cell giving rise to hypothesis on the probable function of the protein. FRDA appears to be due to a loss of function of the protein.

The FXN gene has its homologous in nematode, yeast and mouse (Campuzano et al., 1996; Koutnikova et al., 1997). The FXN gene exons 3-5 encode for the domain of the protein with the highest level of evolutionary conservation (Campuzano et al., 1996).

Themolecular weight of frataxin is a 18 kDa. The N-terminal epitope is not found within the mitochondria, suggesting that the protein goes through a pre-processing through a proteolytic cleavage into its mature form containing the C-terminal that enters the mitochondria. An important finding of the same group, was that the FXN protein was found in the inner mitochondrial membrane, suggesting a rationale for the impairment of cells with a high energy consumption level such as neurons. This finding derived from ransfected and non-transfected cells that were everexpressing FXN gene.

These important findings opened the door to a classification of FRDA under the umbrella of mitochondrial disorders, that appeared to be caused by a loss of function of a nuclear encoded protein (Campuzano et al., 1997) due to instability of normal repeats (NR) from which new expanded repeats are generated.

Pathological changes

The clinico-anatomic correlations in FRDA are represented as a combination of the developmental and degenerative processess in dorsal root columns and the sensory nerves, progressive destruction of the dentate nucleus (DN), atrophy of Betz cells and degenerationof the corticospinal tracts (Figure 1) (Koeppen and Mazurkiewitcz, 2013).

The frataxin protein is synthesized as a precursor of 210 aminoacids imported into the mitochondrion. The mature form is fully functional for cell survival.

Frataxin is an iron-binding and an aggregate formation protein. In addittion, frataxin apparently interacts with ferrochelatase, that is involved in the enzymatic reaction that leads to the final step of heme byosyntheisis by inserting iron into the porphyrin (Foury

and Cazzalini, 1997; Lesuisse et al., 2003). Lastly, frataxin appears to be linked to the mitochondrial aconitase, subunits of complex II of the respiratory chain and several chaperones (Bulteau et al., 2004; Gonzales-Cabo et al., 2005; Shan et al. 2007).

Frataxin binds iron and is required for the synthesis of iron-sulphur clusters and, thereby, for the synthesis of enzymes in the respiratory chain complexes I – III and aconitase (Pastore and Puccio, 2013).

The CNS involvement is viewed as a dying-back neuropathy of the following: long ascending and descending tracts of spinal cord, large sensory fibres of peripheral nerves, posterior sensory fibres of peripheral nerves. In addition, dentate nucleus and optic nerve tracts are involved.

Classification and diagnosis

The first effort in presenting diagnostic criteria was made from Geoffroy and collaborators (1976). They divided their 50 patients with a diagnosis of FRDA into 4 groups as follows: complete typical FRDA, incomplete atypical FRDA, atypical FRDA and no FRDA. They proposed some commendable diagnostic criteria, but since they were not fully applicable to all the FRDA patients due to the early onset of the cohort and the homogeneity of the study population (10 French-Canadian families from Quebec), were subsequently refined (Harding, 1983). She elaborated a classification from her previous works on 90 families with a total of 115 FRDA diagnosed patients (Harding, 1981).

Harding reported early onset (before 25 years of age) FRDA patients with presentation symptoms mainly limb and truncal ataxia, and absent DTR as consistent diagnostic criteria. In addition, she described other symptoms that would eventually develop during the disease progression such as dysarthria, pyramidal signs, and sensory impairment (sense of position and vibration). With the advent of the gene discovery, the suspected FRDA diagnosis was confirmed by genetic detection of pathogenic variants in the FXN gene in

both alleles. This allowed the confirmation of the cases that presented with atypical symptoms such as very early or very late onset, retained or brisk reflexes spasticity or limited progression (Durr et al., 1996; Filla et al., 2000; McCabe et al., 2000).

The clinical diagnosis of FRDA should be suspected in the presence of the combination of the following findings: progressive ataxia (gait and limbs), absent muscle DTR in LL (inconstant finding), dysarthria, an onset generally before 25 years of age and in an autosomal recessive inheritance manner. In addition, skeletal deformities (scoliosis, pes cavus), corticospinal tract (CST) involvement (LL weakness, Babinski), diabetes mellitus or glucose intollerance, hypertrophyc cardiomyopathy, optic atrophy or deafness. In addition, a series of instrumental examinations are important in order to complete the diagnostic process such as cerebral magnetic resonance (MRI) visual evoked potentials, motor and sensory nerve conduction velocities.

Clinical features

The residual amount of FXN protein is reported in the range of 4-29% in patients as compared to the levels of healthy controls, and that these levels were inversely correlated to the GAA repeat size of the short allele (Campuzano et al., 1997).

Early studies reported that the GAA repeat expansion is negatively correlated with the age at onset (AAO) and positively correlated with disease progression (Campuzano et al., 1997, Filla et al., 1996; Durr et al., 1996; Montermini et al., 1997; Lamont et al., 1997; Monros et al., 1997) suggesting a role of GAA repeat expansion in the FXN protein residual levels and subsequently in the disease severity implication. Similarly, positive correlation have been shown between GAA repeat expansion and incidence of cardiomyopathy.

The normal chromosomes have fewer than 33 GAA repeat expnasion. The smallest syntomatic GAA repeat expansion has been reported to be 44. FRDA patients present

usually with 600-900 GAA repeat expansion, with minimum and maximum pathogenic repeat expansion reported to be 70 and 1700, respectively (Pandolfo, 2001).

Regarding the AAO, there have been reported two ages of onset in FRDA. Most of the reports allocate the onset to be early, thus before the age of 25 years old. However, the late onset cases have been reported as well.

Generally, FRDA is classified in two different phenotypic representation, the classical and atypical phenotype. The latter incorporates the low-onset FRDA (LOFA) and very-low-onset FRDA (VLOFA), Acadian type and early onset FRDA.

Classical Phenotype.

The AAO is generally around puberty, reported to be 10.5 ± 7.4 years and 15.5 ± 8 years (Harding, 1981; Filla et al., 1990). The same authors reported a modal age at onset at around 10-12 years and 12-15 years, respectively. Harding (1981) described cases with a very early onset, before age of 5 years old, describing those cases of FRDA patients as the ones that rapidly deteriorate, while other associated early AAO with a larger size of the short allelle (GAAsr), a more severe phenotype, a faster progression of disability and higher incidence of non-neurological features such as cardiomyopathy, diabetes mellitus (DM) and pes cavus (Durr et al., 1996; Schols et al., 1997).

The presenting symptoms are usually associated with the gait and limb ataxia, clumsiness (Harding, 1981; Filla et al., 1990; Durr et al., 1996; Delatycki et al., 1999). Nevertheless, scoliosis and pes cavus might be the first symptom to be observed by the clinician, leading to a necessary further neurological assessment.

The neurological features are mainly represented by gait and lower limb (LL) ataxia. The ataxic signs are of mixed origin, such as spinocerebellar degeneration, peripheral sensory neuropathy, cerebellar and vestibular pathology (Corben and Delatycki, 2012). The upper limb (UL) ataxia is reflected in the impairment of the manual dexterity, difficulty on

handwriting, use of cutlery, washing, carrying objects. The LL ataxia is observed as impossibility or difficulty in the heel-to-shin task performance. Weakness and muscle wasting are usually noted later in life.

DTR are usually absent in the LL, and mostly absent in the UL. The extensor plantar reaction is an early pyramidal sign. The muscle tone is initially normal or reduced, progressively decreasing. While spasticity appears to be associated to LL and is responsible for associated symptoms, such as pain, contractures and discomfort. The sensory system is almost always involved, predominantly with the vibration and joint position sense impairment. Visual system appears to be involved, with mostly fixation instability, less commonly nystagmus and smooth pursuit movements impairment, decreased visual acuity and increased pattern visual-evoked potential latency (Durr et al., 1996; Fortuna et al., 2009). Dysarthria is a common and early symptom, while dysphagia develops in advanced stages and hearing loss appears to be a common but understated problem. The bladder hyperactivity is common in FRDA, on the contrary the bowel problems cause fewer problems (Parkinson et al., 2013).

The non-neurological features involve heart and pancreas (Figure 2). Hypertrophic cardiomyopathy or left ventricle hypertrophy (LVH) either concentric or asymetric septal hypertrophy (Goeffroy et al., 1974; Filla et al., 1990; Durr et al., 1996; McCabe et al., 2000). Some patient present with EKG alterations such as T wave inversion, ST-segment abnormalities or arrhythmias (Dutka et al., 1999; Bourke and Keane, 2011).

Diabetes mellitus (DM) is another non-neurological feature in FRDA that appears to be either due to insulin-resistance or decrease insulin secretion (Finocchiaro et al., 1988). The incidence of DM in FRDA cohorts was reported to be around 6-32% (1976; Harding, 1981; Filla et al., 1990; Schols et al., 1997; Delatycki et al., 1999; McCabe et al., 2000; Durr et al., 1996).

Importantly, skeletal deformities are present in FRDA. Scoliosis is a common feature, occasionally the presenting symptom. It is commonly mild when the AAO is relatively late (Parkinson et al., 2013).

Foot abnormalities are present in about 55-90% of the cases reported and consist in pes cavus, talipes equinovarus and pes planus as well (Harding, 1981; Geoffroy et al. 1976; Ackroyd et al. 1984; Filla et al. 1990; Durr et al. 1996; Schols et al. 1997; Delatycki et al. 1999; McCabe et al., 2000).

Non classical phenotype

There have been, however, described cases with AAO after 25 years of age. In particular, "Late onset FRDA" (LOFA) form has been reported to have a mean age at onset at around 28.8 years (range of 25.5–48) (Bhidayasiri et al., 2005; Arnold et al., 2006). LOFA appeared to have a milder phenotypic representation with retained LL DTR. The latest AAO reported have been allocated at the seventies (Galliman et al, 2008; Stolle et al., 2008) and to our knowledge at 82 years old (Alvarez et al., 2013). This form is usually classified as "Very late onset FRDA" (VLOFA) with a AAO after 40 years old. Another atypical phenotype is FRDA with retained reflexes, known as FARR (Klockgether et al., 1996; Coppola et al., 1999).

Another atypical clinical representation of FRDA was reported in from Richter et al. (1996) in a series of patients deriving from 10 Acadian families in Canada. Their clinical presentation overlapped the classical phenotype, but lacking cardiomyopathy and DM, and eventually displayed retained or increased DTR.

About 98% of FRDA patients have a GAA repeat expansion in a homozigous pattern (Campuzano et al., 1996). In addition 2-4 % of FRDA patients present with either FXN point mutation or deletion. The former mutations might be either truncating or missense, and appear to be responsible for a milder phenotype (Cossee et al., 1999). The latter

presentation is a rare presentation and usually associated with an earlier onset and a more severe phenotype (Zulke et al., 2004; Anheim et al., 2012).

NPS studies

In 1976, Geoffroy et al. (1976) mentioned decreased IQ as a clinical criteria for FRDA. However, despite slowed information processing in FRDA, cognition does not appear to be affected (Corben et al., 2006). From a study of 13 FRDA patients (Mantovan et al., 2006), the IQ profile was characterised by concrete thinking associated to impairment in concept formation and visuospatial reasoning. Other findings of De Nobrega et al. (2007) that related to impairment in phonemic and action fluency, led the authors to claim primary prefrontal or cerebello-prefrontal dysfunction. Further studies, conducted by Corben and collaborators (Corben et al 2010, 2011a, b, c), hypothesised the disruption of cerebro-ponto-cerebello-thalamo-cerebral loops to explain the cerebellar impairmet that were probably causative of the difficulty in accommodating unexpected movements, difficulty in the movement initiation without a direct visual cue, and impairments in the reaction time to incongruent stimuli. Sustained volitional attention and working memory is impaired in FRDA (Klopper et al., 2011). Lately, findings in 36 FRDA patients, confirmed motor and mental speed, conceptual thinking, verbal fluency, acquisition of verbal information, use of semantic strategies in retrieval and action naming deficits. These findings were suggestive of parieto-temporal dysfunction (Nieto et al., 2012). In summary, the cerebro-cerebellar circuits may be functionally important in FRDA, and the eventual interruption is to be regarded as causative in FRDA.

MRI studies

Magnetic resonance imaging (MRI) studies reflec the clincial features. MRI findings show cervical cord atrophy, posterior column atrophy. In early stages, there might be either no involvement of cerebellum or brainstem or minimal atrophy of the superior vermis and

medulla oblongata (De Michele et al., 1995). Additional MRI studies, such as VBM studies conducted by Della Nave et al., (2008) report symmetrical volume loss in the dorsal medulla, inferomedial cerebellar hemispheres, rostral vermis and dentate regions, which appeared to correlated with disease duration and severity. Another study, reported correlations between superior cerebellar peduncle atrophy and clincal severity, AAO and DD (Akhalagi et al., 2011).

Treatment in FRDA

There is no cure for FRDA. There have been many clinical trials that have tried different molecules (Fig. 3). Among them antioxidants have been attempted.

Coenzyme Q10 and vitamin E were used in association, demonstrating an improvement in ATP production for either 6 months (Lodi et al., 2001) and 47 months (Hart et al., 2005). The former findings were oriented toward ATP improvement in the heart and skeletal muscle; the latter confirmed these findings by reporting improvement in bioenergetics and in cardiac function. Conversely, another study high dosage of coenzyme Q10 and vitamine E controlled to low dosage coenzyme Q10 failed to demonstrate any intergroup differences in ICARS score (Cooper et al., 2008).

Idebenone, a short chain analog of coenzyme Q10, was thought lead to left ventricular hypertrophy reduction in some studies (Hausse et al., 2002, Buyse et al., 2003; Mariotti et al., 2003) but it was not confirmed by others (Lagedrost et al., 2011). Likewise, the neurological benefits of idebenone compared to placebo and measured by ICARS from a phase II clinical trial were reported by Di Prospero et al. (2007) and rejected by Lynch et al. (2010) conducted by a phase III study.

Controversial findings were reported in three case reports treated with intra muscle injection of thiamine (Costantini et al., 2013).

Iron chelators, as deferiprone, have been used in an open label study showing apparently reduction of iron in the dentate nucleus (DN) and neurological benefits (Boddaert et al., 2007). Consistently, intraventricular septum thickness reduction was observed from an open label study combining deferiprone and idebeone (Velasco-Sanchez, et al., 2011) but no ataxia score significant change was observed. Attempts to increase the levels of frataxin protein have included studies in cellular models trying different molecules such as hemin, butyric acid, and erythropoietin (Sturm et al., 2005; Sarsero et al., 2003). Clinical studies with erythropoietin as an open label study (Boesch et al., 2007) led to significant decrease levels of oxidative markers, while a six-months placebo-controlled study did not identify any clinical benefit (Mariotti et al., 2012).

An upregulation of FXN expression was tried by using histone deacetylase inhibitors (HDACi) (Herman et al., 2014) as Rai and colleagues (Rai et al., 2008) demonstrated how compound 106, HDACi analogue led to restored frataxin levels in heart and central nervous system (CNS) in FRDA mouse model. A phase I clinical trial of RG2833 was concluded (Gottesfeld et al 2013). Similarly, another molecule, class III HDACi led to increase in frataxin expression in FRDA cell and mouse models (Chan et al., 2013) while a clinical open label study reported findings of increase levels of FXN transcript approximately equivalent to the asymptomatic carriers (Libri et al., 2014). Soragni et al (2014) demonstrated that class I HDACi can induce epigenetic changes, such as increase in FXN mRNA and acetylation of a key residue either in the blood of FRDA patients or in the iPSC-derived neuronal cells and PBMC of treated patients providing proof of concept for epigenetic therapy.

Finally, interferon gamma (yIFN) seems another molecule important in upregulating frataxin levels in cellular and mouse models of FRDA with prevention of dorsal root ganglion (DRG) in dorsal root ganglia and motor performance improvement (Tomassini et

al., 2012), with ongoing phase I clinical trials. Seyer et al. (2014) published their findings of an open label trial of yIFN for 4 months in 12 children with FRDA, demonstrating small significant changes of frataxin levels in erythrocytes, PBMC and platelats and FARS score changes equivalent to a 18 months improvement.

An attempt to try gene therapy in conditional cardiac FXN deletion mouse model, demonstrated the FXN delivered intravenously via an adeno-associated virus vector prevented and reversed cardiomyopathy (Perdomini et al., 2014).

Lately, Corben et al. (2014) have published recommendations addressing almost all the areas of health issues (neurological, heart, scoliosis, diabetes mellitus, genetic issues, pregnancy and quality of life issues) in patients with FRDA. These recommendations are generated from the evidence of systematic reviews, from randomized clinical trials (RCT), from comparative studies with control group or historical control and from case series.

The purpose of this study

This study is intended to provide a multidisciplinary overview of the clinical condition integrating it with a comprehensive MRI protocol on FRDA patients compared to controls. We have designed a longitudinal study in order to be able to describe the disease progression and to search for any potential biomarkers.

CHAPTER 2: Clinical and neuropsychological assessment in the cohort of Friedreich's Ataxia patients

Abstract

Friedreich's ataxia (FRDA) is an autosomal recessive (AR) progressive hereditary neurodegenerative disorder. The prevalence is reported 2-5:100.000 in the Caucasian populations. Around 98% of FRDA patients present with GAA repeat expansion. The clinical diagnosis of FRDA should be suspected in the presence of the progressive ataxia, absent muscle deep tendon reflexes, dysarthria, early onset and an AR transmission. In addition, skeletal deformities, pyramidal involvement, diabetes mellitus, cardiac hypertrophy, optic atrophy atrophy or deafness can be found. Specific neuropsychological profiles including executive and memory deficits, have been detected in FRDA. We performed a multidisciplinary overview of the clinical condition integrating it with an extensive cognitive and neuropsychological assessment. Twenty one patients with a molecularly confirmed diagnosis of FRDA were recruited. The patients were aged >12 years of age and had an early onset and molecularly defined diagnosis of FRDA. All participants gave their written informed consent. All patients underwent a full clinical (neurological and ataxia scoring scales) and neuropsychological assessment (WISC III, WAIS R), specific tests for the attentive, executive and memory functions and MMPI A for the personality assessment. The cohort presents with an early age at onset (AAO) (10.6 ± 4.6, range 4-20). The F:M ration was 16:5. The age at the visit was .9 ±10.3 years (range 12-50) and disease duration was 16.3 ± 8.8 years (range 3-32). FRDA cohort presented as homozygous for the GAA repeat expansion in 96%. The mean GAA repeat expansion in the short allele was 653.7 ± 221 (range 170-946) that correlated negatively with AAO. In most cases the onset was with ataxia, gait clumsiness, and scoliosis, but few with

asymptomatic cardiomyopathy and pes cavus. Vibratory sense was impaired in all the patients, with milder deficits in the other senses. Dysarthria was present in all patients. Muscle strength and tone were impaired in almost all the patients. One of them presented with a spastic ataxia with retained DTR. The pyramidal signs were present in 57%. Nystagmus was present in 61.9%. Half of the patients were wheelchair bound. Few patients developed diabetes mellitus. Cardiac involvement was registered in 76.2%, mostly presented as ventricular, septal or apical hypertrophy, but few with arrhythmias and valve prolapsed. The pulmonary system was involved in 28.6% of the patients (restricted pulmonary involvement, bronchial asthma and a positive history of ab ingestis pneumonia). Dysphagia was present in 80.9%. in addition, Helicobacter pylori positive gastritis and bowel disturbances were reported. The sensory component afferences was involved as three patients complained hearing loss (14.3%) and one of them visual field reduction (4.8%). Interestingly, our cohort presented with a wide of systemic conditions. Half of the patients had normal to superior IQ tot, followed by borderline presentation and few with mental retardation. The the motor impairment (dysmetria, slowness) mostly affected the IQ tot socres. And finally only 10.5 % of the cohort presented with IQ tot values that were allocated within the mental retardation range. The neuropsychological profile assessment of the FRDA patients evidenced impairment in attentive functions at around 47.4% of the cohort, the executive function as phonemic (26.3%) and semantic fluency 21.5, and planning and spatial working memory (57.9%). The personality of the FRDA cohort included mostly concern with bodily symptoms, worries and anxiety, depressive symptoms. Few patients complained hypomania, bizarre behaviours and ideas, awareness of family problems, and very few did confirm hypochondria, low self esteem, anger management difficulties and also aggressive behaviour tendency. Here we report our

experience of a cohort of FRDA patients after an extensive clinical and neuropsychological assessment.

Introduction

Friedreich's ataxia (FRDA) is a hereditary neurodegenerative disorder trasmitted in an autosomal recessiva (AR) manner. FRDA was initially described by Nicholaus Friedreich (1863a). The prevalence is reported 2-5:100.000 in the Caucasian populations. The prevalence in the whole Italian population is reported to be 1:90.909 (Romeo et al., 1983) with an incidence of around 1:25.449. The carrier frequency is 60-1:100. Campuzano et al. (1996) discovered that FXN gene is linked to FRDA. Around 98% of FRDA patients present with GAA repeat expansion in both alleles. The pathological repeat size ranges from 66 to 1700. The FXN gene encodes for the frataxin protein which is entangled in the synthesis of enzymes involved in the respiratory chain complexes I – III and aconitase (Pastore and Puccio, 2013). The neuropathological findings are in line with a degeneration in the dorsal root ganglia (DRG), sensory nerves, progressive destruction of the dentate nucleus, atrophy of Betz cells and degeneration of the corticospinal tracts (CST) Koeppen and Mazurkiewitcz, 2013). Harding (1981) described a large cohort of patients and subsequently refined the diagnostic criteria for FRDA (Harding et al.,1983).

The clinical diagnosis of FRDA should be suspected in the presence of the combination of the following findings: progressive ataxia of gait and limbs, absent muscle deep tendon reflexes (DTR) in the lower limbs (LL), dysarthria, onset before 25 years and an AR transmission. In addition, skeletal deformities (scoliosis, pes cavus), CST involvement (LL weakness, Babinski sign), diabetes mellitus (DM) or glucose intolerance, hypertrophyc cardiomyopathy, optic atrophy or deafness can be found. With the gene discovery, the FRDA can be molecularly confirmed.

Specific neuropsychological profiles including executive and memory deficits, have been detected in FRDA (Mantovan et al. 2006; Nieto et al. 2012) indicating parieto-temporal dysfunctions. The personality of FRDA patients has been characterized by increased irritability, poor impulsive control, reduced defensiveness and a poor-self-presentation (Mantovan et al. 2006).

The aim of this study was to explore the clinical presentation of the FRDA cohort afferent into our centres. We investigated our cohort via a thorough neurological and neuropsychological assessment. This study is intended to provide a multidisciplinary overview of the clinical condition integrating it with an extensive cognitive and neuropsychological assessment.

Methods

Participants

Twenty two patients with a molecularly confirmed diagnosis of FRDA were recruited at the Research Centres "Eugenio Medea" in Conegliano/Pieve di Soligo (Treviso) and Bosisio Parini (Lecco, Drs. Grazia D'Angelo, Erika Brighina) and in Bologna (Dr Valerio Carelli) between 2011 and December 2014. The patients were aged >12 years of age and had an early onset and molecularly defined diagnosis of FRDA. All participants, but two, were native Italians, mostly originating from Central and North Italy. The other two patients were of non-Italian nationality (Albanian and German). One patient had to be excluded from the study due to difficulties in clinical protocol administration as the she had undergone orthopaedic surgery for feet deformities.

Ethic committee approval and patients consent

The study has been reviewed and approved by the Institutional Review Board (IRB) on 07/07/2011 (Prot. No 051/11-CE). All participants gave their written informed consent in accordance with the 1964 Declaration of Helsinki.

Measurement tools

All patients underwent a full clinical assessment including neurological examination from a trained neurologist. Disease severity was assessed by three different ataxia rating scales. The Scale for the Assessment and rating of Ataxia (SARA) (Subramony et al., 2005) includes 8 items regarding upright posture, speech and limb kinetic function (range 0-40). The International Cooperative Ataxia Rating Scale (ICARS) (Trouillas et al., 1997) consists of 19 items divided into 4 subgroups assessing posture and gait, limb movements, speech and oculomotor disturbance (range 0-100). The Friedreich Ataxia rating Scale (FARS) (Schmitz-Hubsch et al., 2006) consists of the following sub-scales: functional staging (0-6), activities of daily living (ADL) (0-36), neurological examination (0-117), PATA rate and 9-hole peg test (9-HPT) for the manual dexterity.

Motor function and movement were examined with the 6-Minute Walking Test (6MWT) (Laboratories, 2002), the modified Ashworth scale (MAS) (Bohannon & Smith, 1987), muscle strength assessed with Medical Research Council (MRC) (Compston, 2010) and also articular and sensory assessment was completed.

The independence in activities of daily living was explored with the functional independence measure (FIM) (Keith et al., 1987) (range 7-126).

NEUROPSYCHOLOGICAL PROTOCOL

The patients and healthy controls underwent a complete neuropsychological assessment weighted specifically for the group ages: 12-16 and 16-50 years (and over). The cognitive functions in the subjects aged 12-16 years were studied through the Wechsler Intelligence Scale for Children (WISC) III (Orsini e Picone, 2006) and in the group of adults and older adolescents was used the Wechsler Adult Intelligence Scale (WAIS) R (Wechsler, 1997).

The neuropsychological protocol was designed to specifically investigate the attentive, executive and memory functions. The attentive functions were explored via a software for

the attention and concentration and the Trail Making Test A-B (Mondini et al., 2011). Memory was tested by direct and inverse span tests, memory prose tests and also the recall of the Rey complex figure for the memory functions. In addition, the semantic and categorical verbal fluency and the Tower of London test (Shallice, 1982) were used to detect the executive functions. Finally, the Minnesota Multiphasic Personality Inventory A (MMPI A) was used to test the personality and to explore eventual psychopathological indexes in the adults.

Statistical analysis

Statistical analysis was undertaken using SPSS V. 21. Descriptive variables were presented as means, medians, modes and standard deviation. Bivariate correlations were used by performing Spearman test.

Results

Clinical data

Twenty one patients with a molecularly defined diagnosis of FRDA were examined.

The mean age at the time of the visit (AAV) was 26.9 ±10.3 years (range 12-50, mode 26 years) (Table 2.1). The mean disease duration was 16.3 ± 8.8 years (range 3-32). The gender ration was F:M 16:5. Patients declared an AAO of about 10.6 ± 4.6 (range 4-20), with a bimodal distribution (10 and 11 years).

All the patients had a molecularly definite diagnosis of FRDA. Twenty of them were heterozygous for the GAA repeat expansion, but one of them had 170 GAA repeat expansion on one allele and a point mutation on the other. The mean GAA repeat expansion in the short allele was 653.7 ± 221 (range 170-946), while the long allele counted for 809.5 ± 245.1 (range 350-1230). GAA repeat expansion correlated negatively with AAO (r2 -0.709, p= 0.001).

The symptoms at onset are as follows: ataxia, gait clumsiness, and scoliosis (7 patients, 33.3%). One patient reported to have had an onset with asymptomatic cardiomyopathy (4.8%), another one pes cavus (4.8%) and one of them with tendency to fall (4.8%).

The clinical features of the patients derived from the administration of a series of ataxia scales, from the neurological examination and other complementary assessments. Table 2.3 presents a summary of the ataxia rating scales. The variables include SARA, ICARS and FARS neurological examination full score. In addition, the subitems of ICARS and FARS are reported for a better characterization of each item or group of subitems. The manual dexterity was assessed through 9-HTP. All the patients but two were right handed. Vibratory sense was impaired in 100% of the patients. Other forms of sensory impairments regarded tactile (n=10, 47.6%), proprioception (n=7, 33.3%), pain (n=5, 23.8) and temperature (n=4, 19%).

Dysarthria was present in all patients but with a range of various severity. Most of the patients had a mild dysarthria as measured by FARS ADL score 0.5 to 1 (n=16, 76%). Tremor and dysmetria were present and not always associated in the same case index. Almost all patients had muscle weakness, mainly in LL. Muscle tone was reduced in the majority of patients, but one who presented with a spastic ataxia. Almost 57% presented with pyramidal signs (Babinski positive in 12 of them) and 3 of them had increased deep tendon reflexes (DTR). Nystagmus was present in 61.9%(n=13).

Twelve patients were wheelchair bound. They started using the wheelchair at a mean age of 22.3 ± 10.9 years with the earliest and the latest at 11 and 49 respectively. Five patients were autonomous in performing the 6 MWT with a mean length distance of 241 ± 147.5 meters (range 51-390). One of the patients performed the 6MWT with the use of a walking aid. Three of them were not able to perform the 6MWT as they were not autonomously deambulant, and another one preferred to avoid it due to tendency of falls.

Regarding the different aids used in their everyday life, as mentioned in the previous paragraph, n=13 were regular wheelchair users (57.1%). Five of them used foot plantar (23%). The orthopaedic corset was used from 5 FRDA patients (23%). Ankle foot orthotics (AFO) was used in 3 cases (14.3%) and 1 patient used a walking aid (4.7%) and another one used an index splint to facilitate herself for the computer use.

Functional independence was measured either with FIM or with ADL section of the FARS. The former presented with a mean of 99.6, ranging from a full autonomy in everyday functionality (126) to an almost complete dependence in all the daily activities (53).

Two patients presented with a diagnosis of Diabetes Mellitus (DM) with years after onset 21 and 14 and were both in treatment with oral hypoglycaemic.

Cardiac involvement was registered in 16 patients (76.2%). Patients mostly presented left ventricular hypertrophy (n=5, 23.8%), septal or apical hypertrophy (n=2, 9.5%), arrhythmias (n=2, 9.5%) and mitral prolapsed in one of them (4.8%).

The respiratory system was involved in 6 patients. Two of them had a restrictive pulmonary condition, and another had reduced FVC. One had a diagnosis of bronchial asthma and another had positive history of ab ingestis pneumonia.

The gastrointestinal system was largely involved as 17 FRDA (80.9%) patients complaints dysphagia, mainly for liquids. One patient had a history of Helicobacter pylori positive gastritis (4.8%) and two of them had bowel disturbances (9.5%) (one stipsi and the other bowel incontinence episodes).

The cohort of patients presented other conditions, systematic involvement. One of them presented a D4-D9 hernia (4.8%), two patients had had trauma (hand and fibular epiphysis fractures) (9.5%). Seven patients had undergone surgery for dorsal lumbar arthrodesis (n=2, 9.5%), knee arthroscopy (n=1, 4.8%), appendicectomy (n=2, 9.5%), ovarian cyst removal and tonsillectomy (n=1, 4.8%).

Pain was common and it was complained as headache by 3 patients (14.3%) and as LL pain due to muscle contractures in another (4.8%).

The sensory component afferences appeared affected as three patients complained hearing loss (14.3%) and one of them visual field reduction (4.8%).

Systemic involvement was observed due to the presence of celiac disease (n=1, 4.8%), kidney stone (n=1, 4.8%), acne vulgaris and seborrhoic dermatitis (n=2, 9.5%). In addition, other patients had been diagnosed with rheumatoid arthritis (n=1, 4.8%), autoimmune thyroiditis (n=2, 9.5%) and iron-deficiency anaemia (n=1, 4.8%).

Interestingly, one patient complained sleepwalking (4.8%), another one presented with a congenital VII cranial nerve palsy and 3 of them with mood swings, apathy and anxiety (14.3%).

The patients were in treatment with various drugs such as idebenone (n=11, 52.4%), deferiprone (n=2, 9.5%), vitamins (group B, D, E, n=8, 38.1%), antispastics (n=2, 9.5%), oral hypoglycaemics (n=2, 9.5%), acetyl salicylic acid (n=2, 9.5%). In addition, they were in therapy with metothrexate (n=1, 4.8%), levotyroxine (n=1, 4.8%) and citalopram, sertraline and amantadine (n=3, 14.3%).

Nineteen patients underwent the NPS protocol assessment. Two of the patients of the cohort did not undergo the NPS protocol for their first language was not Italian as they were of Albanian and German nationality.

Neuropsychological data

The IQ assessment of the FRDA patients was administered from 3 qualified psychologists. The distribution of the IQ components is evidenced in the figure 2.1. Five patients out of 19 (26.3%) had normal values of the three IQ components: verbal (IQ v), performance (IQ p) and total (IQ tot). Other four patients (21.5%) presented with superior to high cognitive potential IQ v; normal (2), border (1) and superior (1) IQ p, whereas the IQ tot ranged

from normal (1) to superior (3) values. Other two patients (10.5%) had normal IQ tot but disarmonic verbal and performace values. Another 10.5 % of the cohort (2) presented with IQ tot values that were allocated within the mental retardation range, both with border level of IQ v and IQ p <70. Finally, four patients (2) (1.5%) had border IQ tot, with dysarmonic IQ v and IQ p.

The neuropsychological profile assessment of the FRDA patients evidenced impairment in attentive functions CPT in 2 patients (10.5%) and TMT-A and TMT-B in 9 patients (47.4%). Executive functions were impaired as demonstrated from altered results in phonemic in 5 patients (26.3%) and semantic fluency in 4 (21.5%), in addition with 57.9% of ToL impairment values. Memory functioning was impaired as measured from altered direct span in 4 (21.5%), inverse span in one (5.3%), and Rey recall figure in two (10.5%). From the MMPI A administration, we observed that 21% of patients (4) had concerns related to bodily symptoms. Worries and anxiety was found present in 21% (4). Depressive aspects of FRDA patients personality were noticed in 16% of the cohort (3). In addition, the following findings characterize our cohort: hypomania in 11% (2), bizarre behaviours and ideas in 11% (2), family problems awareness in 11% (2), hypochondria in 5% (1), low self esteem in 5% (1), anger management difficulties in 5% (1) and aggressive behaviour tendency in 5% (1).

Discussion

The cohort presents with an early AAO. 10.6 ± 4.6 (range 4-20), with a bimodal distribution (10 and 11 years). This findings are in line either for the AAO or for the mode of onset with the previously published literature (Harding, 1981; Filla et al., 1990). The cases that presented the lowest age at onset had larger GAA repeat expansion in the short allele, a severe phoenotype, a fast progression and an important functional impairment as previously reported (Harding, 1981; Durr et al., 1996; Schols et al., 1996). The gender

distribution is wheighted more in towards the female end. Although, there is differences in gender prevalence in other reports. The mean AAV was 26.9 ±10.3 years (range 12-50) and DD was 16.3 ± 8.8 years (range 3-32). All the patients had a molecularly definite diagnosis of FRDA. Almost 96% of them were homozygous for the GAA repeat expansion, but one of them (4%) was heterozygous for GAA repeat expansion and a carrier of point mutation. Interestingly, Campuzano reported that about 98% of FRDA were homozygous for GAA repeat expansion (Campuzano et al., 1996), whereas the remaining of 2-4 % of FRDA patients present with either FXN point mutation or deletion. The heterozygous case index had had an onset at around 18 years old with a wheelchair bound age at around 49 years. The milder point mutation is in fact a good explanation for the milder phenotypes (Cossee et al., 1999). The mean GAA repeat expansion in the short allele was 653.7 ± 221 (range 170-946), while the long allele counted for 809.5 ± 245.1 (range 350-1230) and it correlated negatively with AAO. The GAA repeat correlated negatively with AAO as reported previously (Campuzano et al., 1997, Filla et al., 1996; Durr et al., 1996; Montermini et al., 1997; Lamont et al., 1997; Monros et al., 1997). Most of the FRDA patients had an onset with ataxia, gait clumsiness, and scoliosis, but few of them with asymptomatic cardiomyopathy and pes cavus. These findings are in line with what has been previously published (Harding, 1981; Filla et al., 1990; Durr et al., 1996; Delatycki et al., 1999).

Vibratory sense was impaired in all the patients, whereas in other studies this loss was estimated be around 73-88% (Harding, 1981; Durr et al., 1996; Schols et al., 1997; Delatycky et al., 1999). Other types of sensory deficits such as tactile, temperature and proprioception, were observed but with a smaller impact.

Dysarthria was present in all patients but with a range of various severity, this was reportedly to be in around 90% of previous works (Parkison et al., 2013). Poole et al.

(2015) report a study on nasality in 37 FRDA patients compared to a control group, their findings suggest variability of nasality in FRDA with either hyper- or hyponasality, how perceptual ratings of hypernasality correlate with GGA2 repeat length suggesting probable genetic influence on nasality profile. Vogel et al. (2014) conclude in their review that there is insufficient evidence to determine the effectiveness of any treatment for speech disorder.

Muscle strength and tone were impaired in almost all the patients. One of them presented with a form of spastic ataxia with retained DTR. This was an atypical phenotype known as FARR (Klockgether et al., 1996; Coppola et al., 1999).

The pyramidal signs were present in 57% (Babinski positive) and absent DTR in all but in 14% of them. Other works report DTR presence in ranges from 1-33% (Harding,1981; Delatycky et al., 1999; Durr et al., 1996).

Nystagmus was present in 61.9%(n=13), and it is usually a common early sign (Parkinson et al., 2013).

Almost half of the patients were wheelchair bound by mean age of 22.3 ± 10.9 years. This is quite similar to the age reported in literature (25 years) (Harding, 1981; Parkinson et al.,2013). Despite the wheelchair, patients used other aids in order to comply with a better residual functioning such as foot plantar, orthopaedic corset, AFOs and finger splints.

Only 9.5% had developed DM. The DM incidence is known to account for 10-30% of the FRDA patients (Parkinson et al., 2013).

Cardiac involvement was registered in 76.2%, mostly presented as ventricular hypertrophy, septal or apical hypertrophy, and also few arrhythmias and valve prolapsed. Usually, the FRDA patients develop hypertrophic cardiomyopathy or LVH (Goeffroy et al., 1974; Filla et al., 1990; Durr et al., 1996; McCabe et al., 2000), in addition to some EKG alteration (Dutka et al., 1999; Bourke and Keane, 2011).

The pulmonary system was involved in 28.6% of the patients. The patients presented with restricted pulmonary involvement, bronchial asthma and a positive history of ab ingestis pneumonia.

Dysphagia was present in 80.9%. in addition, other interesting findings such as Helicobacter pylori positive gastritis and bowel disturbances were reported and no bladder involvement. Conversely, Parkinson et al. (2013) reports that bladder hyperactivity as comer and rarer bowel problems.

The sensory component afferences appeared affected as three patients complained hearing loss (14.3%) and one of them visual field reduction (4.8%). Dag et al. (2013) studied OCT in 10 FRDA patients reporting a retinal thinning, a generalized reduction of visual field testing and correlation of ICARS score to the retinal nerve fibre thickness. Fortuna et al. (2009) reported an extensive study in 26 FRDA patients, reporting visual pathway involvement as optic radiation ADC impairment in DTI, different patterns of visual field impairment, reduced retinal nerve fibre layer (RNFT) thickness, abnormal VEP and also correlation with clinical variables, ICARS scores, GAA triplet expansion, AAO and DD.

Interestingly, our cohort presented with a wide systemic involvement such as celiac disease, acne vulgaris and seborrhoic dermatitis, rheumatoid arthritis, autoimmune thyroiditis, iron-deficiency anaemia and sleepwalking.

We assessed nineteen patients NPS protocol. Around 47.8% of the cohort presented with normal to superior IQ total. Six patients had disarmonic IQ values, nevertheless 2 of them had normal IQ tot and 4 were within the border range. We observed, that many of the patients had very good scores in the verbal components, but it was the motor impairment (dysmetria, slowness) that affected the IQ tot socres. And finally only 10.5 % of the cohort presented with IQ tot values that were allocated within the mental retardation range. There are few studies that have dealt with cognitive function in FRDA. Initially, it was mention a

decrease in IQ (Geoffroy et al. 1976), but it was not sustained by Harding in her extensive 115 patients in 1981 (Harding, 1981). Conversely, Wollman et al (2002) reported reduced motor functioning and mental retardation.

The neuropsychological profile assessment of the FRDA patients evidenced impairment in attentive functions around 47.4% of the cohort. The executive function appeared altered in 26.3% and 21.5% in the phonemic and semantic fluency respectively. In addition, 57.9% presented with impaired ToL test values. Memory functions were affected as measured by direct span in 21.5%, inverse span in 5.3% and Rey figure in 10.5%. Previous studies, have reported reduced processing speed of information (Mantovan et al., 2006; White et al., 2000). The latter paper reported in addition visuospatial deficits, impaired verbal learning and executive dysfunctions. A reduced verbal spam and deficit in letter fluency, impaired acquisition and consolidation of verbal information was reported (Wolman et al. 2000), as well as differential impairments in semantic verbal, phonemic, and action fluency performances (De Nobrega et al.,2007).

The personality traits were variably affected. Mostly they interested concern with bodily symptoms, worries and anxiety. Nevertheless, 16% of the cohort complained depressive symptoms. In addition, few patients complained hypomania, bizarre behaviours and ideas, awareness of family problems, and very few did confirm hypochondria, low self esteem, anger management difficulties and also aggressive behaviour tendency. Flood et al. (1987) reported major depression in FRDA patients, whereas Leclercq et al. (1985) and White et al (2000) failed to show any psycho-organic symptoms and mood disorders. Mantovan et al. (2006) reported that the personality traits of FRDA patients were characterized by increased irritability, poor impulsive control, reduced defensiveness and a poor-self-presentation. Ciancarelli et al (2010) reported 29% of her cohort to have mood disorders.

The same authors report their experience of 1 year neuropsychological rehabilitation in the FRDA cohort which apparently contributed to the reduction of cognitive decline.

Da Silva et al. (2013) investigated 22 FRDA patients and reported reduction of grey matter (GM) volumes in medial and orbital region of frontal lobe and anterior cingulated gyri. The Berg Depression Inventory scores inversely correlated with the GM volume of right superior frontal gyrus. Akhalaghi et al. (2013 cognitive deficits) studied 12 FRDA patients found reduced FA, increased ADC and RD in the dentate-thalamic, thalamo-cortical and dentate-rubral tracts. The white matter (WM) changes in the latter correlated with cognitive impairments as assessed by Simon effect.

Actually, the whole range of cognitive impairments in the FRDA patients could be due to the disruption of different neural circuit that provide connection between cerebellum and other central nervous system (CNS) structures. The cerebellar circuitry consists of prevalently of corticoponto-pontocerebellar tracts, cerebellothalamic-thalamocortical tracts, and also of parieto-cerebellar, prefrontal-cerebellar and hypothalamus-cerebellar connection. Cerebellum is known so far to be an important CNS component involved in neurocognitive development, language function, working memory, executive function and the cerebellar internal control models (Koziol et al., 2014).

CHAPTER 3: NEUROIMAGING FINDINGS IN A COHORT OF FRIEDREICH PATIENTS: DTI AND FUNTIONAL MAGNETIC RESONANCE

Abstract

Background: Friedreich's ataxia (FRDA) is a progressive hereditary neurodegenerative condition caused by an autosomal recessively inherited GAA repeat in the FXN gene. In this study we used clinical measures and advanced tractography combined to functional MRI (fMRI) to explore white matter (WM) connectivity and motor dysfunction in a cohort of FRDA patients. Methods: Molecularly defined FRDA patients (n=17) were clinically assessed with the specific ataxia scales. Patients and age matched healthy controls underwent a neuroimaging study protocol on a 3T MRI scanner that included advanced neuroimaging DTI and fMRI. After the pre-processing, a nonlinear monoexponential model was used to calculate fractional anisotropy (FA), mean, radial and axial diffusivity (MD, RD, AD) maps. Non-parametric voxel-based permutations were performed on the WM maps regions of interest (ROI), considering age and sex via a general linear model (GLM) with critical threshold 0.05 while correcting for multiple tests. An fMRI sequence was acquired during a simple block design finger-tapping task. After a standard pipeline pre-process, intra- and intergroup GLM analysis were conducted, considering age and sex variables and also $p < 0.001$ threshold. Results: Our cohort included early onset FRDA patients, mean age at onset 10.65 ± 5.08 (range 4-20 years); F/M: 13/4; mean GAA expansion in the smaller repeat was $651,07 \pm 234.39$ (n=16) and one patients with a single base pair deletion and 170 GAA repeat. Mean age at assessment was 27.82 ± 10.51 years (12-51), mean disease duration was 17.17 ± 8.43 (4-33). The mean age of the control group was 23 ± 4.83 years; F/M= 5/8. From both the voxel-based and ROI-based analysis

altered FA and MD parameters were consistently found in the following four Central Nervous System areas: cerebellar WM (superior, median and inferior peduncles), long sensory-motor pathways (corticospinal and lemnisceal systems, cerebral peduncles), major commissural fibres (splenium and tapetum of the corpus callosum), the thalamic and the optic radiations. The fMRI data were analyzed from 13 patients (mean age 30.05 ± 11.76 years) and 8 controls (mean age 24.5 ± 3.85 years). The finger-tapping task demonstrated intragroup activation of the controlateral motor cortex and the ipsilateral cerebellar cortex both in patients and healthy controls. Intergroup analysis demonstrated a consistent and significantly higher cerebellar cortex activation, in controls compared to the FRDA patients, in particular in the lobules V and VI. Discussion: We show that a comprehensive MRI protocol consistently discriminates FRDA patients from controls. DTI changes in selected areas and BOLD signal in the ipsilateral cerebellar cortex in response to a simple motor task show strong intergroup discriminating power and may prove to be useful paraclinical disease markers. A longitudinal study is undergoing to explore the sensitivity of these indicators to disease progression.

INTRODUCTION

Friedreich ataxia (FRDA) is characterized by a set of motor and sensory deficits which result in ataxic behaviour. The disease is caused by the lack of frataxin protein due to intronic GAA trinucleotide repeat expansion in the FXN gene on chromosome 9 (Campuzano et al., 1996). Age of onset, clinical progression and severity are not uniform among patients, but correlate in various ways with the expansion size (Montermini et al. 1997).

In the last years, some in vivo MRI studies have provided information relative to the damage of cerebellar, cerebral and spinal cord areas involved in FRDA and other genetically determined ataxias (Akhalaghi et l. 2012; Akhlaghi et al. 2013; Jayakumar et

al. 2008; Ormerod et al. 1994; Villanueva-Haba et al. 2001) which could be useful to monitor disease progression.

With the advent of the VBM, it was possible to quantify the degree of atrophy, to monitor it in time and to identify various patterns typical of a specific form of ataxia (Della Nave et al. 2008a). Various studies have evidenced a significant correlation between the degree of the cerebellar atrophy, the severity of the clinical picture and also the duration of the disease (Della Nave et al., 2008a; Della Nave et al. 2008b; Mantovan et al. 2006; Ormerod et al. 1994; Prakash et al. 2009).

There are no quantitative objective biomarkers that show strong and reliable correlation with progression rate and severity. To date there is no effective treatment available for FRDA and the few clinical trials carried out so far reveal the weakness of the poor capacity to detect and document promptly and objectively meaningful changes (Di Prospero et al., 2007). Markers of oxidative damage such as 8-hydroxy-2'-deoxyguanosine (Schulz et al., 2000) have been proposed to document the efficacy of treatment, but showed poor correlation with the clinical variables (Di Prospero et al., 2007). On the other side validated and commonly used clinical severity scales don't show the sufficient sensitivity to capture changes in the short term (6 months - 2 years), making them unfit to reliably monitor any expected treatment-induced changes and in a sufficiently short term. These problems, coupled with the rarity of FRDA, are obstacles that make assessment of treatment efficacy slow and inefficient, thus resulting in further procrastination in the development of an effective therapy.

The advanced neuroimaging techniques such as Voxel-Based Morphometry (VBM), Susceptibility Weighted Imaging (SWI), Diffusion Tensor Imaging (DTI) and functional Magnetic Resonance Imaging (fMRI) could offer on one hand the necessary complement to the description of the neuropathological basis of the disease, and on the other could also

represent an objective indicator of the disease progression that could be used even as paraclinical end-point in therapeutic trials. Surrogate end-points based on neuroimaging indicators have been extensively used in other neurological diseases such as Multiple Sclerosis, and their introduction speeded up significantly the recognition of effective treatments and their longitudinal evaluation (Sormani and Bruzzi, 2013).

Modifications of the fMRI pattern in response to specific tasks involving both the motor and the planning ability have also been demonstrated in FRDA patients, and fMRI based protocols could offer an adjunctive indicator of disease progression or of therapy induced modification (Jayakumar et al. 2008; Mantovan et al.2006). Previously, has been reported a heterogenous pattern of cortical activation following a finger tap motor task in fMRI (Mantovan et al.2006). In additions, another study using a cognitive task (Simon effect) demonstrated a reduction of the BOLD effect in FRDA patients (Georgiou-Karistianis et al., 2012).

Neither of these studies however included any follow-up assessment to demonstrate clinical progression.

DTI is a non invasive neuroimaging technique that allows the study of diffusion process in the brain tissue, in particular to sensitize the MRI signal intensity in relation to water diffusion. The pulsed magnetic field gradient is used principally and the precession of the protons is proportional to the magnet field gradient which is in turn related to precession of the protons (Qiu et al., 2015). The final step of events leads to pulsed magnetic field gradient which leads to signal loss due to the amount of water diffusion derived at each of the location of the spatial domains.

The structural MRI is used to study brain structure, and fMRI is used to study brain function. The fMRI studies the blood oxygenation level dependent (BOLD effects). It measures the deoxyemoglobin levels that lead to a perturbation in the local magnetic field.

The result is the brightness which in turn is an fMRI image linked to the level of local magnetic perturbation. fMRI measures the local increase in brain activity as a sign of the initial use of the local pool of oxygen, which is then followed by a larger increase in regional oxygen delivery than needed due to local area flooded by oxyhaemoglobin, less deoxyhaemoglobin, less magnetic perturbation than at rest with a brighter image as an outcome. The data are preprocessed in order to be cleaned up and to increase the signal to noise ratio (SNR). This process is important for the removal of detrimental effects of head motion, background noise, physiological noise, brain anatomy variability. The latter is corrected by smoothing and normalisation to standard template. The noise is usually related to breathing, heartbeat, machine artefacts and also movements that are induced by movement of the subject (or body segments) when the stimulus appear.

The main objectives of this study regard an attempt to To establish an efficient protocol to obtain, from neuroimaging, objective and quantitative biomarkers for FRDA useful to monitor disease progression and response to treatment. This objective was designed to be pursued through: longitudinal analysis of the patterns of cerebral and cerebellar damage in FRDA using advanced neuroimaging techniques (VBM, DTI, fMRI); and eventual correlation of the clinical (motor and cognitive) data with the neuroimaging ones.

The motor task was selected considering the motor impairment in FRDA patients. The finger tapping task was administered to study the motor cortex activation, motor coordination and precision. The cognitive task consists of the Stroop test (color reading frame) which is useful to study the selective attention, the ability to ignore irrelevant details and the conceptual thinking, in addition to be able to measure the reaction time.

Therefore, this study is intended to provide a multidisciplinary overview of the clinical condition integrating it with a comprehensive MRI protocol in FRDA patients compared to

controls. The initial proposal of the project was to comply with a longitudinal design in order to study the disease progression and to search for any potential biomarkers.

METHODS

Participants, informed consensus and clinical assessments are described in chapter 2.

A control group of healthy controls was considered, matching age and gender characteristics. The control group underwent the MRI protocol and neuropsychological one. They were all free of any significant pathology that would interfere with the study protocol.

Neuroimaging protocol

All the MRI scans were performed with a Philips Achieva system equipped with a 3.0 Tesla magnet. The MRI protocol included diffusion tensor imaging (DTI), fMRI with motor and cognitive task.

DTI data were acquired by means of a 2D T2-weighted EPI sequence (slice thickness = 2mm, acquired matrix=112x112, field of view= 224x224 mm2, final voxel size=2.2x2.2x2.2 mm3, TR=8,645s ,TE=63ms, flip angle= 90°) along 6-15-32 non-collinear directions with repeated acquisitions and multiple b-values (0, 300, 1100 sec/mm2). DTI data were used to characterize the diffusion parameters in the white matter (WM) Moreover, a T2W structural volume was acquired with a 2D Turbo Spin-Echo (TSE) sequence to correct DTI data for the susceptibility artefacts (slice thickness=1.7mm, acquired matrix=112x112, field of view=224x224 mm2, final voxel size=2x2x1.7 mm3, TR=3s ,TE=100ms).

DTI data were analysed using TORTOISE software V.2.0.1 (Pierpaoli et al. 2010) (http://www.tortoisedti.org), a free set of tools developed by the NIH paediatric neuroimaging group. The analysis pipeline can be divided in three phases: preprocessing, tensor estimate and postprocessing.

In the preprocessing phase the acquired data are prepared for the analysis. Firstly, all images are reoriented to the AC-PC plane in order to achieve a common system of reference. Secondly, a motion correction procedure is performed to eliminate misalignment among volumes due to patient motion during the acquisition. Then, images are corrected for B0 susceptibility and EPI distortion artefacts applying a robust registration analysis (Rohde et al., 2004). In this step the structural T2W image is also used. Finally, a visual inspection of the corrected data is performed to detect remaining artefacts and data corruption. Corrupted data may be discarded from the subsequent analyses.

The diffusion tensor estimate is performed on the preprocessed data using the method described in Change et al. (2005). This method allows a robust estimate of the diffusion tensor by iteratively identifying outliers on the data and accordingly updating the fit weights.

In the postprocessing phase the diffusion data are prepared for the comparison among subjects and for the statistical analysis. In particular, a study template is built according to Chang et al (2005) and all subject tensors are moved to the study template using the tensor based registration algorithm included in DTI-TK (Zhang et al. 2007) (http://dti-tk.sourceforge.net). Once the subject tensor is moved to the template space, several diffusion parameters are derived, such as the Fractional Anisotropy (FA), the Mean Diffusivity (MD), the Axial and Radial Diffusivities (AD, RD). Diffusion maps are analysed using both voxel- and ROI-based approaches. Moreover, the deformation fields computed to move each subject tensor to the template are used to perform a diffeomorphic analysis of the white matter (WM) driven from the diffusion data.

In order to quantify eventual statistical differences between two groups, and to investigate eventual disease-related structural differences, the diffusion data of all the subjects were

aligned in a common space. In order to perform this operation, a reference diffusion tensor atlas was created by use of DTI-TK software, leading to the alignement of all diffusion tensors in all the subjects. Notably, the DTI-TK performs non linear rigid, affine and diffeomorphic registrations in a succession manner on the diffusion tensors. By using the spatial transformations calculated on the diffusion tensors, the FA maps were aligned. Three types of tests were performed: Tract Based Spatial Statistics (TBSS) (Smith et al., 2006), voxel-based statistics on permutations and statistical analysis of the regions of interest (ROI) on WM tracts by means of general linear model (GLM).

TBSS follows an hypothesis on two samples based on permutations using the "Randomise" tool (Winkler et al., 2014) of FSL (Jenkinson et al., 2012) comparing the FA values of both groups of the WM. The test was performed with multiple correction tests and with the "Threshold-Free Cluster Free Enhancement" (TFCE) (Smith & Nichols, 2009), that leads to the automatic elimination of eventual less significative clusters. The significative threshhold accepted was 0.05 after 10.000 casual permutations.

For the regional statistics, we used definite ROIs deriving from the WM of the John Hopkins University atlant including FSL (Moriet al., 2005), measuring the mean FA value for every single person. The ROIs used correspond to the principal WM tracts either above or below the cerebellar tentorium. The mean FA values calculated were used as a Y vector, therefore a linear regression was performed following the linear model (Matlab, The MathWorks, Inc., Natick, Massachusetts, United States):

The intergroup statistical significant difference for ROIs values were considered   when p-values reached the level of <0.005 with multiple test corrections.

fMRI

Functional data were acquired by means of a T2-weighted EPI sequence of 178 volumes (TR = 2 sec, FOV = 128 x 128 x 40, voxel size 1.875 x 1.875 x 3.5 mm3) covering the whole brain and cerebellum. Functional images were acquired both during cognitive and motor tasks, and also during rest.

The cognitive task was used to investigate brain activation during executive and attentive functions (Stroop test), while the motor stimulus was selected to study brain activation during a task that implies manual coordination and precision (Finger tapping test). Resting state data were processed in order to measure functional connectivity.

During the Finger-tapping task, subjects were asked to press the buttons of an hand-shaped response-pad with a precise order, from thumb to small finger, and with the best possible accuracy. A block-design paradigm with 6 repetitions (20s of stimulus per each hand + 16s of rest) for each hand were used, for a total duration of 5 minutes and 40 seconds. The Endinburg inventory was administered to all patients and healthy controls in order to test the handedness. (Oldfield, 1971).

The block-design task of the Stroop-test consisted in 30 alternating blocks of colour identification trials such as congruent and incongruent colour/word blocks. During colour identification blocks, subjects would view a series of 10 stimuli ('XXXX') and would be instructed to identify the font colour of each stimulus as quickly as possible (e.g., 'XXXX' in blue). Four colours were – red, blue, yellow and green – and mapped to response keys for, respectively, the index and middle fingers of the right and left hand. During congruent and incongruent colour/word blocks, subjects would view a series of 20 colour names presented in a congruent or incongruent font colour and would be asked to identify the font colour. Each stimulus was displayed for 1000 ms; a 12-second rest interval occured half-way through the task.

Colour identification requests and congruent/incongruent stimuli were randomly presented through MRI compatible goggles. Subjects registered their responses using response pads with their left and right hands. The total paradigm would last 7 minutes.

Before the exam each subject was instructed about how to perform tasks in the correct way with a short training.

The functional data were analysed using Matlab 7.11 (The Mathworks Inc., Natick, MA/USA), and Statistical Parametric Mapping (SPM8) software, Welcome Department of Imaging Neuroscience, London, UK). Preprocessing of fMRI data will include different steps. A slice timing correction of the shift between slices in the range of (0, TR) seconds, to obtain the time-course desired. In the next step we performed the realignment of the volumes obtaining also a mean volume of the whole sequence; this volume was normalized on the Montreal Neurological Institute (MNI) space using the standard EPI template included in the SPM package. Then we obtained a transformation matrix to apply to all the single volumes to normalize the entire sequence on the standard space, re-sampled at the voxel size of 2x2x2 mm3. To check the activations on the morphological T1-weighted volume we also performed the segmentation process with the use of segmentation parameters to normalize morphological data. Finally we smoothed functional data with 6-mm isotropic FWHM Gaussian kernel, in order to attain and to compensate for the residual macro-anatomical variations among the subjects. A random effect analysis was used (single-case analysis) at the point when we collected a sufficient number of patients. The experimental conditions were specified as interest regressors. Linear contrasts to the parameter estimates of the experimental conditions was applied at the level of each single subject in order to obtain a t-statistic for every voxel. The Random Effects Analysis at the level of the group analysis occured, when the linear contrast images were inserted in a one-sample t-test analysis in order to create SPM{T} maps, indicating the

specific and significant activations for every contrast at the level of group analysis. A statistical cut off value of p<0.05 was used, corrected for multiple contrasts at cluster levels with a height threshold at the level of each voxel of p<0.001 (not corrected).

T1-WEIGHTED IMAGES

Anatomical images were acquired with a T1-weighted 3D Turbo Field Echo (TFE) sequence (TR=8.3 ms, TE=3.9 ms, 150 sagittal slices with no gap, FOV=240x240 mm2, voxel size 1x1x1 mm3) as anatomical reference for fMRI data and for gray/white matter (GM/WM) segmentation and volumes calculation.

T1 image analysis include several preprocessing and quantification steps. Firstly, intensity artefacts due to the bias field inhomogeneities are corrected using N4ITK tool (Tustison et al., 2010) included in ANTs (http://stnava.github.io/ANTs/, Advanced Normalization Tools). Then, the brain is extracted from the images and it is segmented into WM, GM and cerebro-spinal fluid (CSF) using the ATROPOS (Avants et al., 2011) tool included in ANTs. From the segmented images of the brain, WM and GM volumes are derived.

Subsequently, images are elaborated using the tools included in FreeSurfer software suite (http://surfer.nmr.mgh.harvard.edu). More precisely, the following steps are performed: surface generation, topology correction, surface inflation, registration to a spherical atlas, cortical parcellation and thickness calculation (Fischl et al. 2000).

Voxel-based morphometry (VBM)

The VBM was performed to assess eventual intergroup differences in the cortical thickness of patients and healthy controls. We have faced some problems due to the volume registration and segmentation, which le to difficulties in preliminary analysis.

RESULTS

In this study we report the MRI findings regarding the MRI data acquired from 30 subjects who voluntarily underwent MRI scan at the Scientific Institute "Eugenio Medea" in Bosisio Parini (Lecco).

Our cohort included early onset FRDA patients with a mean age at onset 10.65 ± 5.08 years (range 4-20 years); F/M gender ratio: 13/4; mean GAA expansion in the smaller repeat was $651,07 \pm 234.39$ (n=16) and one patients with a single base pair deletion and 170 GAA repeat. The mean age at assessment was 27.82 ± 10.51 years (range 12-50), mean disease duration was 17.17 ± 8.43 years (range 4-33). The mean age of the control group was 23 ± 4.83 years; F/M gender ratio = 5/8. The fMRI data were analyzed from 13 patients (mean age 30.05 ± 11.76 years) and 8 controls (mean age 24.5 ± 3.85 years).

ROI-based analysis

From an initial analysis of the FA values, significant differences in FRDA patients compared to healthy controls were found (Table 3.1). The FA values of controls are significantly higher when compared to the FRDA patients.

We further analysed the WM of the bundles by considering the MD values. Significant differences found in FRDA patients compared to healthy controls are demonstrated in table 3.2. The statistical analysis has taken into account the differences in age and gender distribution of both cohorts.

In summary, the areas that showed FA and MD impairment are assembled as follows:

- Cerebellar WM (superior, midldle and inferior peduncles)

- Motor and sensory long tracts (lemniscus, CST and cerebral peduncles)

- Major commissural bundles (splenium of corpus callosum, tapetum)

- Thalamic and optic radiations

Voxel-based analysis

The voxel-based analysis has confirmed the ROI-based analysis, demonstrating FA and MD variations corresponding to the above mentioned bundle voxels. The principal differences were evidenced at the corpus callosum, that demonstrated diffusive WM structural alterations. However, this finding should be cautiously analysed due to the possible partial volume influx on the data. Figures 3.1-3.5 demonstrate voxels corresponding to the FA impaired areas such as optic radiation, the CST, and cerebellar involvement (middle and superior peduncles).

Finger tapping data

In this section of the study, the two groups consisted of 13 FRDA patients (mean age = 30.05 ± 11.76 years) and 8 healthy controls (mean age = $24,5 \pm 3,85$ years).

An initial Fixed Effect analysis was performed on each subject via GLM by using the movement parameters as confounds. These data were subsequently used to perform the group Random Effect Analysis. Age and gender were used as regressors. The intergroup differences were calculated through a two-tailed test.

By considering the FRDA group we have demonstrated that the finger tapping task led to the expected and evident cortical activation in line with the healthy control group, the controlateral motor cortex and ipsylateral cerebellar cortex. The figures demonstrates activation of controlateral motor cortical and omolateral cerebellar areas activated during Right (Figure 3.6 and 3.7) and Left hand (Figure 3.8 and 3.9) finger tapping task in FRDA patients and healthy controls.

By considering an intergroup analysis of the differences in cortical activation between FRDA patients and healthy controls, emerged that the cerebellar cortex activation, in particular lobules V and VI, was higher in the patients group when compared to the healthy control one (Figure 3.10 and 3.11).

Stroop test

We performed the analysis of data derived from Stroop test with analogous methods to the previous task.

Unfortunately, the small number of patients and due to the presence of movement artefacts in a consistent part of the cohort did not allow to generate any data. The good quality data were distributed as follows: 9 FRDA patients and 6 healthy controls. Both groups were small enough to produce significant results. Therefore, it is necessary to provide a larger sample in order to be able to perform valid intra- and intergroup analysis.

Resting state data

The resting state fMRI data analysis is ongoing by means of ICA.

TBSS

The TBSS statistical analysis are ongoing.

Voxel-Based Morphometry (VBM)

The main hypothesis to test is whether there is any differences in the intergroup cortical thickness. The VBM analysis is ongoing.

DISCUSSION

We report the DTI and fMRI data from a cohort of FRDA patients confronted to a control group. The patients were all homozygous for GAA triplet repeat, but one that was heterozygous for the expansion and presented with a deletion.

The cohort of patients had an early onset of FRDA, with an age at MRI that ranged from 12 to 50 years but with a mean age at around 27 years old. The disease duration ranged from 4 to 33 years. The control group younger than the patients had a mean age of 23 years. Regarding the DTI, we analysed 17 FRDA patients, whereas for the fMRI analysis only 13 of them. The control group size was 8 adult subjects.

From the DTI analysis (either ROI-based or voxel-based) emerged that there is a significant reduction of FA and MD values in FRDA patients in 4 major CNS areas. There

is a prevalent involvement of cerebellar peduncles (SCP, MCP and ICP) (FA/MD). The long sensory tracts FA is prevalently impaired in the medial lemniscuses, CST and cerebral peduncles, with MD additionally impaired in the medial lemniscuses. The FA and MD are impaired as well in the major commissural bundles such as corpus callosum (CC) (body, splenium and tapetum). And finally, FA and MD values regarding thalamic and optic radiation are impaired in FRDA patients compared to healthy controls. Prakash et al. (2009) studied a group of SCA1 patients finding out significantly decreased FA in all the three cerebellar peduncles and this decrease correlated with disease severity. Akhalaghi et al., 2011 demonstrates that the cross-sectional area of SCP was significantly reduced in FRDA patients and correlates positively to AAO, and negatively to FARS score and DD. Other studies reported atrophic CNS areas in FRDA patients. Pagani et al. (2010) reported WM atrophy in their 16 FRDA patients in the following areas: central portion of the medulla oblongata, dorsal upper pons, SCPs, the central portion of the midbrain, the medial portion of the right cerebral peduncle, the peridentate region, bilaterally, and the optic chiasm. These findings were found to correlate with the clinical status of the patients. Chevis et al. (2013) demonstrated that spinal cord area in their 33 FRDA patients was smaller than the healthy controls and negatively correlated with the FARS scores. Della Nave et al (2008) by mean of TBSS found decreased FA in medulla, cerebellar hemispheres and small segments of occipitofrontal and inferior longitudinal fasciculus in 14 FRDA patients. In addition, Della Nave et al. (2011) reported increased MD in 14 FRDA patients in the decussation of the SCPs. Zalesky et al. (2013) reported an extended study in 13 FRDA patients indicating the WM connectivity disruptions of the cerebello-cerebral circuitry, either in motor areas (supplementary motor area, putamen and pallidum) or in non-motor areas (cingulated cortex, hippocampus and frontal cortex). Additionally, they imply for the disruption of the connectivity between brainstem and cerebellum. While

Fortuna et al. (2009) reported an increased ADC in the optic radiations. Rizzo et al. (2011) reported increased MD in medulla, cerebellar hemispheres, vermis and peduncles, brainstem and optic radiations. Corben et al. (2014) reported that the reduction in the magnetization transfer imaging ratio in the SCP and no alteration in the CC in their cohort of 10 FRDA patients, was indicative of SCP myelination scarcity. Synofzik et al (2011) reports hyperechogenicity of the dentate nucleus in a cohort of 34 FRDA in most of the patients and observe this finding even in the patients with a short DD.

The fMRI findings with the finger tapping task evidenced, from an intragroup analysis, a high activation of the controlateral motor cortex and ipsilateral cerebellar cortex. This finding was consistent either with left or right hand, and also consistently present in both cohorts. An intergroup analysis compared the activated areas in both groups during the finger tapping task. From this analysis emerges that there is a higher cerebellar cortex activation in FRDA patients, and in particular in the lobules V and VI. The lobule V is involved in sensorymotor tasks, in motor activation and sematosensory activation. In addition, the lobule VI is involved in sensorymotor tasks, language, spatial tasks, executive functions, emotions (Grimaldi and Manto, 2012).

Previously published functional works report a variety of findings.

Jayakumar et al. (2008) have performed a fMRI study with a set of supination/pronation tasks in SCA1 patients, suggesting a decoupling of sensorimotor cortical and cerebellar areas, therefore a probable rupture of

cortico-cerebellar loops. Interestingly, from an fMRI study in healthy volunteers (Liu et al., 2011) with a finger tapping task found out three regions involved in sustained negative BOLD response, mainly frontal, somatosensory and occipital. They suggested that the findings imply more of a suppression of neuronal activity rather than blood steal event. Mantovan et al. (2006) report heterogeneous cortical activation in FRDA during self-paced

finger movements. Other groups have provided even cognitive tasks. In particular, Georgiou-Karistianis et al. (2012) by using the Simon effect in fMRI implied a reduction in functional brain activation, reduced functional connectivity between cortical and subcortical regions. This implies a possible disruption of cortico-cerebellar loops and ineffective engagement of cognitive and attention regions.

A limitation of this study is the sample size. This is due to the fact that FRDA is a rare condition and despite the not indifferent number of patients recruited (n=22), some of them were in a very advanced stage of their disease which explains why their MRI scan data were almost "dirty" of movement artefacts. An insufficient sample size did not allow a statistical analysis for the fMRI cognitive task data. In addition, the cognitive task provided within the MRI protocol appeared to be not easy to all the patients. Some of them stated that they did not understand the task despite they were provided with a pre-MRI training. The recruited patients ranged either in severity of disease (FARS stage 2-6) or in AAV (12-50 years). In addition, the DTI and fMRI analysis lack of the clinical scales correlation.

Von Hohenberg et al. (2013) performed DTI in 12 FRDA and found out significant correlations between radial diffusivity (RD) and FARS scores and also with the number of GAA repeat expansion, suggesting the DTI as an informative biomarker in this condition. Mascalchi, (2013) in Letters writes in response to Vedolin et al. (2012) pointing out that visually assessed MRI in early onset FRDA are normal, no atrophy or reduction in cerebellar size can be found and this is confirmed by Della Nave et al. 2008 (Brain WM....) by VBM study. Nevertheless, Mascalchi points out points out the occurrence of microstructural changes in FRDA. These changes could be figured out with morphommetry or DTI computational measures which lead to the findings that superior cerebellar peduncles and this correlate to neurological severity, in addition damage in the

deep cerebellar nuclei such as dentate nuclei are observed and confirmed. Probably SCP could be a good biomarker for FRDA but however it required proper computational tools, and in addition even the iron deposition in the dentate nuclei observed in T2 acquisition and postprocessing. Santner et al. (2014) have tested the effect of two months treatment rhuEPO in 9 FRDA patients with scans pre- and post-treatment. They found out an increase in VBM in the grey matter of the thalami (pulvinar) in post when compared to pre, and this correlated with ataxia scores. In addition there was an increase in the posterior parietal cortex. But this study has a small sample and it is still difficult to generalize or compare these findings to others due to the fact that images are acquired in different scanners. Solbach et al. (2014) suggest to cautiously consider iron content in DN as a biomarker in FRDA trials, due to the fact that they found atrophy of the cerebellum and DN in their cohort (14FRDA/14controls) but normal iron content.

Our results support the evidence that DTI and fMRI techniques may provide reliable quantitative biomarkers that could be used in longitudinal studies for prognostic and therapeutic clinical trials.

Further work is needed to identify which is the best MRI technique that is more sensitive to detect the most efficient biomarker of FRDA at different stages of disease. Probably, even a composition of MRI techniques might provide an appropriate array of measures suitable to complement the clinical assessment.

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Figures of chapter 1

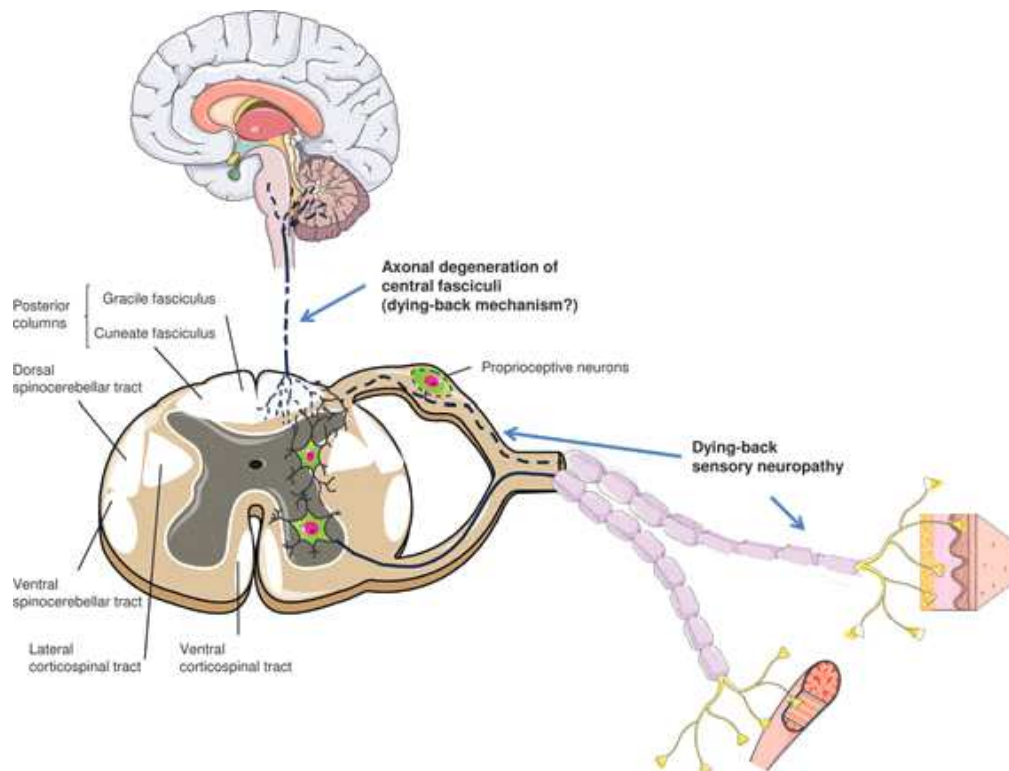**Figure 1**: Schematic representation of the central and peripheral nervous sysem involvement in FRDA (Gonzales-Cabo and Palau, 2013).
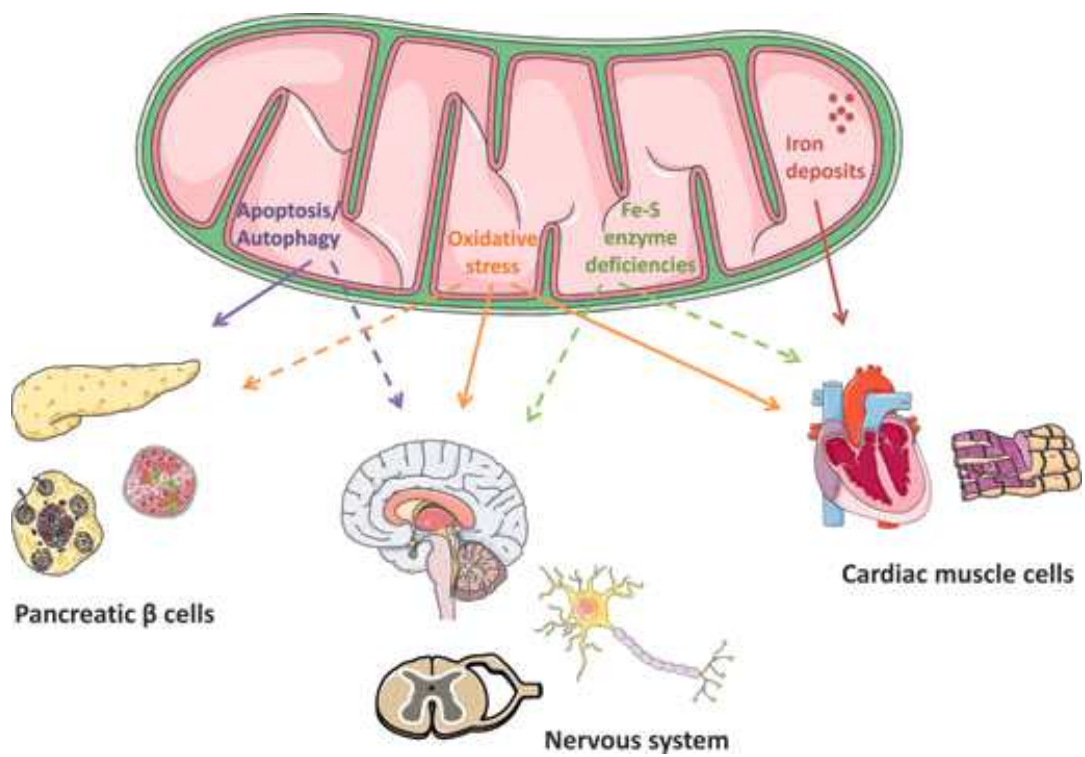
Figure 2: Schematic representation of pathological mechanisms of damage and the corresponding organ damage (Gonzales-Cabo and Palau, 2013).
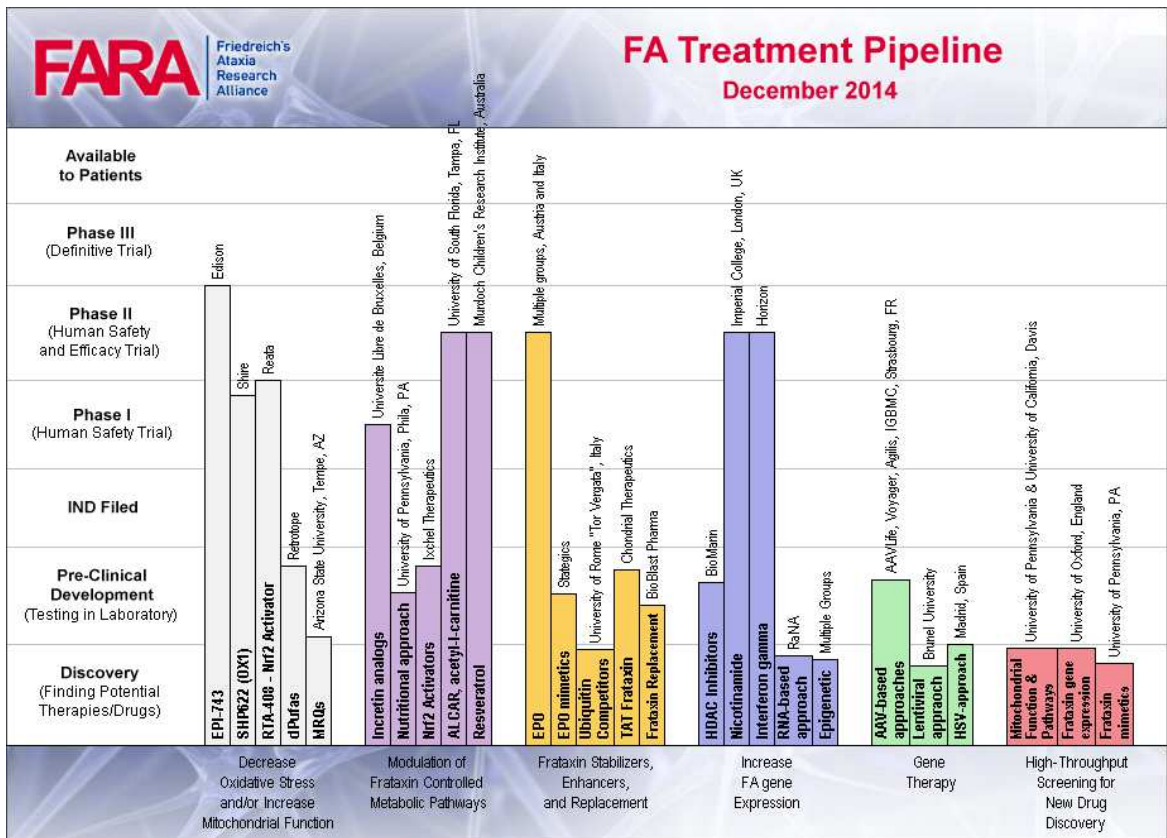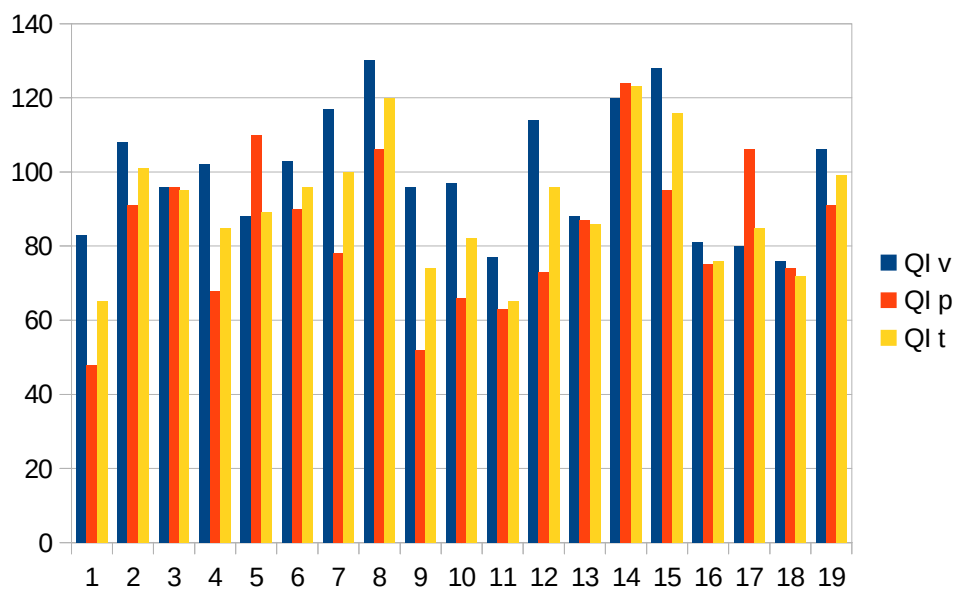
Figure 3 FRDA treatment pipeline. (http://www.curefa.org/pipeline.html).

Figures chapter 2.

Figure 2.1. IQ distribution in the FRDA cohort.

Tables chapter 2.

Table 2.1: participants clinical and genetic features.

Table 2.1

Characteristics (n=21)	
GAAsr (n=18)	
mean (SD)	653.7 (221)
range	170-946
GAAlr (n=17)	
mean (SD)	809.5 (245.1)
range	350-1230
Gender	
female (%)	16 (76.2)
AAO	
mean (SD	
range	
AAV	
mean (SD	
range	
DD	
mean (SD	
range	

GAA: guanine ade... AAV: age at the visit, DD disease duration.

Table 2.2. C... and additional ones.

Patient's code	Presenting Symptoms	Babinski	DTR	NY	6MWT (m)	W-d-age	Aid	Skeletal	DM	Heart	Respiratory (+)	GI	Other	Drugs
p.1.c	Gait clumsiness	-	-	-	NA	16	Wheelchair, R index splint. Orthopedic corset	Club foot, Scoliosis, Hamp	+ (21)	+ (LV)	+ (restrictive)	Dysphagia	DL arthrodesis	Idebenone, Beta-blocker, Baclofen, Thiamine, Sitagliptin, Metformin
p.2.b	Ataxia	-	+	-	NA	NA						Dysphagia.	Headache. Sleepwalking	
p.3.c	Scoliosis	-	-	+	278	NA		Mild pes cavus, Scoliosis		+ (LV)			Headache	
p.4.c	Gait clumsiness	-	-	-	NA	22	Wheelchair	Pes cavus, Hammer toes, Scoliosis				Dysphagia.	Appendicectomy	Citalopram
p.5.c	Gait clumsiness	+	-	+	360	NA		Pes cavus, Depressed R shoulder, Kyphosis, Claw feet		Arythmias			Congenital VII c.n. palsy, Knee arthroscopy	
p.6.c	Scoliosis	-	-	+	NA		Wheelchair	Pes cavus, Scoliosis		+, Arythmias			Headache	Almotriptan, Cholecalciferol
p.7.c	Clumsiness	+	-	+	NA	NA	Foot plantar, Orthopedic corset	Pes cavus, Scoliosis, Hump, LL heterometry		+		Dysphagia.	LL trauma (fibular epiphyseal fractures)	Idebenone, Thiamine, Tochopherol
p.8.c	Scoliosis, Gait clumsiness	+	-	+	390	NA	Foot plantar, Orthopedic corset	Skoliosis		+ (LV), Mitral prolapse				Idebenone
p.9.c	Ataxia	+	-	+	NA	19	Wheelchair, AFO	Pes cavus, Scoliosis, Pelvis and hip joints heterometry		+ (LV)	+ (reduced FVC)	Dysphagia, Gastritis HP+.	Sensorineural hearing loss, Coeliac disease, Ovarian cyst removal	Deferiprone
p.10.c	Scoliosis		-	+	NA	23	Wheelchair	Club foot, Scoliosis, Kyphosis				Dysphagia.	Visual field reduction, Kidney stones, L hand fractures	Deferiprone, alpha1-blocker
p.11.c	Ataxia		-	+	NA	16	Wheelchair			+ (LV)		Dysphagia.	Mood swings	Sertraline

Legend: NA: non applicable, GI: Gastrointestinal, NY: nystagmus, DTR: deep tendon reflexes (-absent, + present, ++ brisk), DM: diabetes mellitus (years after onset), W-d-a: Wheelchair dependency age, Heart involvement: (+ hypertrophy), R: Right, L: left, AFO: ankle foot orthosis, V ventricle.

Table 2.2. (Continues)

Patient's code	Presenting Symptoms	Babinski	DTR	NY	6MWT (m)	W-d-age	Aid	Skeletal	DM	Heart	Respiratory (+)	GI	Other	Drugs
p.12.c	Tendency to fall	+	++ (clonus)	-	NA	12	Wheelchair, AFO	Pes cavus, Hammer toes, Hump, Scoliosis	+ (14)	+		Dysphagia,	R LL pain	Idebenone, Baclofen, Sitagliptin, Niacin.
p.13.b	Gait clumsiness	+	-	+	NA	11	Wheelchair, Orthopedic corset	Pes cavus, Kypho-scoliosis		+	+ (restrictive)	Dysphagia,	Arthrodesis	Idebenone, beta1-blocker, Calcifediol
p.14.c	Ataxia	+	-	-	NA	NA	Wheelchair, Foot plantar	Pes cavus, Mild scoliosis, Kyphosis, Lordosis				Dysphagia, Bowel incontinence	Acne vulgaris (face, trunk), D4-D9 hernia	
p.15.c	Scoliosis	+	++	+	51(walking aid)	NA	Walking aid	Scoliosis		+		Dysphagia,	Spastic-Ataxia, Anxiety, Apathy	Idebenone, Tizanidine, Venlafaxine
p.16.b	Ataxia	+	-	-	NA	49	Wheelchair	Pes cavus, Scoliosis, Kyphosis.			Dyspnea	Dysphagia, Stipsi, Dyspepsia	Sensorineural hearing loss, Appendicectomy, Pectoral ghirdle, Tonsillectomy	Idebenone, Tochopherol, Oxybutinin, Acetylsalycilic acid
p.17.b	Scoliosis	-	-	+	NA	34	Wheelchair	Pes cavus, Hammer toes, Kypho-scoliosis.		Arythmias		Dysphagia,	Rheumatoid Arthritis, Autoimmune thyoidits, Mood swings, Iron-deficiency anemia	Idebenone, Methotrexate, Omeprazole, Folic Acid, Thiamine, Cholecalciferol, Acetysalycilic acid, Etoricoxib, Lipoic acid
p.18.c	Ataxia	+	-	-	NA	25	Wheelchair	Pes cavus, Moderate scoliosis		+	Bronchial asthma	Dysphagia,	Hypothyroidism, Hypoacusis	Idebenone, Amantadine, Levothyroxine, Terfenadine, Acetylsalycilic acid.
p.19.c	Ataxia, Pes cavus, Scoliosis	+	-	-	NA	NA	AFO, Foot plantar, Orthopedic corset	Pes cavus, Scoliosis, LL heterometry		+ (Septal, V)		Dysphagia,		Idebenone, Tochopherol
p.20.c	Cardiac hypertrophy	+	-	+	NA	18	Wheelchair	Scoliosis		+ (Septal, Apical, LV)	Ab ingestis Pneumonia	Dysphagia, Modified consistency	Folliculitis (trunk), Seborroic dermatitis	
p.21.c	Clumsiness	-	-	+	126	NA	Foot plantar	Pes cavus, Scoliosis, Hump				Dysphagia,		Idebenone

Table 2.3 Ataxia rating scales scores.

Table 2.3

Clinical scale measurements			
	mean (SD)	median (range)	mode
SARA	21.4 (7.8)	23 (8-32)	8
ICARS posture	25.9 (9.3)	31 (8-34)	33
ICARS kin func	22.3 (9.2)	24 (9-43)	14
ICARS speech	2.5 (1.2)	2 (1-5)	2
ICARS oculomot	2.2 (1.4)	2 (0-5)	3
FARS stage	4 (1.3)	5 (2-6)	5
FARS ADL	17.3 (7.3)	17.5 (3-32.5)	17,5
FARS kin func	23 (8.6)	25 (8-34.5)	25
FARS stab	22 (5.8)	24 (10.5-28)	27
FARS NE	62.7 (18.4)	69 (31.33-92.5)	69,5
9-HPT R	130 (135.6)	80.2 (43.4-613.1)	43,38
9-HPT L	138.2 (153.1)	90 (40.4-719)	40,42
FIM (n=17)	99.6 (21.4)	103 (53-126)	103

TABLES CHAPTER 3

Table 3.1 Significant differences of FA.

White Matter bundle	Group(p)
Middle cerebellar peduncle	0,000754
Body of corpus callosum	0,000074
Splenium of corpus callosum	0,000011
Corticospinal tract R	0,000094
Corticospinal tract L	0,000047
Medial lemniscus R	0,001008
Medial lemniscus L	0,00002
Inferior cerebellar peduncle R	0
Inferior cerebellar peduncle L	0
Superior cerebellar peduncle R	0
Superior cerebellar peduncle L	0
Cerebral peduncle R	0,00027
Cerebral peduncle L	0,000726
Posterior thalamic radiation (include optic radiation) R	0,000267
Posterior thalamic radiation (include optic radiation) L	0,000022
Sagittal stratum (include inferior longitidinal fasciculus and inferior fronto-occipital fasciculus) L	0,00044
Tapetum R	0,00021
Tapetum L	0,000001

L: left, R: right.

Table 3.2. Significant differences of MD.

White Matter Bundle	Group(p)
Middle cerebellar peduncle	0.000083
Body of corpus callosum	0.000061
Splenium of corpus callosum	0.000001

Medial lemniscus R	0.000099
Medial lemniscus L	0.000028
Inferior cerebellar peduncle R	0.000007
Inferior cerebellar peduncle L	0.000003
Superior cerebellar peduncle R	0.000000
Superior cerebellar peduncle L	0.000000
Posterior thalamic radiation (include optic radiation) R	0.000045
Posterior thalamic radiation (include optic radiation) L	0.000023
Tapetum R	0.000004
Tapetum L	0.000001

L: left, R: right.

FIGURES CHAPTER 3
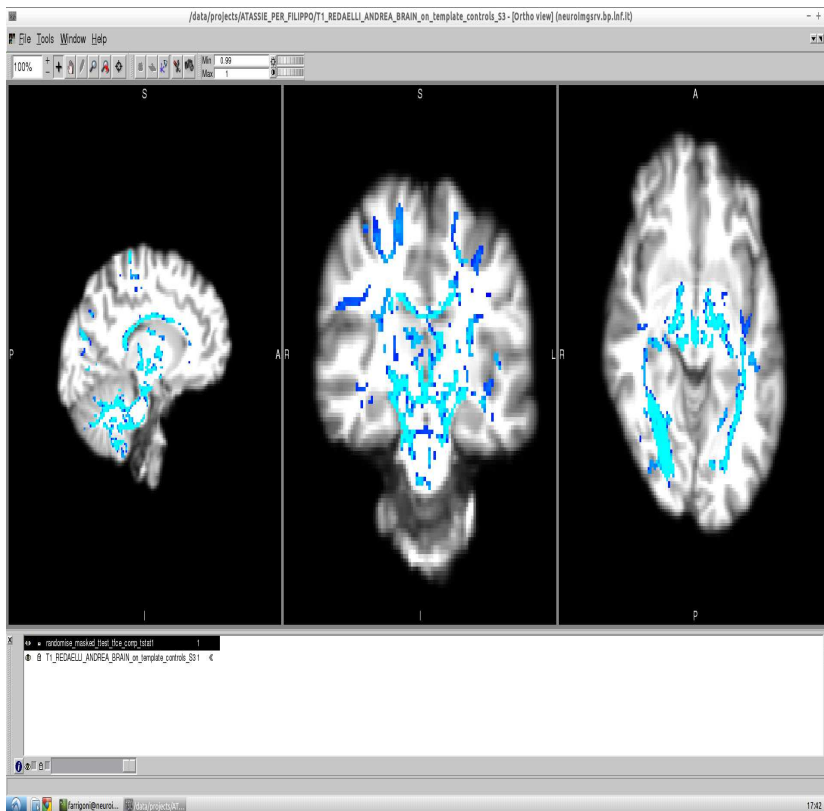
Figure 3.1. Optic radiations.
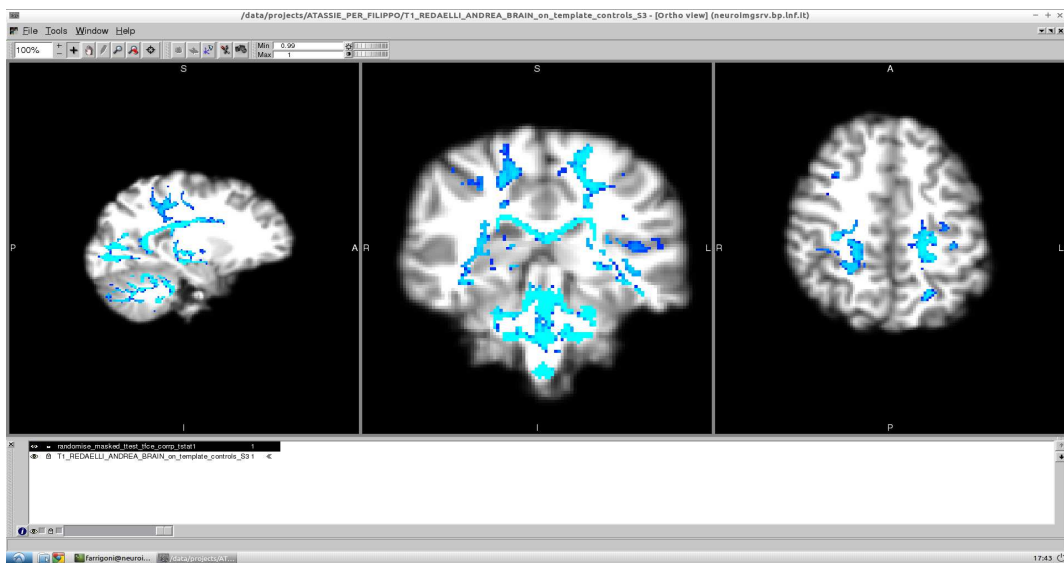
Figure 3.2. Corticospinal tracts.
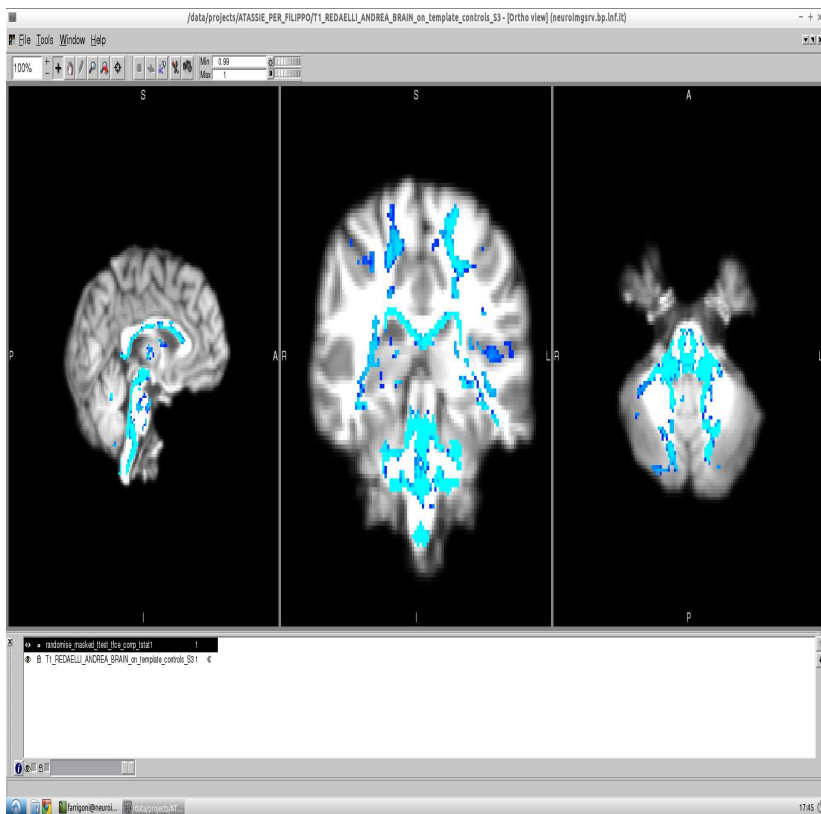
Figure 3.3 Corticospinal tracts.
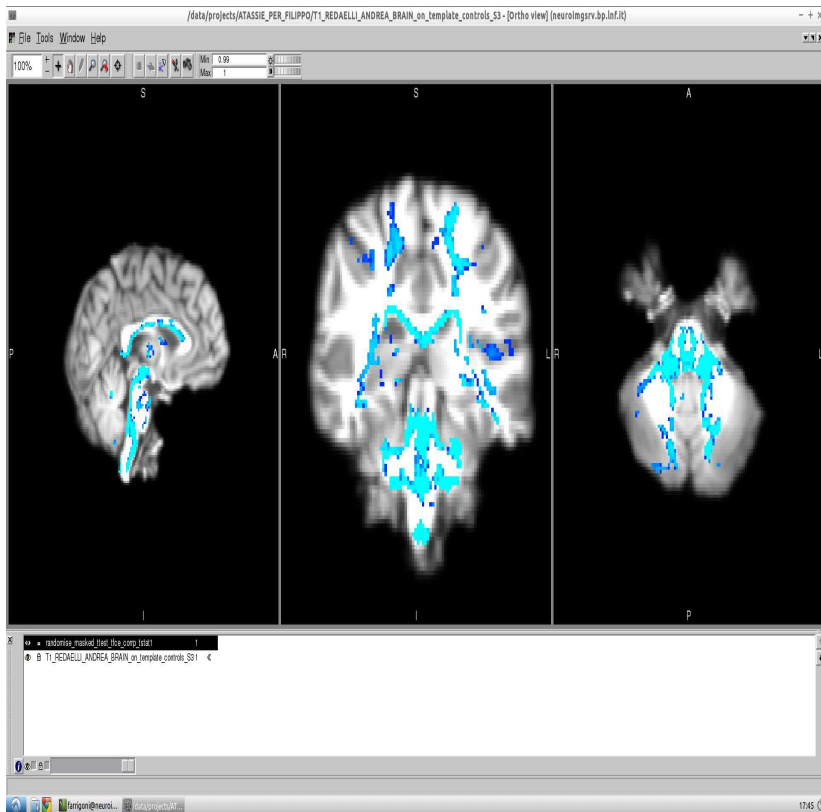
Figure 3.4. Median cerebellar peduncles.
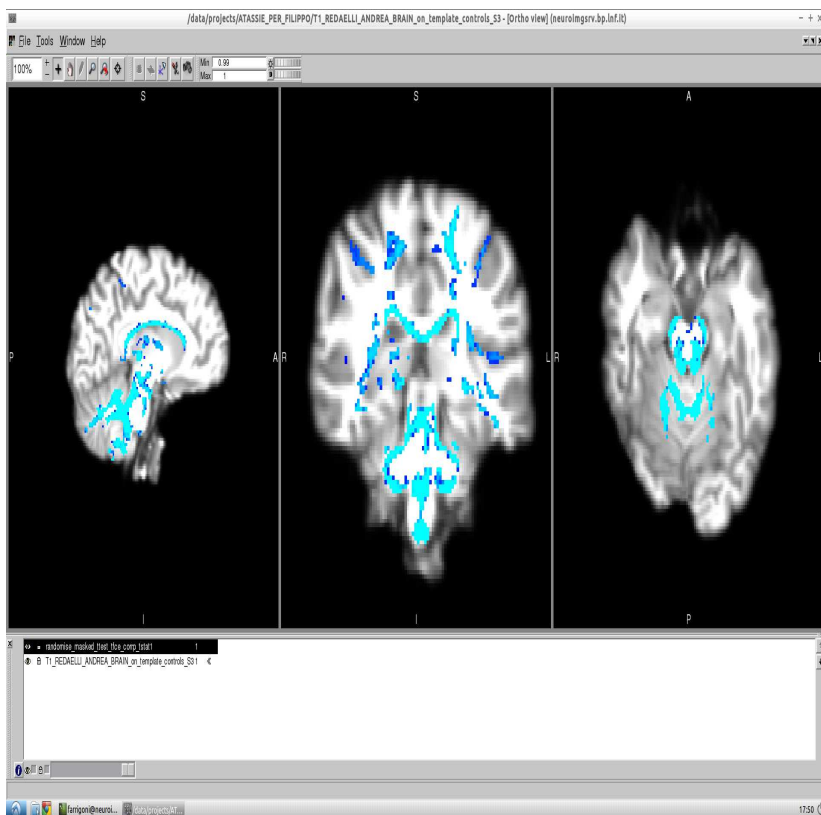
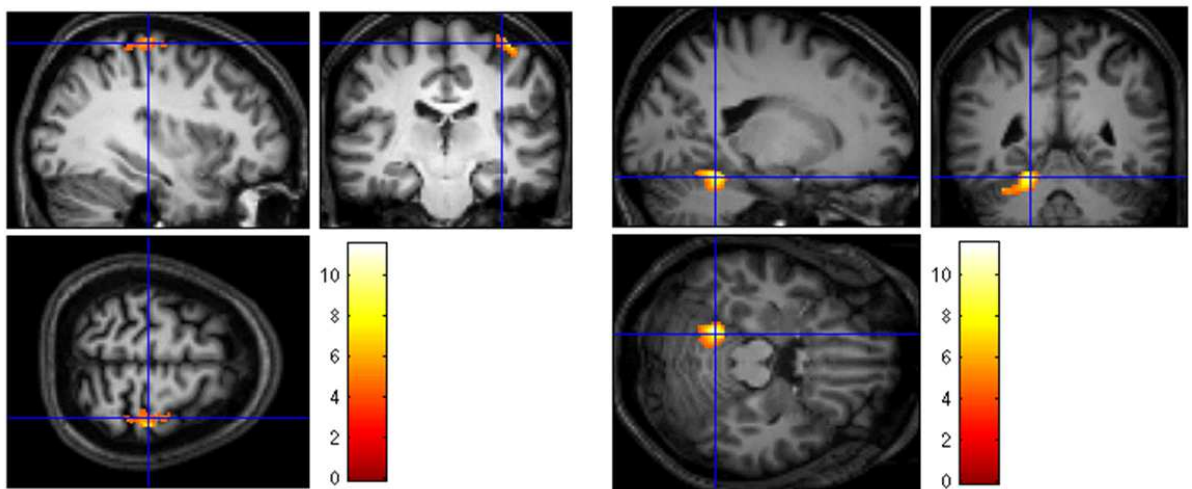**Figure 3.5**. Superior cerebellar peduncles
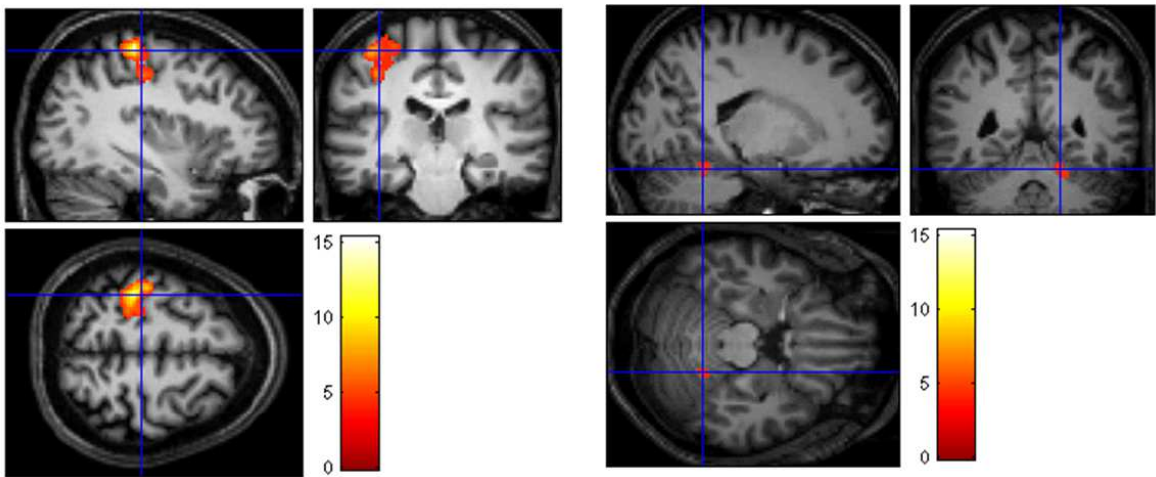
Figure 3.6. The figure demonstrates activation of controlateral motor cortical and omolateral cerebellar areas activated during Right hand finger tapping task in FRDA patients.
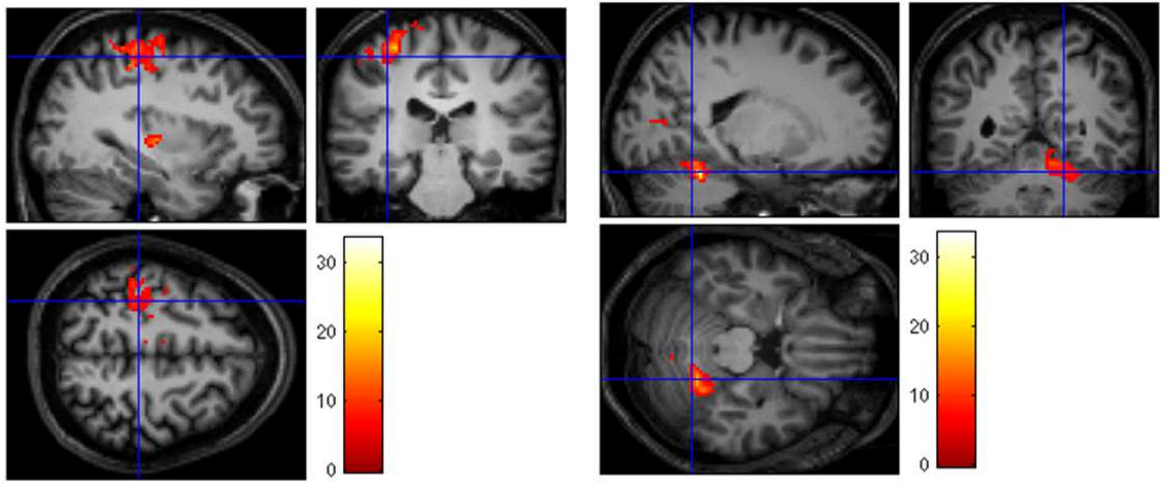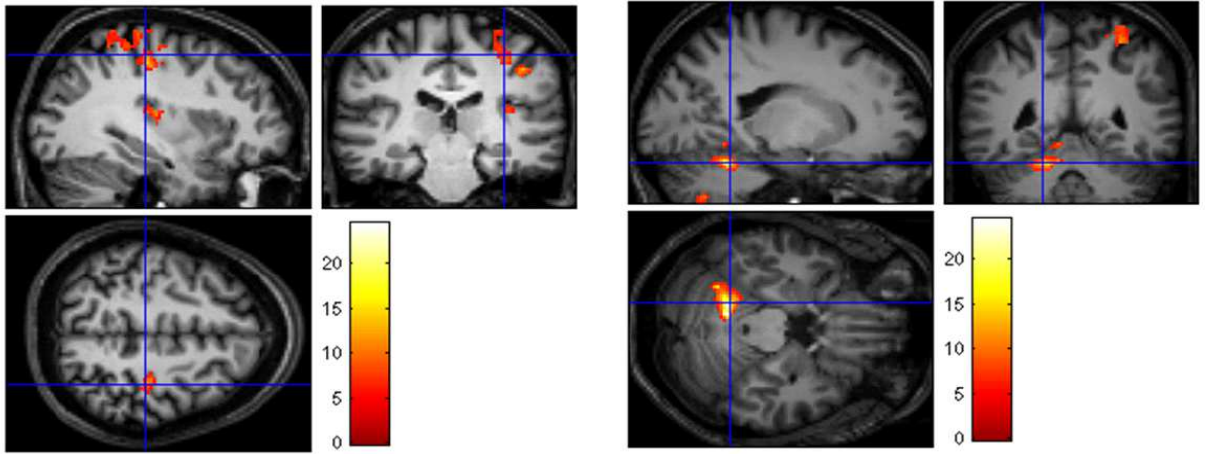
Figure 3.7. The figure demonstrates activation of controlateral motor cortical and omolateral cerebellar areas activated during Right hand finger tapping task in healthy controls.
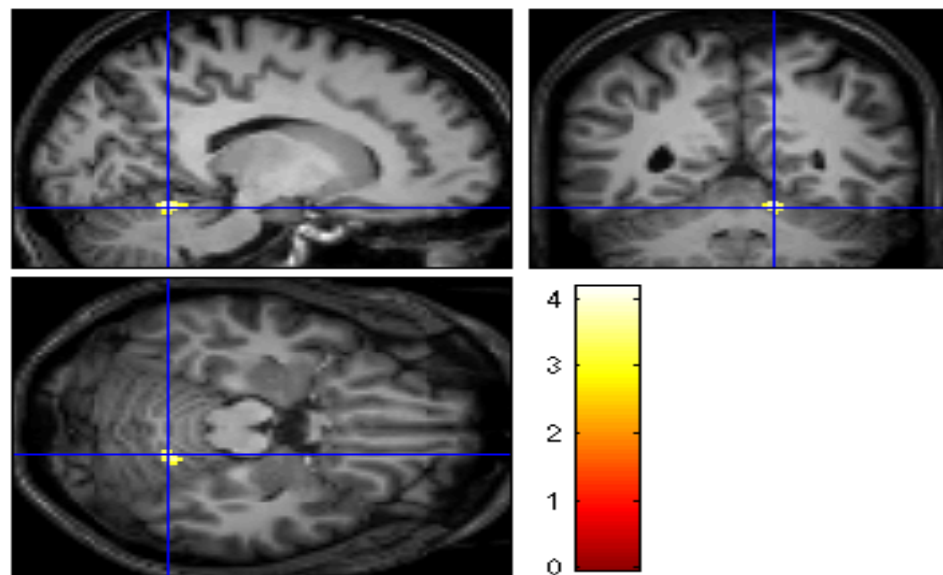

Figure 3.8. The figure demonstrates activation of controlateral motor cortical and omolateral cerebellar areas activated during Left hand finger tapping task in FRDA patients.

Figure 3.9. The figure demonstrates activation of controlateral motor cortical and omolateral cerebellar areas activated during Left hand finger tapping task in healthy controls.
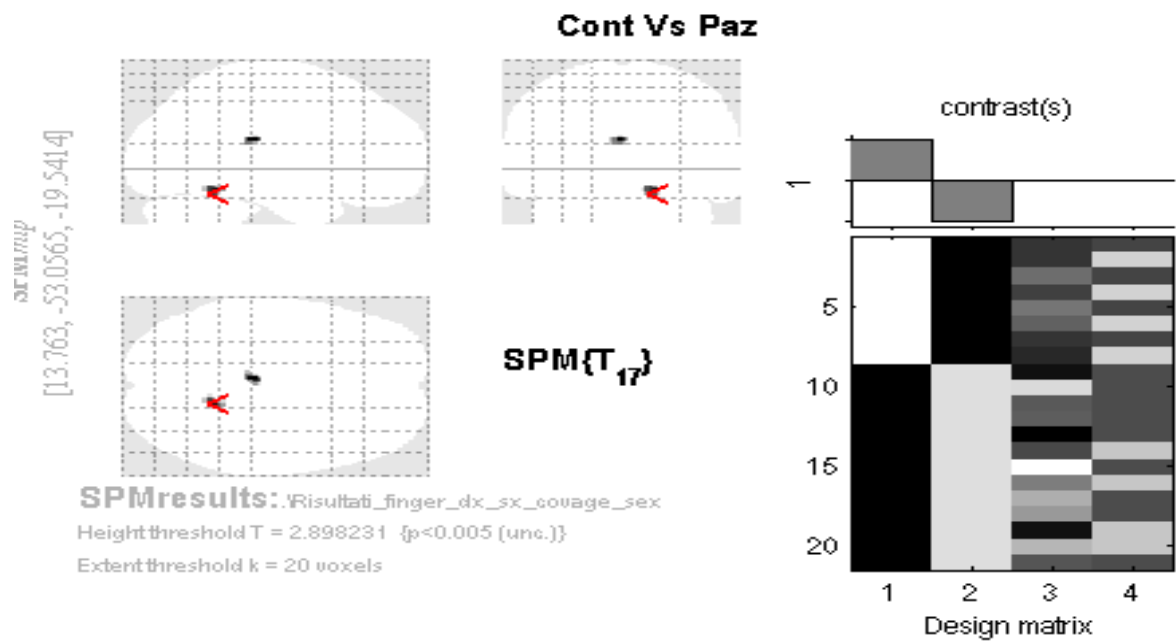
Figure 3.10. Right hand finger tapping task. The figure demonstrates areas more active in healthy controls compared to the FRDA patients.
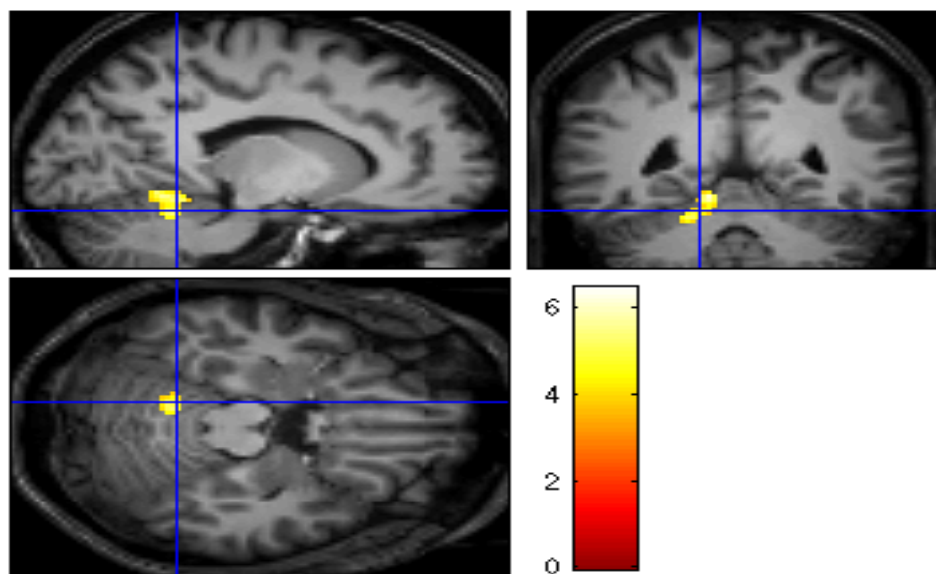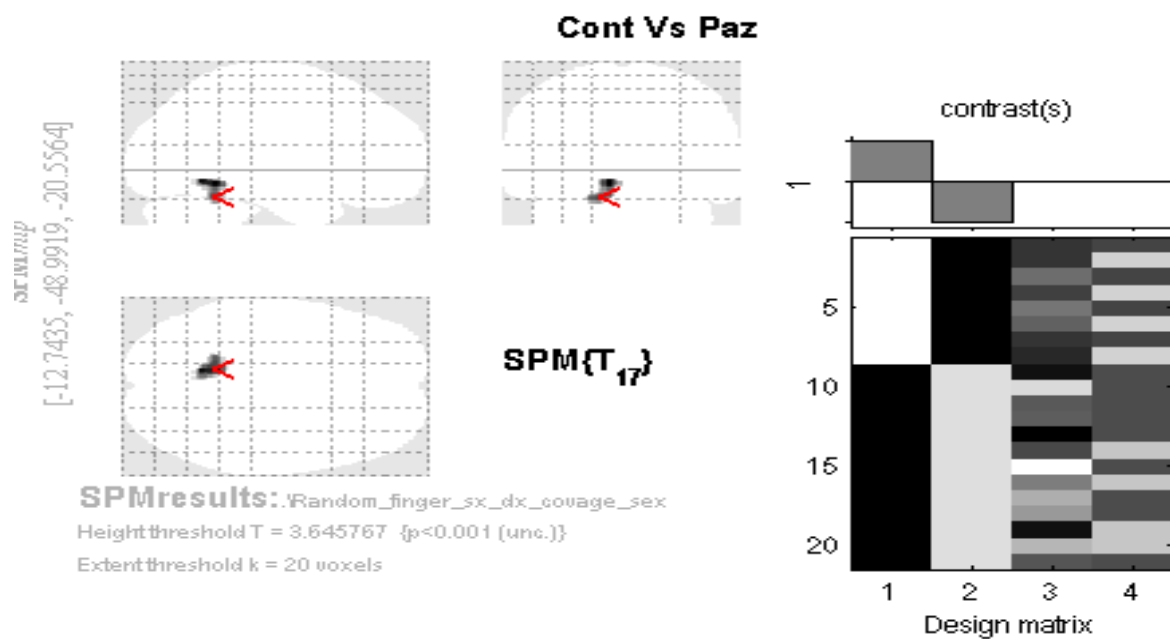
Figure 3.11. Left hand finger tapping task. The figure demonstrates areas more active in healthy controls compared to the FRDA patients.