Outcome measures in hereditary ataxias: analysis of clinical scales and evaluation of new tools to assess disease progression in Friedreich ataxia

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Summary

Introduction:

In Europe, a disease is considered rare when its prevalence is lower than 1:2.000. Cumulatively rare disease affects 3.5-5.9% of the world population. To date, 6-7000 rare diseases have been discovered and new diseases are added each year, it is estimated that 80% of these diseases have a genetic etiology. Most of these conditions present with neurological manifestations (Reinhard et al., 2020), while rare neurological diseases represent almost 50% of all rare diseases (Federico, 2013). Rare diseases are associated with unmet needs due to the lack of diagnosis and treatment measures as well as the difficulty to develop such measures, mostly related to the low number of subjects suffering from each disease. In Europe, as response to this challenge, the European Reference Network for Rare Neurological Diseases (ERN-RND) has been launched, and cerebellar ataxias are one of the disease groups ERN-RND is focusing on.

Hereditary ataxias

Hereditary ataxias are a heterogeneous group of rare genetic diseases sharing incoordination of gait and balance deficit as main feature. Incoordination of hands, dysarthria, eye movement impairment, sensory disturbances, pyramidal involvement and cognitive decline in various association are often part of the clinical picture (Figure 1). Hereditary ataxias can be divided according to the mode of inheritance (autosomal dominant or recessive, X-linked or mitochondrial) and the specific gene implied in the pathogenesis, or based on the clinical phenotype (e.g. Spinocerebellar ataxias – SCAs, Episodic ataxias – EAs, Spastic ataxias – SPAXs). Clinical overlap is frequent and a remarkable number of genes can produce hybrid phenotypes, ranging from pure cerebellar ataxia to pure spastic paraparesis. From here on hereditary ataxias will be discussed according to inheritance mode.
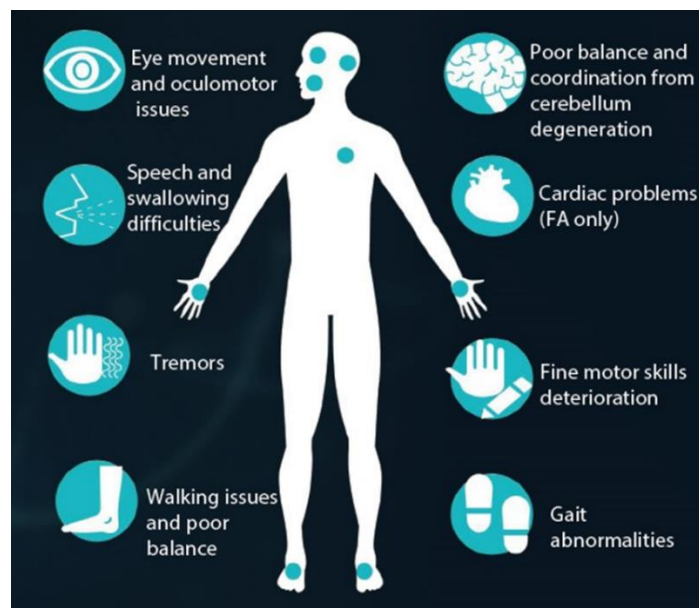

Figure 1. Graphical representation of main symptoms experienced by people with ataxia. From https://lacaf.org/en/ataxias/symptoms/

Autosomal dominant cerebellar ataxias (ADCA). ADCA have a worldwide prevalence estimated between 0 and 5.6/100.000, with SCA3 being most common subtype worldwide (Ruano et al., 2014); regional prevalence of SCA subtypes can vary regionally: for example SCA2 is more prevalent in Cuba (González-Zaldívar et al., 2015), SCA7 is the most frequent subtype in Venezuela (Paradisi et al., 2016), SCA36 is most prevalent in northern Spain, while in Italy SCA1 and SCA2 appear to be the most frequent forms (Klockgether et al., 2019)(Figure 2). CAG repeat expansions ADCA (SCA1, SCA2, SCA3, SCA6, SCA7, SCA12, SCA17, DRPLA) account for about 50% of ADCA diagnosis (Coutelier et al., 2017); mutation in other genes like SCA 8, 28, 36 are each responsible for less than 1% of ADCA (Aydin et al., 2018). Clinically, ADCA are marked by the presence of gait ataxia and incoordination, impairment of eye movements or visual problems and dysarthria. Additional features such as pyramidal, extrapyramidal signs, ophthalmoplegia and cognitive impairment are features in specific SCAs. Age of onset is variable, more frequently in adulthood. Disease course is also variable, although progression usually occurs over decades. In pre-genetic era Harding developed a classification that is still useful in the clinical practice (Harding, 1982), dividing ADCA based on clinical phenotype into 3 categories. ADCA type 1 encompasses cerebellar ataxia with variable additional signs; this list is in continuous expansion and includes so far more than 20 genes, including the most frequent forms such as SCA1-2-3 (Perlman, 1993). ADCA type 2 refers to cerebellar ataxia with macular degeneration, that was later identified as SCA7 (Perlman, 1993). ADCA type 3 describes "pure" cerebellar ataxia, such as SCA 5 (Perlman, 1993). Some ADCAs have characteristics features that may help distinguish them: for instance, SCA 14 may have myoclonus and dystonia (Chen et al., 2003) and SCA 36 may present with lingual fasciculation and sensorineural hearing loss (Kobayashi et al., 2011).

Episodic ataxias are also dominantly inherited. They are part of a larger group of diseases called channelopathies and they mostly manifest before adulthood (Jen et al., 2007). EAs are characterized by episodic attacks of imbalance/vertigo, dysarthria and diplopia lasting from minutes to days, and can be associated with other paroxysmal neurological manifestations as migraines, epilepsy or dystonia. In some patients with EA a progressive deterioration of cerebellar function may coexist, especially in older age (Graves et al., 2014).
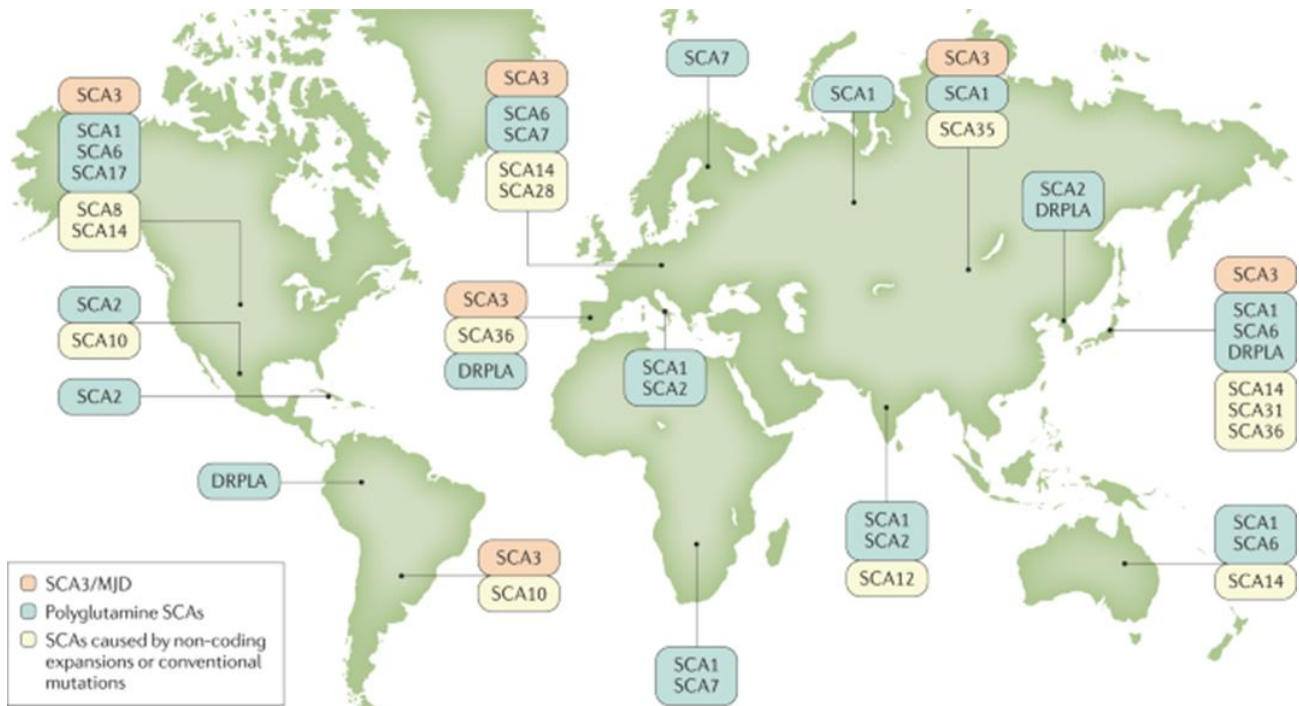
Figure 2. Global epidemiology of autosomal dominant cerebellar ataxias. From https://www.nature.com/articles/s41572-019-0074-3

Autosomal recessive cerebellar ataxias (ARCA). Several recessive multi-system or metabolic disorders may present with ataxia as main feature, making it difficult to isolate and classify this rapidly growing group of disorders in a clear way, despite recent classification proposals (Beaudin et al., 2019; Rossi et al., 2018). Recessive ataxias prevalence ranges from 0 to 7.2 cases/100.000 (Ruano et al., 2014). As with ADCAs, ethnic and regional specificities are an essential element to consider in recessive ataxias. Friedreich ataxia (FRDA) is the most common autosomal recessive ataxia and will be discussed separately since it is the focus of this PhD project. Prevalence of the other autosomal recessive ataxias is far lower compared to FRDA. Clinical manifestations are similar to ADCAs, although none of the autosomal recessive ataxias reported up to now presents exclusively with a pure cerebellar phenotype. Age of onset is variable from early childhood to old age, even for the same disease a high variability is reported. Besides FRDA this group includes: cerebellar syndromes with motor neuron involvement (e.g. SACS, SPG7, SYNE1), cerebellar syndrome with intellectual disability or cognitive decline (e.g. ANO10, ITPR1), cerebellar syndromes with polyneuropathy (e.g. ABHD12), cerebellar syndromes with extrapyramidal involvement and oculomotor apraxia (e.g. ATM, APTX, SETX) and metabolic or mitochondrial syndromes (e.g. POLG, AFG3L2). This classification takes into account the most frequent phenotype for each gene, but significant overlap between these categories is frequent. Recently, RFC1 gene biallelic AAGGG expansions have been identified as a frequent cause of cerebellar syndrome with sensory impairment and vestibular areflexia, a condition known as CANVAS (Cortese et al., 2019). Based on heterozygote carrier frequency of RFC1 AAGGG repeat expansions of 0.7-4%, the estimated prevalence ranges from 1:20,000 to 1:625 (Akçimen et al., 2019; Cortese et al., 2019), possibly making RFC1 spectrum disorder the most frequent cause of recessive ataxia.

X-Linked Hereditary Ataxias. X-linked inheritance of cerebellar ataxia occurs, but is quite rare. The only exception is represented by fragile X tremor ataxia syndrome (FXTAS) (Zanni and Bertini, 2011). FXTAS occurs in individuals who have an FMR1 premutation (55-200 CGG repeats) and is characterized by late-onset cerebellar ataxia, intention tremor and cognitive impairment. Because of potential repeat instability upon transmission, women with alleles in this range are considered to be at risk of having children with Fragile-X syndrome.

Ataxias with mitochondrial inheritance. Ataxia associated with mutation of mitochondrial DNA is generally part of complex phenotypes, manifesting with a combination of seizures, deafness, diabetes mellitus, cardiomyopathy, retinopathy, and short stature (Da Pozzo et al., 2009). Conditions showing this pattern of inheritance include NARP (neuropathy, ataxia, and retinitis pigmentosa), MERRF (myoclonic epilepsy with ragged red fibers) and Kearns-Sayre syndrome (Finsterer, 2009).

Digenic inheritance ataxia. Recently, a new form of ataxia has been identified as being the result distinct mutations occurring in *TBP* and *STUB1* genes (Magri et al., 2022). For the disease to manifest, intermediate alleles in TBP gene (CAG range 41-46) have to present in combination with heterozygous STUB1 mutation. Presence of either TBP 41-46 expansions or STUB1 variants individually is not associated with the disease, as opposed to previously thought. This is the first case of digenic-inherited ataxia. Clinical picture includes ataxia, cognitive decline and extrapyramidal manifestations, such as chorea and dystonia.

Measuring ataxia

Clinical scale are the basic tools for measuring neurological impairment in ataxia. Quality of a clinical scale is defined by its psychometric properties. Acceptable scale should not display relevant floor or ceiling effects. Linearity should be confirmed by regression analysis, applying a linear model. Influence of random errors due to different raters (inter-rater reliability), or to "noise" in repeated measurements under identical conditions (test-retest reliability) is evaluated as intraclass correlation coefficient (ICC). ICC values exceeding 0.8 are considered good. Cronbach's α (ideal >0.8) measures internal consistency, that describes how much items of the scale or of a sub-scale section are measuring the same feature. Construct validity is assessed via principal component analysis (PCA) and determines how much variability is explained by observed variables in respect to unobserved variables. In other words, construct validity assesses whether a scale tests what it is intended to test. There are several ataxia rating scales, the first scale proposed was the International Cooperative Ataxia Rating Scale (ICARS) (Trouillas et al., 1997), that was later followed by the Scale for the Assessment and Rating of Ataxia (SARA) in 2006 (Schmitz-Hübsch et al., 2006). Both these scale were created to rate motor aspects of cerebellar dysfunction in a population of Spinocerebellar ataxias. ICARS also incorporate some further elements (oculomotor, bulbar and sensory function) of the neurological examination that are absent in SARA. Friedreich Ataxia Rating Scale (FARS) was developed in 2005 (Subramony et al., 2005) to measure FRDA severity and progression and, similarly to ICARS, also rates

non-ataxia functions. FARS scale also includes the evaluation of daily living activities (ADL), that is applied also in non-FRDA ataxias. Both FARS and SARA scale have been tested for remote, video assessment (Tai et al., 2021). INAS (Inventory of Non-Ataxia Signs) scale was developed to quantitatively assess non-ataxia signs that contribute to disability and may interfere with ataxia (Jacobi et al., 2013). Less used scales, not specific for cerebellar ataxia, include Functional Independence Measure (FIM, (Keith et al., 1987) and the Modified Barthel Index (MBI, (Shah et al., 1989). FIM explores physical, psychological and social function while MBI is a measure of activities of daily living. A relevant aspect of the disease is also the assessment of health related quality of life (QoL). QoL questionnaires focus on patients' view of disability and symptoms and their impact in routine activities. QoL instrument adopted in FRDA include generic instruments like the Medical Outcomes Study 36 item Short Form Health Survey (SF36) and the EuroQol (EQ-5D, (Rabin and de Charro, 2001). SF36 examines general aspects shared by many diseases, and also includes symptom specific questionnaires (Epstein et al., 2008; Wilson et al., 2007). EQ-5D includes a self-rating of health-related quality of life on a 100-point visual analogue scale and a self-rated questionnaire. Friedreich Ataxia Impact Scale was specifically developed for use in FRDA as patients reported outcome to assess QoL (Cano et al., 2009). Quantitative outcome measures have also been developed over the years to increase reliability and sensitivity of ataxia measurement. For ataxia, a number of tests are in use that measure performance in specific tasks. These tests produce metric, continuous data that are more easily analysed. Due to the objective nature of the tests inter-rater reliability is usually higher compared to clinical scales. Assessments include simple time measurements, like the time needed to complete a 25-foot (ft) walk or 8 meters (T25FW and 8MW), syllable repetition rate (PATA), pegboard tests (9HPT), and low-contrast visual acuity (LCVA). More elaborated measures are derived from quantitative computerized assessment of gait, balance and upper limb functionality. The scores obtained reflect performances in the respective tasks only, so composite scores have been generated to provide a more global assessment of ataxia severity. Composite scores include the Ataxia Functional Composite Scale (AFCS) (Tai et al., 2017), the Spinocerebellar Ataxia Functional Index (SCAFI) (Schmitz-Hübsch et al., 2008) and the Composite Cerebellar Functional Score (CCFS) (Tezenas du Montcel et al., 2008).

Friedreich ataxia overview

Friedreich ataxia (FRDA) is a progressive neurodegenerative ataxia first described in 1863 by German physician Nikolaus Friedreich. It is the most common hereditary ataxia in the Caucasian population, with a prevalence of 2-4:100.000 and is inherited in an autosomal recessive manner. In Europe a prevalence gradient is present, with a prevalence of 1/20.000 in southern Europe and of 1/250.000 in northern and eastern Europe. Carrier frequency is estimated between 1/60 and 1/110 (Epplen et al., 1997)(Figure 3).
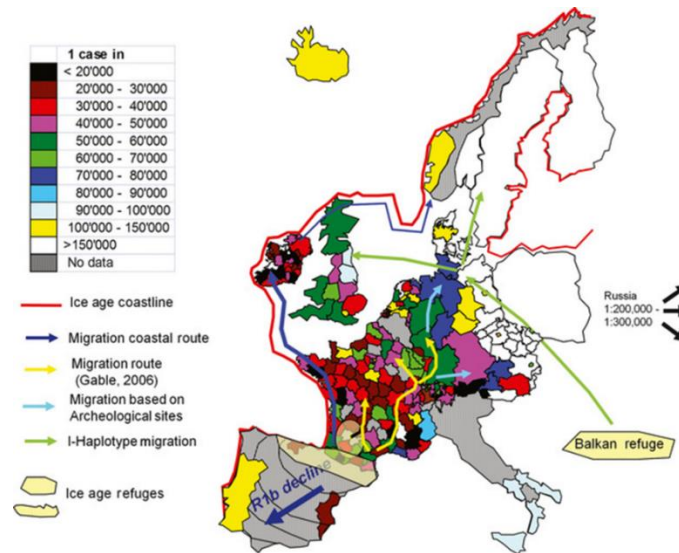
Figure 3. Summary of FRDA prevalence data in Europe. From https://pubmed.ncbi.nlm.nih.gov/23859338/

In the majority of cases the disease is caused by an abnormal GAA repeat expansion in the first intron of the *FXN* gene, encoding frataxin (Campuzano et al., 1996). GAA tracts containing less than 40 repeats are considered within normal range, the pathological threshold seems to be 70; triplet repeats in FRDA patients are most commonly between 600 and 900. About 1–3% of FRDA carry a compound heterozygous expansion and a point mutation or deletion of the frataxin gene (Gellera et al., 2007). The expanded GAA repeats lead to reduced quantities of FXN transcript (Bidichandani et al., 1998), with frataxin protein levels 70-95% lower compared to controls (Chutake et al., 2014). Complete loss of function of frataxin results in embryonic death in mouse models (Cossée et al., 2000) and has never been found in humans. Interestingly, however, frataxin protein and mRNA levels in FRDA patients partially overlap with controls and carriers (Saccà et al., 2011). A negative correlation exists between the length of GAA repeats in the shorter allele (GAA1) and levels of FXN transcript and frataxin protein (Chutake et al., 2014). The expanded GAA repeat has been shown to form non-canonical DNA structures (Li et al., 2019), resulting in the formation of a repressive chromatin state, gene methylation and silencing that are relevant causes of FXN transcriptional deficiency (Delatycki and Bidichandani, 2019). Frataxin is a mitochondrial protein produced from the most abundant FXN transcript derived from exons 1–5a (Campuzano et al., 1996). Its expression reflects the main sites of involvement in FRDA as it is highly expressed in dorsal root ganglia, spinal cord, cerebellar dentate nuclei, cerebral cortex, pancreas, heart, liver and skeletal muscle (Campuzano et al., 1997)(Figure 4). The 210 amino acid polypeptide undergo cleavage to the final 130 amino acid mature protein by mitochondrial processing peptidase (Schmucker et al., 2008). Different isoform of frataxin are also known, some of them tissue-specific, like frataxin isoform E, that is present in erythrocytes which do not have mitochondria (Guo et al., 2018). Frataxin is located in the mitochondrial matrix and is part of a complex that assembles iron-sulfur clusters (ISC) (Shan et al., 2007). ISC are necessary cofactors in Krebs cycle and for mitochondrial respiratory complexes I, II, and III. Frataxin deficiency hence results in a reduced mitochondrial ATP

production. Other, less understood, functions of frataxin involve iron metabolism, heme synthesis, regulation of apoptosis and as antioxidant agent (Pastore and Puccio, 2013). As a result of frataxin deficiency and reduced ISC biosynthesis, iron is translocated to the mitochondria, accumulates and is oxidized (Pandolfo and Hausmann, 2013). Production of reactive oxygen species, resulting in greater oxidative stress burden, leads to increased mitophagy, impaired cytoskeletal dynamics, abnormal calcium homoeostasis, altered lipid metabolism and cell death, possibly also via ferroptosis (Cotticelli et al., 2019). Pathological intramitochondrial iron deposition has been reported in cellular and animal FRDA models, as well as in cardiomyocites and in the dentate nuclei of the cerebellum in subjects with FRDA (Ward et al., 2019).
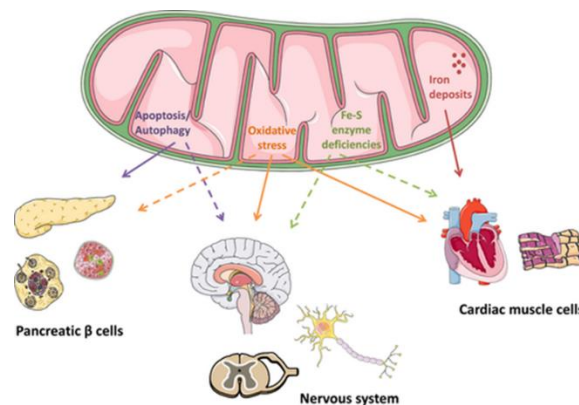

Figure 4. Schematic representation of the biological pathways altered in FRDA as consequences of frataxin deficiency in mitochondria. From https://onlinelibrary.wiley.com/doi/full/10.1111/jnc.12303

Clinically, FRDA presents as a multisystem disorder, involving central and peripheral nervous systems, myocardium, musculoskeletal system and endocrine pancreas. Friedreich, more than 150 years ago, correctly identified the core of the typical phenotype: poor balance, leg weakness, decreased walking capabilities, impaired coordination, dysarthria, nystagmus, impaired sensation, kyphoscoliosis and foot deformities. He also described the presence of degeneration of dorsal spinal cord and of fatty degeneration in the myocardium and noted the hereditary nature of the condition (Pearce, 2004). Age at onset is between 10 and 16 years in the majority of subjects, with typical onset being before age 25 (Harding, 1981). After the identification of the genetic abnormality underlying FRDA, it has been shown that about 25% of FRDA patients can present onset after 25 years of age, in some cases up to the seventh decade (late onset FRDA) (Bhidayasiri et al., 2005). Gait instability is the most common presenting symptom, in a smaller proportion of patients scoliosis or cardiac abnormalities are firstly discovered while neurological signs may or may not be observed (Indelicato et al., 2020). The neurological phenotype is broad, but gait instability, limb incoordination, dysarthria and lower limb areflexia are found in all typical cases. The ataxia is of mixed origin, resulting from spinocerebellar degeneration, peripheral sensory neuropathy, cerebellar and vestibular involvement (Delatycki and Corben, 2012). Pyramidal signs may be present. Abnormalities are apparent with using MRI, showing mild atrophy of the cerebellar dentate nucleus and of the superior

vermis and superior cerebellar peduncle, prominent atrophy of the dorsal columns causing and decreased spinal cord diameter especially in the cervical region (Rezende et al., 2019). As disease progresses, patients develop increasing dependence on walking aids, initially relying on intermittent support of walls and other people, and later needing sticks and wheeled walkers. Ambulation is typically lost after 15 years from disease onset (Rummey et al., 2020a). In advanced stages truncal ataxia results in difficulties in sitting. Limb ataxia leads to reduced independence in daily activities such as handwriting, washing, dressing and eating. Weakness and muscle wasting are relatively late signs, much more prominent in the lower limbs and resulting from pyramidal degeneration and motor neuropathy. Muscle weakness and wasting is reported in 25-80% of patients according to disease stage (Dürr et al., 1996; Filla et al., 1990; Harding, 1981). Sensory loss is invariably present in typical onset FRDA, with particular impairment in perception of vibration and joint position. Neurophysiological studies show severe axonal neuropathy and abnormal central conduction, which do not appear to progress significantly over time. Abnormalities of eye movements manifest mainly as fixation instability and square wave jerks, found in 70-100% of patients (Fahey et al., 2008; Schöls et al., 1997). Smooth pursuit movements may have reduced velocity, and may be interrupted by saccadic intrusions in 12–30% of cases (Dürr et al., 1996; Harding, 1981). Saccadic velocities are often normal but dysmetria is very common (Fahey et al., 2008). Despite the common occurrence of these abnormalities, their impact on patients' impairment is limited. Decreased visual acuity is less common than eye movement abnormalities and usually presents in advanced stages. Approximately 20% of patients develop visual impairment, though all patients have reduced retinal nerve fibre layer thickness on optical coherence tomography (Dürr et al., 1996; Fortuna et al., 2009; Harding, 1981). Dysarthria is present in more than 90% of individuals, and progresses with disease duration, leading to impaired intelligibility and anarthria in advanced cases. Mild dysphagia is again a common symptom (27-74% of patients (Dürr et al., 1996; Filla et al., 1990; Schöls et al., 1997) and can lead to severe consequences in advanced disease, such as aspiration pneumonia, one of the most frequently recognized cause of death. Sensory degeneration can also affect auditory nerves leading to hearing difficulties (Rance et al., 2012). Hearing impairment is frequent, affecting up to 39% of patients (Schöls et al., 1997), typically manifests as impaired speech understanding in conditions of background noise and can be socially disabling. Symptoms of bladder hyperactivity, such as urinary urgency and secondary urinary incontinence, are common in FRDA (prevalence 23%-41%, (Delatycki et al., 1999; Dürr et al., 1996); bowel symptoms appear to be less frequent. Cognition is not affected in FRDA, though FRDA patients show an overall impairment in cognitive tests measuring working memory, fluencies and visuo-spatial functions compared to healthy controls (Corben et al., 2011). Cognitive performances could reflect cerebellar pathology, as a correlation between cognitive deficits and atrophy of specific area of cerebellar cortex has been identified in FRDA (Cocozza et al., 2018). In late stages of the disease hallucinations and delusions may appear, as a result of a deafferentation syndrome secondary to visual and hearing loss (Fichera et al., 2022).

Non-neurological manifestations of FRDA include cardiomyopathy, diabetes mellitus and skeletal abnormalities. Evidence of some degree of cardiac involvement is present in the majority of cases of FRDA although the patients are very often asymptomatic. ECG is abnormal in 60-93% of subjects (Pousset et al., 2015; Reetz et al., 2018), with repolarization abnormalities – T-wave inversion or flattening in lateral or inferior leads, ST-segment depression or elevation – being the most reported findings. On echocardiography increased end-diastolic septal and posterior wall thicknesses, and left ventricular concentric hypertrophy are reported in 45-60% of patients (Pousset et al., 2015; Reetz et al., 2018). Ejection fraction is generally within normal range, though longitudinal data suggests that in 20% of patients a decline in function with age and disease duration may develop leading to cardiac failure, along with a progressive increase in left ventricular dilatation (Regner et al., 2012). Evolution of cardiomyopathy seems to be correlated with the size of the GAA1 repeat (longer GAA1 tracts are associated with worse outcome) (Pousset et al., 2015). In the advanced stages of disease supraventricular tachyarrythmias, most commonly atrial fibrillation, can manifest and may worsen systolic function. Diabetes mellitus was recognized as a manifestation of FRDA more recently compared to other features (Thoren, 1962), prevalence of impaired glucose tolerance ranges between 1% and 32% (Dürr et al., 1996; Reetz et al., 2018). Mechanism underlying diabetes in FRDA is unclear, but seems to involve both peripheral insulin resistance and decreased insulin secretion due to pancreatic beta cell dysfunction (Cnop et al., 2013). Scoliosis is common (33-100% of patients, (Reetz et al., 2018; Rummey et al., 2021) and involves typically thoracic and lumbar tracts. It is usually mild and in half of cases non-progressive, especially in patients with onset in later age. Hyperkyphosis can be associated to scoliosis in 24-36% of patients. Foot abnormalities are common as well, and are present in 55 to 90% of cases (Delatycki et al., 1999; Dürr et al., 1996; Filla et al., 1990; Harding, 1981; McCabe et al., 2000; Schöls et al., 1997). Among feet abnormalities pes cavus is the most represented, though talipes equinovarus and pes planus occur, either singly or in combination.

Late-onset FRDA (LOFA) is defined as age at onset >25 years. Ataxia remains the main findings, but the phenotype is generally milder compared to typical onset FRDA. LOFA is also associated to smaller GAA expansions (Bhidayasiri et al., 2005). Neurological progression evaluated via ataxia rating scales is less pronounced in LOFA, and disease duration before wheelchair dependence is longer than in typical cases (Rummey et al., 2020a). Sensory neuropathy is less pronounced and can be absent in up to 50% of LOFA. Spasticity and sustained reflexes are present in 22-40% of LOFA, but are absent in typical FRDA patients. LOFA patients also display a lower frequency of the non-neurological features of the disease: diabetes is reported in 3.5-6% of patients, heart disease in 11-20%, scoliosis in 14-40% and foot deformities in 25-35%; abnormal ECG, however, is found in almost all cases (Bhidayasiri et al., 2005; Reetz et al., 2018). Atrophy of the superior vermis rather than the cerebellar hemispheres has been shown in some LOFA patients (Bhidayasiri et al., 2005).

Measuring Friedreich ataxia

In the past 15 years a great deal of effort has been dedicated to the development of ideal outcome measure to assess FRDA disease stage and progression. Potential outcome measures should have excellent reliability, reflect the changes in clinical severity of the disease and linearly progress from the initial to the most advanced phases. Finally, potential outcome measures should be easily and widely applicable to clinical practice. So far, no ideal outcome measures has emerged for the assessment of FRDA. Many outcome measures have been proposed over the years, and their use in subjects with FRDA will be discussed below. Main psychometric properties of most used clinical scales and functional tests are summarized in Table 1, while a summary of the most important studies assessing outcome measures in FRDA is reported in Table 2 at the end of the section.

1. Clinical Scales

1.1 ICARS

ICARS is a 19–items scale that requires at least 20 minutes for completion. Score ranges between 0 and 100, with higher scores reflecting higher disability. ICARS score is determined by the sum of four subscores that refer to posture/gait, limb ataxia, dysarthria and oculomotor function. The scale was first used in 77 FRDA patients by Cano and coworkers (Cano et al. 2005). The study revealed that items of each subscale shared a common theoretical construct. Some items ("finger-finger test" and "fluency of speech" items) did not reach the recommended item-total score correlation of 0.3. Posture/gait subscale showed the highest correlations between item and subscale, except for one item, "quality in sitting". For the other subscales item-subscale correlations were of the same magnitude of item-other subscale correlation, providing less support to grouping in subscales. Cronbach's α and test-retest ICCs were high (>0.85) for total ICARS and for posture/gait and limb ataxia subscales, while the two remaining subscales failed reaching criteria for reliability. Intercorrelations between subscales ranged 0.30-0.75, meaning that subscales captured related yet distinct constructs. No ceiling or floor effects were reported. In a 2009 study (Bürk et al., 2009) ICARS was evaluated in 96 FRDA patients. Cronbach's α was 0.69 when considering all four ICARS subscales. All items, except 3, were significantly correlated to the ICARS total score (mean r=0.72). PCA identified four factors with an eigenvalue greater than 1. ICARS properties were reassessed in a subsequent large cross-sectional study on 603 FRDA patients (Metz et al., 2013). The item-subscale correlations ranged from 0.37 to 0.94, the highest correlation was seen for the posture/gait items followed by speech and kinetic functions items. Oculomotor subscale items had the lowest correlation; items assessing lower limb function correlated better with the posture/gait subscale than with their own subscale. Cronbach's α varied from 0.6 (oculomotor subscale) to 0.94 (posture/gait subscale and total ICARS). ICARS total sum score did not show significant floor or ceiling effects. However, the authors found evidence for ceiling effects in "posture/gait"

and "lower limb" items, leading to reduced sensitivity in advanced stages. Items in the oculomotor subscale were more prone to floor effect, as it was the "quality of sitting" item. Authors showed that after a linear increase in the first 20 years of disease duration the scale reached a plateau. No correlation of ICARS sum scores and disease duration was present after >20 years of disease duration.

<u>1.2 FARS</u>

Unlike other hereditary ataxias FRDA is characterized by relevant sensory dysfunction, and FARS scale was developed and validated specifically for addressing this peculiarity (Subramony et al., 2005). FARS is a 35–item scale (range 0-159) calculated as the sum score of an examination score derived from neurological assessment (FARSn, 25 items, maximum score 125), a functional ataxia staging score (range 0-6) and an assessment of the ADL (9 items, range 0-36). FARSn is composed of bulbar (subscale A, range 0-11), upper (B, 0-36) and lower limbs (C, 0-16), peripheral nerve (D, 0-26) and posture/gait (E, 0-28) subscores. FARS assessment takes about 30 minutes. In the validation study (Subramony et al., 2005), all items and subscales showed inter-rater ICC>0.75, except bulbar and peripheral scores. Rater bias was present for disease stage, ADL, upper limb coordination, peripheral nerve scores, and total FARSn. Subsequently, Fahey and colleagues (Fahey et al., 2007) evaluated 43 FRDA patients at baseline and after a 12-months interval, showing a significant decline over 12 months. Disease duration was significantly correlated with FARS. Burk and colleagues (Bürk et al., 2009) evaluated FARSn properties along with ICARS and SARA scales. No ceiling or floor effects were detected. For the five subscales of FARSn (A-E) internal consistency was high (Cronbach's α=0.86), single items were significantly correlated to the total score (mean r=0.69), though 5 items showed weak correlations (r<0.4 for facial atrophy, action myoclonus, weakness, finger to finger test and deep tendon reflexes items). PCA identified five different factors for FARSn. Over the years FARS scale has been applied in longitudinal observational studies in both American (Friedreich Ataxia Clinical Outcome Measure Study – FA-COMS) and Australian cohorts. A 5-years quantitative characterization of FARS scale in a large population of FRDA was provided by Patel and colleagues (Patel et al., 2016). Baseline assessment involved 802 patients, 290 patients reached the 5-years follow-up. A modified version of FARSn (mFARS, max score 93) was also assessed in this study, score for this version does not include items A1 and A2 (facial and tongue atrophy), and the whole peripheral nervous system subscore. FARSn score was correlated with disease duration (r=0.59), disease stage and ADL (r=0.84). Subsequently, Rummey and colleagues re-evaluated FARSn and mFARS psychometric properties (Rummey et al., 2020b, 2019a). Mean item-total correlations for mFARS subscales were ≥0.58, while subscales A and D of FARSn showed mean values ≤0.42. Cronbach's α values were satisfactory for the upper and lower limb, as well as for the posture/gait subscales (α>0.85). Clearly lower α coefficient was reported for the modified bulbar subscale of the mFARS (0.65), though improved from the subscale in FARSn (0.53). All items in the mFARS subscales correlated (r>0.3) with their subscores, indicating a common construct. Ceiling effects were apparent in subscale C (heel-shin tap and heel-shin slide, 21% of observations showing the maximum score) and for subscale E

(upright stability), corresponding to the number of non-ambulatory patients (32%). In the 9-item subscale E, all 6 stance items revealed a distinct bimodal distributions: a patient could either perform the test well (score 0) or be unable to complete the task. Floor effects were only visible in the bulbar subscale for both FARSn and mFARS. PCA identified in mFARS 3 components with eigenvalues >1, items in FARS E and C loaded strongest in component 1, FARS B loaded into component 2, component 3 included items of FARS A. The remaining 3 remaining items of the FARS E with high amounts of "unable" loaded into another component. Test-retest reliability was excellent for both FARSn and mFARS (ICC>0.9). The subscores showed similar results with ICCs ranging between 0.73 (bulbar) and 0.95 (upright stability). ADL scale is 9-items scale (score range 0-36) part of the extended FARS scale, that is currently used to assess the functional impact of FRDA disease progression even as a stand-alone scale. Although extensively adopted in both observational and interventional trial in FRDA, its psychometric properties have not been reported. In the original publication by Subramony and coworkers (Subramony et al., 2005), excellent inter-rater reliability was reported (ICC=0.94). The ADL subscore correlates with disease duration, disability score and neurological scales (r>0.80) (Reetz et al., 2015; Subramony et al., 2005).

1.3 SARA

SARA is 8-item semi-quantitative scale for the assessment of cerebellar ataxia (Schmitz-Hübsch et al., 2006) and does not include the evaluation of any extracerebellar features. This scale was initially meant to be used in the assessment of SCAs (Schmitz-Hubsch et al., 2010; Schmitz-Hübsch et al., 2006; Weyer et al., 2007), where longitudinal metric properties were validated (Schmitz-Hubsch et al., 2010). Compared to previous scales is more compact and its administration takes about 10 minutes. The eight items composing the scale are: gait (score 0-8), stance (0-6), sitting (0-4), speech disturbance (0-6), finger chase (0-4), nose-finger test (0-4), fast alternating hand movements (0-4), and heel-shin slide (0-4); maximum score is 40. For limb testing arithmetic mean of both sides is considered for sum scores. Burk and coworkers provided psychometric data in a FRDA population (Bürk et al., 2009). No floor or ceiling effect was reported, inter-rater reliability of whole SARA scale was high (ICC=0.99), although items rating upper limb functions showed considerably lower values (ICC 0.51-0.59). Internal consistency for the eight items was 0.89. All SARA items were significantly correlated to SARA total score, mean item-total score correlation was 0.71. SARA items also showed good convergent and divergent validity. In PCA analysis SARA items loaded on a single factor with an eigenvalue of 5.24 explaining 66% of the observed variance. SARA score was significantly correlated with disease duration (r=0.72). SARA scale is applied to monitor neurological progression in the ongoing longitudinal European cohort of FRDA patients (EFACTS) (Reetz et al., 2021, 2016, 2015).

The INAS scale was intended to measure neurological signs that are not covered by SARA (Jacobi et al., 2013). It consists of 30 items that rate: osteotendineus reflexes, extensor plantar reflex, spasticity, paresis, muscle atrophy, fasciculation, myoclonus, rigidity, chorea/dyskinesia, dystonia, resting tremor, vibratory sense, saccades and pursuit, visual acuity. It also includes a section on reported symptoms (double vision, dysphagia, urinary dysfunction, cognitive impairment, muscle cramps, handwriting impairment, speech problems and episodic vertigo). INAS count was defined by the presence/absence of 16 non-ataxia abnormal signs (areflexia/hyper-reflexia, extensor plantar response, spasticity, paresis, atrophy, fasciculations, myoclonus, rigidity, chorea, dystonia, resting tremor, sensory impairment, brainstem oculomotor signs, urinary dysfunction, cognitive impairment). Each sign is rated as present if at least one item related to the sign is positive, leading to a value ranging from 0 (absence of non-ataxia signs) to 16 (most severe extracerebellar involvement). Psychometric properties of the scale have been assessed in SCA patients (Jacobi et al., 2013) in a longitudinal study. Inter-rater reliability of the total INAS count was high (ICC=0.88), 5/30 items had only moderate (r=0.40–0.60) reliability. ICC was also high for test-retest reliability (r=0.89). At 2-years follow-up INAS count change was 0.56±1.78 in the whole group, SRM reached the criterion of small effect (0.31). Floor effect was present for 2.1-3.4% of subjects; ceiling effect did not occur. INAS psychometric properties have not been evaluated in FRDA patients. INAS scale has been applied in conjunction with SARA scale to monitor disease progression in EFACTS study, but it showed poor ability to identify worsening of the disease (Reetz et al., 2021, 2016).

1.5 SF-36, EQ-5D, FAIS and PROM-Ataxia

Patient-reported measures provide information on patients' quality of life, daily functioning, symptoms, and may capture aspects of their health and well-being that are not covered by physicians evaluation. In a recent study in SCA3 patients, Maas and colleagues showed a discordance between patient-reported and clinician-based outcomes, indicating that these measures genuinely evaluate distinct aspects of disease (Maas and van de Warrenburg, 2021). The SF-36 is a generic measure of health related quality of life and is composed of 36 items categorized into eight dimensions: physical function, role physical, bodily pain, general health, vitality, social function, role emotion and mental health. The SF-36 has been updated to version 2 in 1999 (SF-36V2; (Jenkinson et al., 1999)), this version reduced ceiling and floor effects by changing from dichotomous response categories to five-point response categories. In FRDA both versions have been applied in cross-sectional and longitudinal studies. Wilson and colleagues found that FRDA have worse perception of their health status and quality of life compared to control population; similar findings were reported by Epstein et al. in a US cohort (Epstein et al., 2008). Subsequent studies showed that SF-36V2 physical component subscale was significantly correlated with FRDA clinical parameters (disease duration, FARS score, age at onset), however no significant changes in the SF-36V2 were seen over 1 or 2 years despite increased disease severity seen on FARS scale (Tai et al., 2017; Xiong et al., 2020). EQ-5D

includes a questionnaire on five dimensions (mobility, self-care, usual activities, pain and discomfort, and anxiety and depression) plus a self-rating of health-related quality of life on a 100-point visual analogue scale. Psychometric properties of both scales were assessed by Riazi et al. (Riazi et al., 2006). EQ-5D scores did not span the entire scale range, Cronbach's alpha was lower than 0.6 for the unweighted items. Visual analog scale mean score (64.3) was above midscale (50). Longitudinal variations over time were non-significant. Low sensitivity to change was reported by Reetz et al. as well (Reetz et al., 2016). FAIS scale was initially developed as a 126-items scale and validated in 307 FRDA patients (Cano et al., 2009). This 126-items scale includes six subscales with three response options (speech, body movement, upper limb, complex tasks, self-perception, isolation) and two subscales with four response options (lower limb, mood). FARS score, onset age and disease duration correlated significantly with FAIS subscales measuring symptoms and physical functioning, SF-36V2 and FAIS scales were also well correlated (Tai et al., 2015). Poor responsiveness was reported in a 2-years longitudinal study (Tai et al., 2015). The most recent tool developed in the field of patient reported outcome is PROM-Ataxia, developed in 2021 by Schmamann and coworkers (Schmahmann et al., 2021). The questionnaire is composed of 70 items, divided into three sections (Physical, Activity of daily living and Mental domains) and assess patient' experience of ataxia. Performances of the scale were tested in 78 subjects with ataxia due to different causes. PROM-ataxia score was correlated with measures of ataxia severity (such as SARA and BARS) and with ADL part of FARS scale. The ten most correlated items were included in a short version of PROM-ataxia. PROM-ataxia has not been tested in other cohorts of subjects with ataxia.

1.6 Comparatory studies

A few papers have compared performances of the aforementioned clinical scale in cross-sectional and longitudinal studies. Burk and coworkers compared the performances of SARA scale to FARSn and ICARS in a cross-sectional study on 96 FRDA patients (Bürk et al., 2009). SARA, ICARS and FARSn scores were significantly correlated between them (r>0.9 in all cases), with disease duration (SARA r=0.71, ICARS r=0.70, FARSn r=0.68) and with ADL (r>0.9 in all cases). Psychometric properties were calculated for all three scales and are reported in the previous sections. Due to the cross-sectional nature of this study responsiveness to change was not calculated. In the first EFACTS work (Reetz et al., 2015), are reported unpublished data (provided by Schulz JB) on 53 FRDA patients evaluated longitudinally for 12 months; standardized response mean (SRM) was reported to be higher for SARA (0.40) compared to ICARS (0.30) and FARS (0.30). Fahey and colleagues compared sensitivity to change and responsiveness of FARS to ICARS, FIM and MBI in 76 FRDA patients (Fahey et al., 2007). FARS sum scores (FARSn + ADL score + functional disability stage), FARS examination scores and ADL scores were significantly correlated with MBI, FIM and ICARS scores (r>0.85 in all cases). The FARS functional disability subscore showed the highest correlation with FIM, while FARSn correlated best with ICARS scores. In 43 participants a second evaluation with the same measures was performed after a 12-months interval. The mean change was 9.5±9.1 points on FARS, 5.0±6.8 on ICARS,

3.1±6.0 on FIM and 1.9±6.2 on MBI. Progression over time proved significant for all scales except MBI, with total FARS showing the largest effect size (0.34). Sensitivity to change and effect size were similar between FARSn, mFARS in a longitudinal observational study with 597 FRDA subjects completing at least first follow-up (Patel et al., 2016). Direct comparison of mFARS and SARA scale in a homogenous cohort is not present in the current literature.

2. Functional Measures

2.1 AFCS

The AFCS was specifically designed for FRDA as a modified version of the Multiple Sclerosis Functional Composite (Lynch et al., 2006). It is composed of T25FW, 9HPT and LCVA. T25FW was chosen since it was identified as the most accurate measure of ambulation in another study (Fahey et al., 2007) comparing T25FW to 6-minutes walk and timed up-and-go tests and using step activity monitoring as a quantification of ambulatory capacity. For AFCS, Z-scores are then calculated for each test and the arithmetic mean of the three tests (Z3) is considered as the final score; composite scores from the $9HPT^{-1}$ and the $T25FW^{-1}$ scores are designated Z2. AFCS was at first examined in 155 FRDA patients (Lynch et al., 2006). Nineteen (12%) and 79 (52%) of these patients were unable to perform 9HPT and T25FW due to FRDA severity. Of the 73 patients who completed the T25FW, 43 used no assistive device, 8 used unilateral assistance, and 22 used bilateral assistance. All the tests composing the scores showed excellent test-retest reliability (ICC 0.97-0.99). Data were skewed and significant ceiling effect was present for LCVA. Each performance measure correlated significantly with disease duration and severity evaluated with ADL, FARS, and functional disability score, Z2 and Z3 scores correlation coefficients were higher than those of individual measures. Subsequent studies also evaluated AFCS performances in longitudinal studies. Tai and colleagues (Tai et al., 2017) compared performances of AFCS to clinical scales in a longitudinal study. Scores for 9HPT, Z2 and Z3 significantly worsened over the first, second and third year. T25FW changes were significant at year 2 and 3, while LCVA change did not reach significance at any timepoint. This may suggest that Z3 is redundant, as change over time is driven mostly by changes in the 9HPT and T25FW. In this cohort of patients AFCS did not outperform the FARS score in terms of sensitivity to change over a 12 month period. This finding partially contrasts with results from Friedman and colleagues (Friedman et al., 2010) who showed that, despite having higher standard deviations compared to FARS, Z2 and Z3 measure change over time more linearly. The authors also suggested that FARS and Z3 are more useful for testing non ambulatory patients compared to Z2. In a 5 year longitudinal study (Patel et al., 2016) Z2 and Z3 were not more sensitive to change compared to FARS and mFARS in a large FRDA cohort (812 subjects at baseline, 290 at year 5), moreover Z3 was not superior to Z2. Composite scores seemed to progress more linearly with disease severity compared to individual performance outcomes. Another subsequent stud (Hamedani et al., 2018) in 432 patients followed up for a median of 5 visits showed significant changes over time for LCLA. The

mean difference in acuity compared to baseline at 100%, 2.5%, and 1.25% contrast, demonstrated a linear pattern of decrease, particularly at low contrast.

2.2 SCAFI

SCAFI was initially proposed as a functional measure for the assessment of dominant ataxias and tested in 412 SCA patients (Schmitz-Hübsch et al., 2008). Similarly to AFCS, SCAFI is composed of three different functional tests: 8MW (roughly corresponding to T25FW), 9HPT and PATA scores. PATA rate refers to how often the subject can repeat the syllables "PATA" within 10 seconds, the test is performed twice and mean value is considered for analyses. Results of each subtest (PATA, $9HPT^{-1}$, and $8MW^{-1}$) are transformed into Z-scores, SCAFI score is the arithmetic mean of the Z-scores from the 3 subtests. All subtest Z-scores as well as SCAFI were linearly correlated with SARA score, correlation was highest for SCAFI and lowest for PATA. In a 1-year follow-up study on 171 patients from the same cohort (Schmitz-Hubsch et al., 2010) SRM reached the criterion of moderate effect for 9HPT (-0.67), PATA (-0.24) and SCAFI (-0.48) scores; 8MWT did not show significant changes over time. It has to be noted that PATA scores showed weaker correlation with FRDA disease features compared to other functional measures (Friedman et al., 2010; Lynch et al., 2006). SCAFI was chosen as functional composite measures in the ongoing EFACTS study. A 2-years follow-up report showed that SCAFI Z-score could be calculated for 579/605 subjects at baseline; mean annual change was calculated to be −0.04, corresponding to a SRM of 0.05-0.13 (Reetz et al., 2016).

2.3 CCFS

CCFS was developed as a reliable and easily performed quantitative functional test of cerebellar dysfunction. CCFS is composed of 9HPT and click test, both tasks are carried out only with the dominant hand. Time to complete the tests are transformed into Z-scores, and values adjusted by age group. An electronic device is used to acquire test times automatically and to calculate the score. The final CCFS score is calculated as $\log_{10}[7+(Z$ pegboard dominant hand$)/10+4\times(Z$ click dominant hand$)/10]$ (Tezenas du Montcel et al., 2008); higher values indicate more severe impairment. It was validated by Tezenas du Montcel and coworkers (Tezenas du Montcel et al., 2008) in a cross-sectional study enrolling 141 patients with ADCA, 53 patients with spastic paraplegia and 123 controls. In this study, the CCFS score was significantly higher in ADCA patients compared to controls and ADSP patients and was correlated with disease duration. Performances of CCFS were then tested in 146 FRDA patients, 77 patients with SCA and 48 controls by Filipovic Pierucci et al. (Filipovic Pierucci et al., 2015). ICC was high (0.92) in subjects undergoing test-retest. The authors also proved that CCFS is applicable in children older than 7 years with minor corrections of the formula. Tanguy Melac et al. (Tanguy Melac et al., 2018) evaluated the performances of CCFS in comparison to SARA scale in a cohort of 383 FRDA patients, 205 SCA patients and 168 controls. Both CCFS and SARA scores differed between conditions, with higher scores in patients with FRDA compared to SCA and controls. CCFS and SARA increased with disease duration in FRDA, ceiling effect

was observed only for SARA. In FRDA patients the relationship between SARA and CCFS scores was not linear for SARA scores below 10 or above 24, the best model for was a sigmoid model with both floor and ceiling effects. CCFS score was correlated with disease characteristics like disease duration – r=0.38, age at onset – r=-0.36, and number of GAA1 repeats – r=0.30. CCFS lacks longitudinal data in FRDA; in SCA patients CCFS score significantly worsened by 0.0197±0.0614 points in 1 year (SRM=0.32) (Chan et al., 2011).

Table 1. Main psychometric properties of most used outcome measures

	Inter-rater reliability	Test-retest reliability	Mean annual change	Sensitivity to change (SRM, 1 year)	Ref. (for SRM)
Rating Scales					
FARS	0.95	0.94	0.5-3.1	0.28-0.51	Patel et al., 2016
mFARS	-	0.95	1.91-2.62	0.34-0.48	Patel et al., 2016; Rummey et al., 2022
ICARS	-	0.95	0.3-2.1	0.73	Tai et al., 2015
SARA	0.99	-	0.72-0.91	0.32-0.59	Reetz et al., 2016, 2021
FARS-ADL	0.94	-	0.82-1.03	0.35-0.40	Reetz et al., 2016, 2021; Rummey et al., 2022
Functional Tests					
AFCS	Not relevant	0.97-0.99	-0.07 - -0.44	0.01-0.49	Patel et al., 2016
SCAFI	Not relevant	-	-0.06 - -0.03	0.09-0.39	Reetz et al., 2016, 2021
CCFS	Not relevant	0.73	-	-	-

2.4 Quantitative computerized motor assessments

In the last few years computerized assessment instruments have been developed to increase the sensitivity of motor assessment in FRDA. Arcuria and colleagues (Arcuria et al., 2020a) tested an APP-based assessment of upper limb impairment, that can be used with Android or Apple operating systems on a touchscreen device. Performances of this instrument were assessed in 36 FRDA patients and 92 healthy controls along with CCFS, 9HPT and SARA scale. The task (12-RSACT) consists in touching 12 red squares appearing consecutively, randomly on the screen at different positions. Intra-rater and test-retest reliability were excellent, as indicated by ICC=0.87 and 0.98 respectively. 12-RSACT correlated with disease duration (r=0.42), SARA score (r=0.77), CCFS score (r=0.87) and 9HPT time (r=0.72). Sensitivity to change of this instrument has not been tested so far. Robotic assessment of upper limb performances has been tested by

Germanotta et al. (Germanotta et al., 2015) in a small pilot study on 14 FRDA patients and 18 healthy controls. Evaluation consisted of planar reaching movements performed with the robotic system. Several measures could be extracted from the test performed, test-retest ICC values ranged from 0.69 to 0.97, according to the different variables tested. According to the authors duration of movement (kinematic), normalized jerk (smoothness) and number of submovements were the most discriminative indices and were also moderately correlated with SARA score. A similar approach was developed by Corben and colleagues (Corben et al., 2021), that tested the Ataxia Instrumented Measure–Spoon (AIM-S), which consists of a spoon equipped with a wireless motion capture device. The tool was used to measure ataxia of the upper limb during a simulated self-feeding task. The study enrolled 40 FRDA subjects and 20 control participants, 30 individuals with Friedreich ataxia completed also a second assessment 48 weeks later. Sensitivity of the AIM-S to detect deterioration in upper limb function was greater than other measures like 9HPT and mFARS. Q-Motor assessment, originally developed to characterize motor impairment in Huntington's disease, was tested by Hohenfeld and colleagues in FRDA (Hohenfeld et al., 2019). Q-Motor tasks included a lift task, a pronate/supinate task and a speeded finger tapping task. Twenty-nine FRDA patients and 23 healthy controls were tested. A number of measures are extracted from these tests and were overall correlated with clinical variables as well as with age of onset in FRDA patients. These measures also differentiated patients from controls. Only few Q-motor variables showed a time effect at a 1-year follow-up. For comparison, in Huntington's disease, clear progression of Q-Motor performances was detectable after 1 year in symptomatic patients and over 3 years in asymptomatic premanifest gene carriers (Tabrizi et al., 2013).

3. Balance and activity measurement

No gold standard for balance and gait measurement exists. Instrumented outcomes have the potential to measure gait, balance and real-life activity decline in FRDA. A number of studies in degenerative ataxias have proven that quantitative measurement of balance and gait parameters may be more sensitive to change than clinical scales (Ilg et al., 2016; Shirai et al., 2019). This approach was also tested in homogeneous cohorts of subjects with FRDA in small, pilot studies (Milne et al., 2014; Zesiewicz et al., 2017). These studies showed that gait and balance parameters, such as gait speed, step cadence and postural stability indices were significantly correlated with disease duration, clinical scales and traditional functional measures, like T25FW, warranting further investigations. In a recent study (Milne et al., 2021) 61 individuals with FRDA were assessed with GAITRite for spatiotemporal gait parameters, Biodex Balance System Postural Stability Test for static and dynamic postural stability, and SenseWear MF Armband for measuring daily walking and activity levels. Most of the quantitative measure showed significant deterioration during follow-up, with SenseWear and Biodex Balance System providing more responsive variables than clinical outcomes. SenseWear Armband daily step count had the largest effect size over 6 months (SRM= −0.615), while the postural stability test medial–lateral index had the highest SRM (0.829)

over 12 months. However, these outcomes were limited by large variability and significant floor effects (inability to perform postural stability test) at follow-up. An interesting approach was tested by Arcuria and colleagues (Arcuria et al., 2020b), using a smartphone app (APP-Coo-Test) to evaluate balance performances. The APP was installed on a smartphone placed over sternum, and detected the oscillation of the trunk via smartphone's triaxial accelerometers. Performances of the APP were tested in 40 subjects with ataxia (17 of them with a diagnosis of FRDA). APP measurements were highly correlated with SARA scores and intra-rater and test–retest reliability of the APP measurements were excellent.

The use of smartphones or similar easily and widely accessible devices may be useful to provide real-life monitoring in FRDA, and could integrate clinic-based assessments. Patients and caregivers need to travel to specialist clinics and have to cope with reduced mobility and fatigue. Moreover in-clinic assessments are conducted at infrequent intervals and sometimes in conditions that do not reflect typical patients' activity. Real life activity monitoring from wearable sensors, can provide high-frequency data and quantify day-to-day variability. A first attempt to test this hypothesis has been provided by Mueller and coworkers (Mueller et al., 2021) in a cross-sectional study in children with and without FRDA. Home-based digital endpoints included hand drawing assessment with a digital pen, speech assessment with a recorded oral diadochokinesis (PA-TA-KA) test, and gait and balance assessment with 5 wearable sensors. Remote monitoring proved to be feasible and well accepted by subjects. Several parameters discriminated between groups or correlated strongly with mFARS and ADL total scores. Test–retest reliability reached a maximum ICC of 0.77, compared with 0.95 for mFARS.

4. Wet Biomarkers

Novel therapeutic strategies in FRDA aim to restore frataxin levels. Therefore, development of sensitive and specific assays to detect active frataxin levels as biomarker is the most obvious choice for these therapies. Some of these treatments have no in-vivo effect on the levels of biologically active 14 kDa frataxin isoform (Nabhan et al., 2015). A number of assays have been developed to quantify levels of this isoform on platelets. Nevertheless, analyzing frataxin on a routine basis is still challenging in clinical settings and is not yet sufficiently reliable to be considered a viable biomarker (Blair et al., 2019). This has led to the consideration of other biomarker, such as neurofilament light chain levels (NfL). Nfl from serum, plasma or CSF have been investigated in many neurodegenerative diseases, including Alzheimer, Parkinson and Huntington disease, and have proven valuable in tracking disease progression. NfL are cytoskeletal proteins located in both the peripheral and central nervous system, that leak into the interstitial space, CSF and plasma as axons are damaged and die. Studies evaluating serum NfL in FRDA patients found that serum NfL is elevated in patients with FRDA, compared to controls and carriers (Clay et al., 2020; Hayer et al., 2020). However, NfL levels were not correlated with clinical severity or genetic characteristics, and were highest in young children with FRDA, decreasing with age as the disease progresses. After 25 years of age NfL levels

overlapped with control values. This is somewhat opposite to the trend found in normal population, where NfL levels increase with age. Interestingly, Nfl levels remained relatively stable over 1–2 years in FRDA subjects (Hayer et al., 2020). The understanding of NfL evolution in FRDA is still at its beginning, and further studies are needed.

5. Neuroimaging

Neuropathology changes in the dorsal root ganglia and dorsal horns of the spinal cord, dentate nuclei and the spinocerebellar and corticospinal tracts can be also assessed using neuroimaging techniques, such as Magnetic resonance imaging (MRI). Early and progressive volume loss in the dentate nuclei has been reported by different studies (Ward et al., 2019). Spinocerebellar and dentatothalamic tracts volumetric changes in the brainstem also correlate with disease severity, onset age, and disease duration. Other studies have found mild cortical thinning of cerebral cortex, especially in the primary motor and somatosensory cortices. More robust data have been provided by a cross-sectional multicentric study enrolling 248 individuals with FRDA and 262 healthy controls. This study (ENIGMA, (Harding et al., 2021) identified significant reduction in volumes in FRDA subjects compared to controls in many different areas. Brainstem, dentate nuclei, and cerebellar peduncles (superior and inferior) showed the greatest reductions in volume relative to controls; these features also appear early in the disease course. Correlations between brain structure volumes and disease severity and duration were observed across cerebellar gray and white matter, both for motor and non-motor functional regions of the cerebellar cortex. White matter abnormalities, particularly in corticospinal pathways, emerge as intermediate disease features, while cerebellar and cerebral gray matter loss, seemed to occur later in the disease course. These findings may guide subsequent longitudinal studies to identify sensitive and robust biomarkers for FRDA.

Table 2. Summary of studies assessing outcome measures in FRDA

Year	Journal	First Author	N. of FRDA subjects (baseline)	GAA1	Age	Onset	Disease duration	Outcome measures	Design
Clinical scales									
2006	Neurology	Lynch DR	155					FARS; T25FW; LCLA; 9HPT; ADL; PATA; SF36	Cross-sectional
2007	JNNP	Fahey MC	76	709±194	32.2±12.5	15.5±6.8	16.7±10.6	FARS; ICARS; FIM; MBI	Longitudinal (1 year)
2007	Neurology	Fahey MC	20					25MWT; TUG; 6MWT; Stepwatch	Cross-sectional
2009	Mov Disord	Burk K	96		29.1±13.3	16.0±10.0		SARA; ICARS; FARSn; PATA; T25FW; 9HPT	Cross-sectional
2010	Mov Disord	Friedman LS	236					FARS; ADL; 9HPT; PATA; LCLA; 25MWT	Longitudinal (2 years)
2012	J Child Neurol	Regner SR	410	662±247		13.7		FARS	Longitudinal (2 years)
2012	JNNP	Tai G	147	668±221	29.0±12.7	13.7±7.2	15.3±10.4	FARS; ICARS; FIM; MBI	Longitudinal (up to 12 years)
2012	Mov Disord	Marelli C	84		36.0±13.6	18.7±11.1	17.3±9.2	SARA	Longitudinal (up to 4 years)

2013	Brain	Metz G	603	670±226	27±13	14.5±9.1	14.3±9.4	ICARS	Cross-sectional
2015	Lancet Neurol	Reetz K	592	684	32.0	13.0	17.0	SARA; INAS; SCAFI; ADL; EQ5D3L	Cross-sectional
2016	Ann Clin Trans Neurol	Patel M	812	636±241	30.1±15.3	13.7±9.9	16.3±10.7	FARS; mFARS; T25FW; 9HPT; LCLA; ADL	Longitudinal (5 years)
2016	Lancet Neurol	Reetz K	605	590±270	37.9±13.9	15.5±10.4	18.2±10.3	SARA; INAS; SCAFI; ADL; EQ5D3L	Longitudinal (2 years)
2018	JNNP	Tanguy-Melac A	383	550	33.1±14.6	17.8±11.3	15.3±9.0	SARA; CCFS	Cross-sectional
2019	Eclinical Medicine	Rummey C	1021	190-750	14-59	8-34	5-28	FARS	Longitudinal (5 years)
2019	Neurol Genet	Rummey C	1011	628±252	25.3±14.5	13.2±9.4	12.1±9.9	mFARS	Cross-sectional
2020	Neurol Genet	Pandolfo M	54	649±226	24.9±13.3	13.6±9.7	16.9±9.0	SARA; ADL	Longitudinal (up to 8 years)
2020	Ann Clin Trans Neurol	Rummey C	405					mFARS; FARSn; FARSn-117	Cross-sectional
2021	Lancet Neurol	Reetz K	602	591±269	33.7±13.9	15.5±10.4	\	SARA; INAS; SCAFI; ADL; EQ5D3L.	Longitudinal (4 years)
2021	Mov Disord Clin Pract	Tai G	19		36.9±15.0	19.0±10.0	17.0±10.0	mFARS; SARA	Cross-sectional

Year	Journal	Author	N					Measures	Design
2022	Neurology	Rummey C	1115	690 (500-800)	21 (14-34)	11 (7-16)	\	mFARS; ADL; T25FW; 9HPT	Longitudinal (up to 18 years)
Functional Measures and quantitative assessments									
2015	J NeuroEng Rehabil	Germanotta M	14	260-930	15.3	5-20	1-12	InMotion Arm Robot; SARA	Cross-sectional
2017	J Neurol	Tai G	122	656±233	31.5±13.9	14.5±8.9	16.9±10.9	FARS; T25FWT; 9HPT; LCLA	Longitudinal (3 years)
2017	Gait posture	Zesiewicz TA	8	433	29.4±9.0	19.5±8.1		FARS; GAITRITE; Biodex balance	Longitudinal (2 years)
2018	J NeuroEng Rehab	Bionnechere B	27	608±306	26±12.2		15.0±7.4	SARA; ADL; CCFS; automated upper limb evaluation (Kinect)	Cross-sectional
2019	Cerebellum	Hohenfeld G	23	508±266	31.0±14.1	16.6±8.1		Q-motor; SARA; INAS; SCAFI	Longitudinal (1 year)
2019	J neurol	Arcuria G	36		45.6±13			CCFS; APP-coo-test; SARA	Cross-sectional
2020	J Neurol	Arcuria G	18					APP-coo-test; SARA; BBS; stabilometry	Cross-sectional
2020	J Neurol	Hayer SN	99		38.4±13.1			Plasma Nfl light and heavy chain	Longitudinal (2 years)
2020	J Neurol	Clay A	85	650 ± 234	30.7±16.1	12.7±9.1		Plasma Nfl	Cross-sectional

2021	Ann Clin Trans Neurol	Mueller A	13	766±163	13±	8±2	4±3	mFARS; ADL; 9HPT; T25FW; PATA. Home digital devices	Cross-sectional
2021	Cerebellum	Milne SC	61					SenseWear MF armband; SARA; FARS; mFARS; GAITRITE	Longitudinal (1 year)
2021	Cerebellum	Corben LA	40	613±216	32.8	15.8±8.1	16.3±11.1	Ataxia Instrumented Measure–Spoon; mFARS; 9HPT; BBT	Longitudinal (48 weeks)
2021	Ann Neurol	Harding IH	248	656±233	31.1±14.0	16.7±9.2	14.5±9.8	MRI volumetric measures	Cross-sectional

Table 1. Data are shown as mean±s.d. or as median(inter-quartile range). Age, onset and disease duration are expressed as years. FARS: Friedreich Ataxia Rating Scale; FARSn: neurological part of FARS; mFARS: modified FARS; SARA: Scale for the Assessment and Rating of Ataxia; ICARS: International Cooperative Ataxia Rating Scale; T25FW: timed 25-feet walk test; LCLA: Low Contrast Letter Acuity; 9HPT: 9-Hole Peg Test; ADL: Activities of Daily Living part of FARS scale; PATA: PATA fluency test; SF36: Short Form Health Survey-36; EQ-5D: European Quality of life questionnaire; INAS: Inventory of Non-Ataxia Signs; FIM: Functional Independence Measure; MBI: Modified Barthel Index; AFCS: Ataxia Functional Composite Score; SCAFI: SCA Functional Index; CCFS: Composite Cerebellar Functional Score.

Aims:

The aims of the present project are to test existing outcome measures in a large cohort of FRDA subjects and to identify newly developed tools that can be used in natural history studies and in interventional trials, in combination with clinical evaluation, to rate disease severity and progression.

Material And Methods:

To test existing and newly developed outcome measures in subjects with FRDA, we enrolled a large cohort of subjects followed at the Ataxia clinic of the Fondazione IRCCS Istituto Neurologico "Carlo Besta".

All patients are yearly assessed and their clinical data are recorded in our in-house database.

In addition, the majority of the patients gave their informed consent for the participation into the European Natural History Study for Friedreich ataxia, and their clinical data are included into the European Registry, established in 2010, by the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS) (Reetz et al., 2021, 2016, 2015).

The Neurological Institute Carlo Besta is a participating site of the EFACTS Registry with >200 participant patients included so far.

Data collected during the annual include:

- Demographical variables: year of birth and age at baseline, first symptom reported and age at first symptom onset, age of onset of gait instability, disease duration, date and reason for termination of data collection;

- Disability and disease "milestones": age of first presentation of FRDA-related comorbid conditions such as diabetes mellitus, scoliosis, pes cavus, cardiomyopathy, vision and hearing loss; age at intermittent and permanent support for ambulation and age at ambulation loss (i.e. subject wheelchair bound).

- Genetic variables: GAA expansion size on both alleles. Genetic testing was repeated by a centralized laboratory (Laboratoire de Neurologie Experimentale of the Université Libre de Bruxelles, Belgium) for all EFACTS sites and GAA expansion size estimated. We decided to use centralized GAA expansion estimate in order to minimize variability due to different techniques used across various local laboratories. GAA1 and GAA2 are defined as the allele with shorter and longer GAA expansion, respectively, for each subjects. For heterozygous patients point mutation is reported.

- Outcome measures: the primary outcome measure is the SARA scale. The activities of daily living (ADL) part of FARS, INAS inventory and functional measures (8MWT, 9HPT, PATA and SCAFI score) serve as secondary outcomes. Disability stage was rated based on the "spinocerebellar degeneration functional score", score range 1 (no functional handicap but signs at examination) to 7 (confined to bed) (Reetz et al., 2015).

During the PhD project data from the 4-year follow-up longitudinal study have been published in:

Reetz K, Dogan I, Hilgers RD, Giunti P, Parkinson MH, Mariotti C, Nanetti L, Durr A, Ewenczyk C, Boesch S, Nachbauer W, Klopstock T, Stendel C, Rodríguez de Rivera Garrido FJ, Rummey C, Schöls L, Hayer SN, Klockgether T, Giordano I, Didszun C, Rai M, Pandolfo M, Schulz JB; EFACTS study group (Fichera M). Progression characteristics of the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS): a 4-year cohort study. Lancet Neurol. 2021 May;20(5):362-372. doi: 10.1016/S1474-4422(21)00027-2. Epub 2021 Mar 23. PMID: 33770527.

We have also assessed comorbidites and complications in our cohort of longitudinally followed typical onset FRDA. Data collected led us to identify delusions and hallucinations as a complication of combined visual and sensory loss in subjects with advanced FRDA. A paper on this topic has been published in:

Fichera M, Castaldo A, Mongelli A, Marchini G, Gellera C, Nanetti L, Mariotti C. Comorbidities in Friedreich ataxia: incidence and manifestations from early to advanced disease stages. Neurol Sci. 2022 Sep 2. doi: 10.1007/s10072-022-06360-w. PMID: 36053339.

A) Properties of SARA Scale

To evaluated the properties of SARA scale we analyzed baseline and 8-year follow-up SARA scores of 207 FRDA patients longitudinally evaluated between September 2010 and December 2019.

In order to analyze a homogeneous FRDA population, we excluded the late onset FRDA cases (onset >24 years, N=37) that are known to have a less severe phenotype and a slower disease progression.

Number of follow-up visit completed until December 2019 ranged from 0 (only baseline visit) to 8, with a mean number of 4.5 follow-up visit completed.

The assumption of normality for SARA total and subscale distributions was assessed using the Shapiro-Wilk test. The yearly progression rate was estimated using linear mixed effects regression models (LMEM) with restricted maximum-likelihood estimation method, with random effects on slope including baseline scores as fixed effect. Additional LMEM was used to compare progression rates in subgroups by adding interaction term between time and group. Standardized response mean (SRM) was calculated as: mean change (follow-up − baseline score)/standard deviation of the change. Statistical analyses were conducted using SAS, version 9.4 (SAS PROC GLIMMIX for LMEM).

B) SARA-FARS comparison

We tested both SARA and FARS scales in a consecutive series of 99 FRDA patients at various stages of disease progression. The two scales were performed subsequently, starting with FARS, by the same

experienced rater during the same visit. To minimize patient's discomfort, items assessing the same feature (e.g. speech, gait) were evaluated simultaneously. Correlation between SARA and FARS total and subitems scores were assessed using two-sided Spearman or Pearson's correlation tests according to data normality. In addition, we evaluated skewness of their distributions, and floor and ceiling effects. To identify clusters of intercorrelating items a principal component analysis was performed.

To test the possible effect of physical activity and fatigability on SARA score and FARS part E measures the two scales were repeated in a subset of patients (N=18) after a maximal exercise test using a cycle-ergometer. Both evaluations were carried out by the same rater in the same day, at different times. Scores obtained in the two different conditions were compared using two-sided paired Wilcoxon rank test. Statistical analyses were conducted using JMP, version 11.

C) Posturography

Subjects with FRDA were included in this study regardless of their ambulatory status. Trunk sway was assessed using Delos Postural Proprioceptive System© (DPPS, Delos srl, Turin, Italy) (De Carli et al., 2010), that consists of an angular speed detector, applied over sternum. The evaluations were carried out at baseline and at 1-year and 2-years follow-up interval during routine neurological assessments. Each postural evaluation consisted of 4 different conditions. These included seated position without support of feet with arms outstretched (Sitting) and stance in different conditions of increasing difficulty: natural position with feet apart (Natural), with feet together (Feet-together) and Tandem stance. During each trial subjects were asked to maintain the position for at least 20 seconds, correct placement was assured by visual observation of the examiner. All the trials were carried on with eyes open and without shoes. The trial conditions were selected in order to mimic the clinical scoring system included in the SARA scale. For each test the software calculates the closeness of the angle from the median x-y axis, for both the x (medio-lateral) and y (antero-posterior) axis. Lower values indicate better performances. Correlation between SARA, demographical and posturography variables were assessed using two-sided Spearman or Pearson's correlation tests according to data distribution. T-test or Wilcoxon paired rank test were performed to assess statistical differences between baseline and follow-up. Statistical analyses were conducted using JMP, version 11.

D) Wet biomarkers

Transcriptome profile induced by FXN overexpression in FXN-deficient lymphoblastoid cells revealed the BC005240_1 transcript, which corresponds to HS-1-associated protein X-1 (HAX-1), as the most upregulated gene. Quantitative Reverse Transcription-Polymerase Chain Reaction and western blot analysis performed on lymphoblasts from FRDA patients showed that low frataxin mRNA and protein expression correspond to reduced levels of HAX-1. In order to evaluate HAX-1 expression as a potential biomarker in FRDA, FXN and

HAX-1 expression were analyzed in PBMCs from FRDA patients and non-related healthy controls. Laboratory analyses were conducted at the Laboratory of Signal Transduction of the "Tor Vergata" University of Rome. FRDA patients were recruited from our Ataxia clinic. Inclusion criteria were (a) genetic diagnosis of FRDA, (b) age ≥ 18 years and (c) available echocardiography and electrocardiogram evaluations. Exclusion criteria were the presence of active substance abuse, hematological disorders or FRDA-unrelated major comorbidities requiring chronic pharmacological treatment. All the enrolled patients gave informed consent prior to the inclusion in the study.

In a second set of experiments, the effect of microRNAs (miRNAs) modulation on FXN and HAX-1 expression was tested. FRDA patients were classified according to the age at onset of the neurological symptoms, as early-onset group (EOG; onset < 14 years; 12 subjects), and intermediate-onset group (IOG; onset between 15 and 24 years; 14 subjects) and late onset group (LOFA, onset >24 years). The expression of 84 miRNAs was first investigated in pooled plasma sample from 3 EOG FRDA patients (mean age 25.6±6.7 years), 4 IOG patients (mean age 40.3±9.5 years), 4 LOFA patients (mean age 45±13.5 years), and matched healthy subjects. hsa-miR223-3p was the only shared miRNA expressed in all FRDA subgroups and resulted up-regulated in all FRDA groups as well. Based on these results, hsa-miR223-3p expression was analyzed in 37 FRDA patients from the previous cohort. As for the previous part, laboratory analyses were conducted at the Laboratory of Signal Transduction of the "Tor Vergata" University of Rome.

Mann–Whitney test, T test, Wilcoxon test and Kruskal–Wallis test were used for data analysis when appropriate. For parametric and non-parametric distribution, expression data are represented as mean with range. We used Pearson or Spearman correlation tests depending on the distribution of the data (parametric or non-parametric). For all analysis, significance was set at $P \leq 0.05$. ROC curves were performed to assess the diagnostic potential of hsa-miR223-3p. AUC was used to quantify the probability that the prediction will be correct after the test variable is observed.

E) Actigraph

Remote monitoring maximizes the collection of "real world" information outside clinical visits and could integrate in-clinic physician assessment in clinical trials. We tested remote monitoring in a cohort of 25 subjects with FRDA and 13 age- and sex-matched healthy controls. We excluded subjects with FRDA that were completely wheelchairbound (i.e. SARA gait score=8). Healthy subjects were recruited among partners and community. All FRDA subjects underwent clinical evaluation with SARA and FARS/mFARS scales and functional tests (CCFS, 8MWT). At the end of the visit the activity monitoring devices were positioned by study personnel. Subjects were given instruction, both orally and written, on how to reposition properly the devices once removed. ActiGraph GT3X (ActiGraph LLC, Pensacola, FL) was adopted for this study. Two ActiGraph were worn by subjects, one on the non-dominant wrist and the other over L5 vertebra on the back. Subjects were asked to wear both accelerometer during waking hours for the next 7

days. In addition, patients were asked to fill-in a wear time log and to provide information regarding occurrence of falls. At the end of the registration period all study materials were returned by mail. All study participants gave written informed consent to participate in this study.

ActiGraphs collected data at a sampling rate of 30 Hz. The ActiLife (version 6.13.4) software was used to extract the raw accelerometer data from the ActiGraph. The 30-Hz data were summarized in 10-s epochs and raw accelerometer files were processed to identify non-wear periods. Non-wear periods were identified using the non-wear time classification algorithm reported by Choi et al. (Choi et al., 2011), files were then visually inspected and correction were made to fit data with subjects' log. Days with less than 8 h of total wear time were excluded from the analysis. Accelerometer data are expressed as activity counts per 10 s. An activity count is based on the vector magnitude, i.e. the squared sum of the recorded data on the 3 axes (vertical, forward/backward and sideways). To summarize daily activity, activity count were used to define the following variables: (1) average active expense (Kcal); (2) %active; (3) MET (Metabolic Equivalent of Task estimates) score; (4) daily step count. %active defines the % of time spent by subjects in sedentary, light, moderate-to-vigorous and very vigorous activities. Both %active and MET score reflect the average daily activity of a subject.

Repeated Measures ANOVA (RM-ANOVA) was used to detect inter-group differences between healthy controls and subjects with FRDA and between wrist-worn and waist-worn Actigraphs using Group as inter-subjects factor and Location as intra-subject factor. Mann–Whitney test or T test were used for as post-hoc tests according to data distribution to identify differences between healthy controls and FRDA. Spearman or Pearson correlation tests were used to correlate activity data and clinical and demographical variables. For all analysis, significance was set at P ≤0.05. Statistical analyses were conducted using JMP, version 11.

Results

A) Statistical properties of SARA Scale

We analyzed the statistical properties of SARA scale its subitems scores in 170 typical onset FRDA patients.

Figure A-1 summarize baseline characteristics and displays the number of subjects performing follow-up visits for the whole cohort of subjects enrolled in the study.
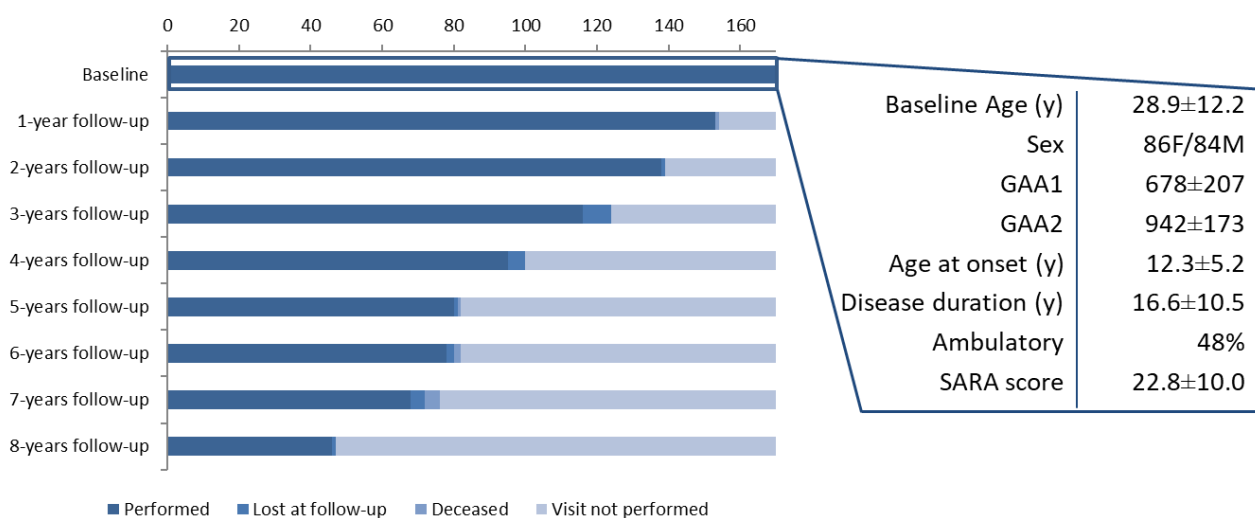

Baseline Age (y)	28.9±12.2
Sex	86F/84M
GAA1	678±207
GAA2	942±173
Age at onset (y)	12.3±5.2
Disease duration (y)	16.6±10.5
Ambulatory	48%
SARA score	22.8±10.0

Figure A- 1. Baseline demographic and genetic characteristics of the whole sample fo FRDA patients. Number of subjects with valid data at each timepoint are displayed on the left graph. Data in the right-sided table are displayed as mean±s.d.

Cross-sectional analyses

SARA total score at baseline showed a bimodal distribution, with two modes at 9 and 33 points, respectively. Non-normal distribution of scores was confirmed by Shapiro-Wilk test (p<0.001). None of the items composing the scale showed a normal distribution (Shapiro-Wilk test p<0.001 in all cases), as seen in Figure A-2. Non-normal data distribution was confirmed also at follow-up examinations (data not shown). Low floor (1%) and ceiling (6%) effects were identified for the total SARA score. Relevant ceiling effect was present for Gait (42%), Stance and Heel-to-shin items (48%), while floor effect was present for Sitting (15%) item.
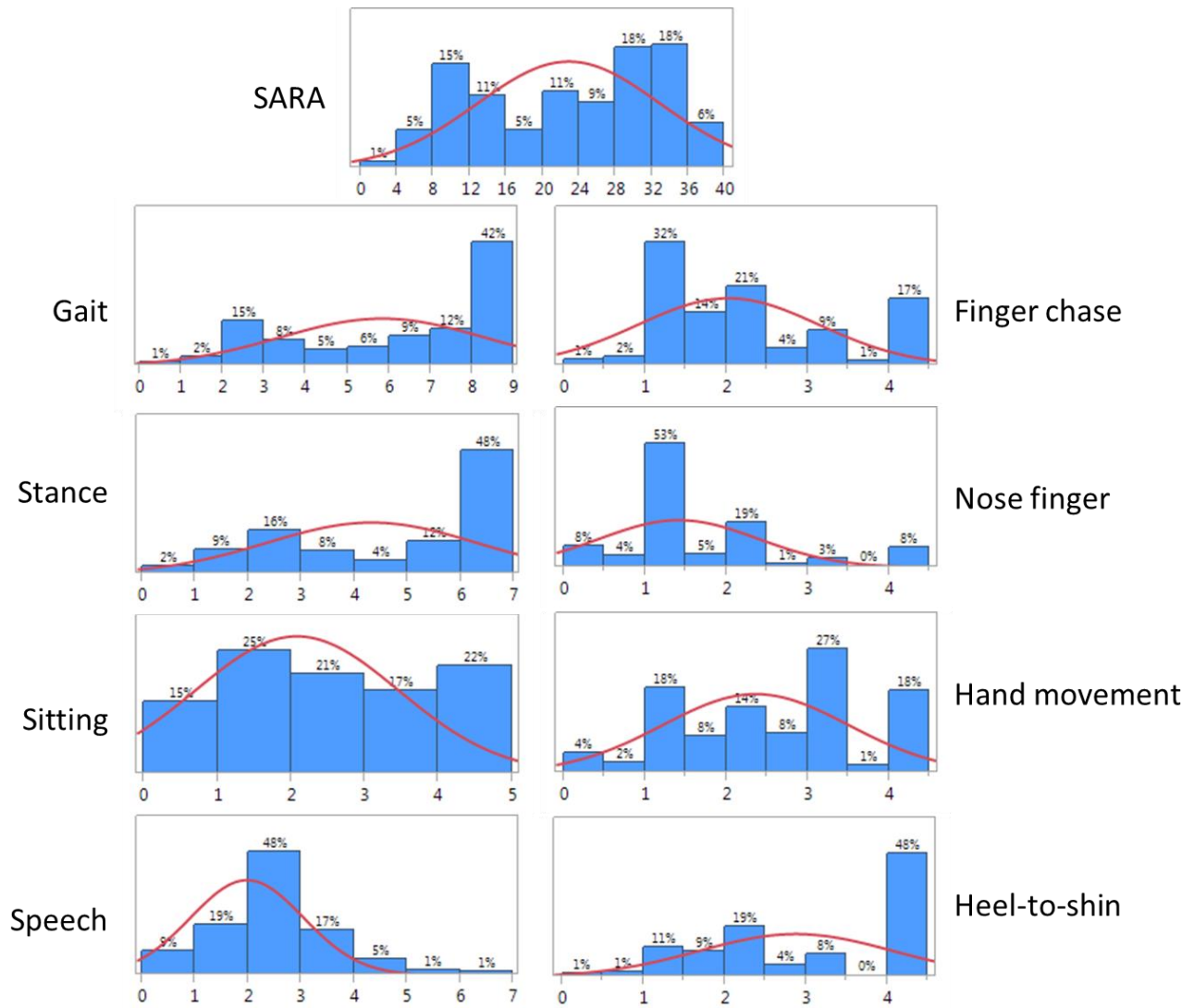
Figure A- 2. Data distribution for total SARA score and items score at baseline

Item contribution to the total SARA scores is not homogenous and varies according to the SARA total score itself (Figures A-3 and A-4). Gait and Stance items (14/40 points of total SARA score) drive SARA progression in the first half of the total SARA score. They reach their maximum score and plateau around 25 points of total SARA score, when the patient becomes wheelchair bound. Similarly, Heel-to-shin item contribution to the total SARA score progression is maximum before loss of ambulation. The sitting item has a more linear progression, and contributes to the increase in SARA score even after loss of ambulation. Dysarthria item score increases when total SARA score is below 15-20 points and after 30-35 points. Very few subjects at baseline scored 6/6 points on this item (i.e. anarthria). Items assessing upper limb dysmetria and tremor contribute to the increase in SARA score after ambulation is lost, and rapidly increase in advanced phases, when upper limb movements become impossible. For these items, a relevant contribution of muscular weakness may mask the correct measurement of limb ataxia. The alternating upper limb movement item shows abnormalities relatively early, but continues to contribute to total SARA score even after loss of ambulation.
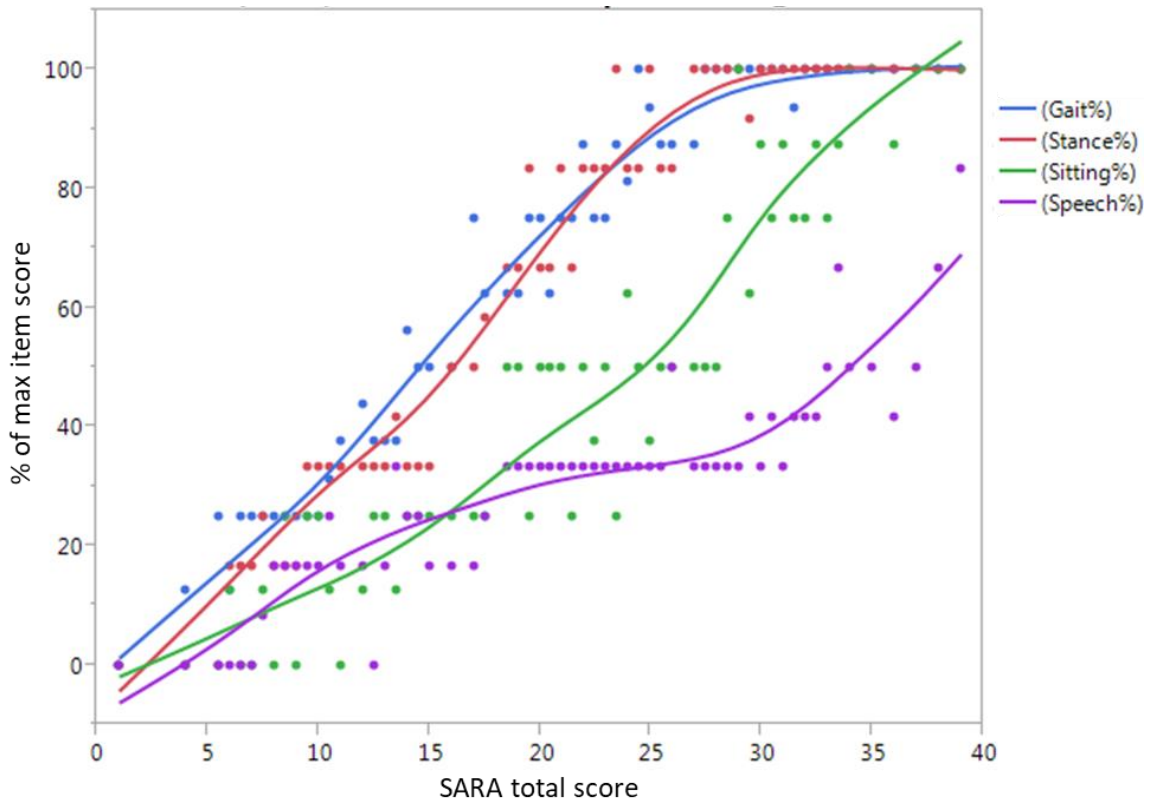
Figure A- 3. Items score, as % of maximum theoretical score, as a function of total SARA score. Dots represent median score.
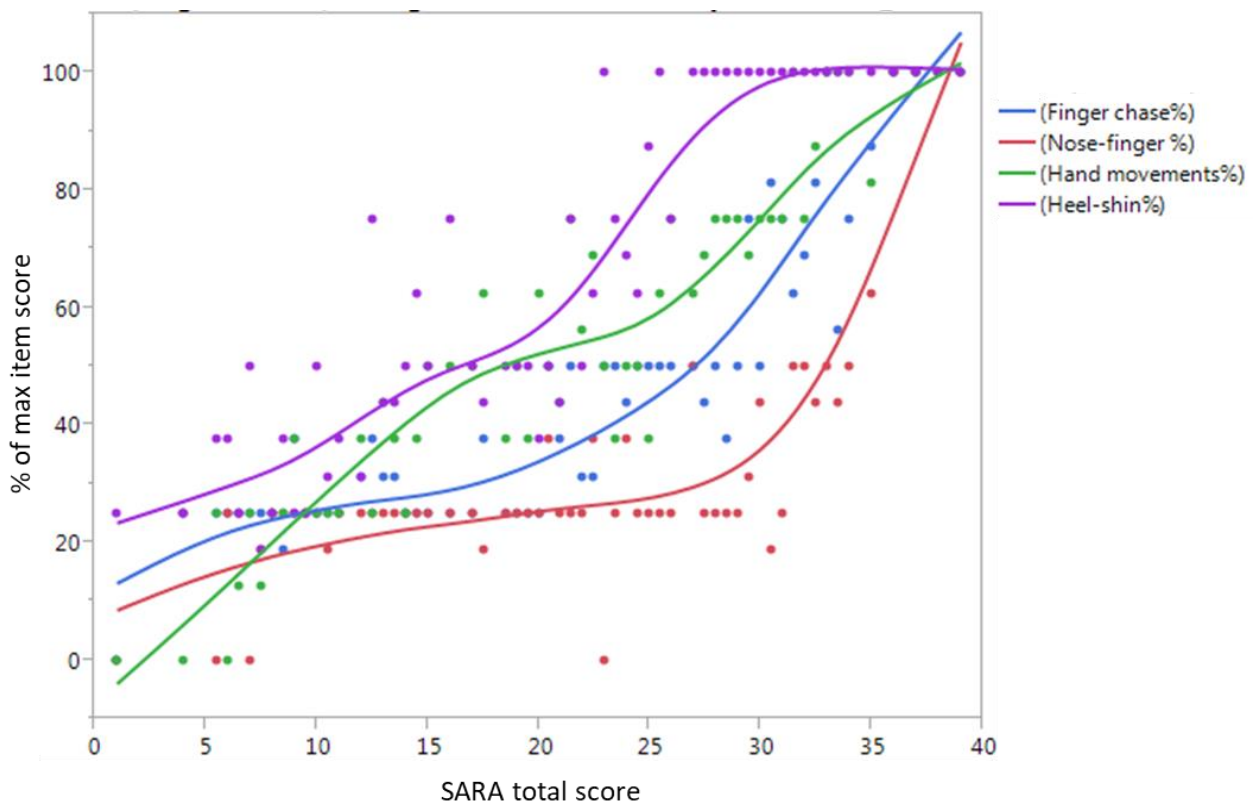

Figure A- 4. Items score, as % of maximum theoretical score, as a function of total SARA score. Dots represent median score.

Longitudinal analyses

We limited our longitudinal analysis to the fourth follow-up since the reduced number of subjects completing subsequent evaluations would have negatively impacted statistical power. Due to the non-linear increase of SARA score we decided to divide subjects into 4 groups according to baseline SARA score: 0-10 points, 10.5-20 points, 20.5-30 points and 30.5-40 points. LMEM analysis showed a significant main effect for the time factor (p-value <0.0001) and a significant interaction effect between time and baseline score (p-value < 0.0001) (Figure A-5). Steeper slope was identified for subjects with baseline SARA score 0-10, with a progressive lowering of the slope coefficient with increasing baseline score. We calculated SRM (i.e. mean change/s.d. of change) for the total SARA score and for each item at follow-up 1 to 4, according to patients' stratification already adopted in previous analyses (Table A-1). SRM coefficient increased with longer observation. Overall, higher SRMs were identified, at each follow-up, for subjects with baseline SARA between 10.5 and 20. Items contributing mostly to total SRM in this cohort were Gait, Stance and Heel-to-shin. In the 10.5-20 baseline SARA score cohort, considering only a sum of these 3 items SRMs were 0.91, 1.36, 1.73 and 1.73 at 1, 2, 3 and 4 years follow-up, respectively. As expected, SRMs for the SARA baseline 30.5-40 cohort was the lowest and did not improve with longer observation period.
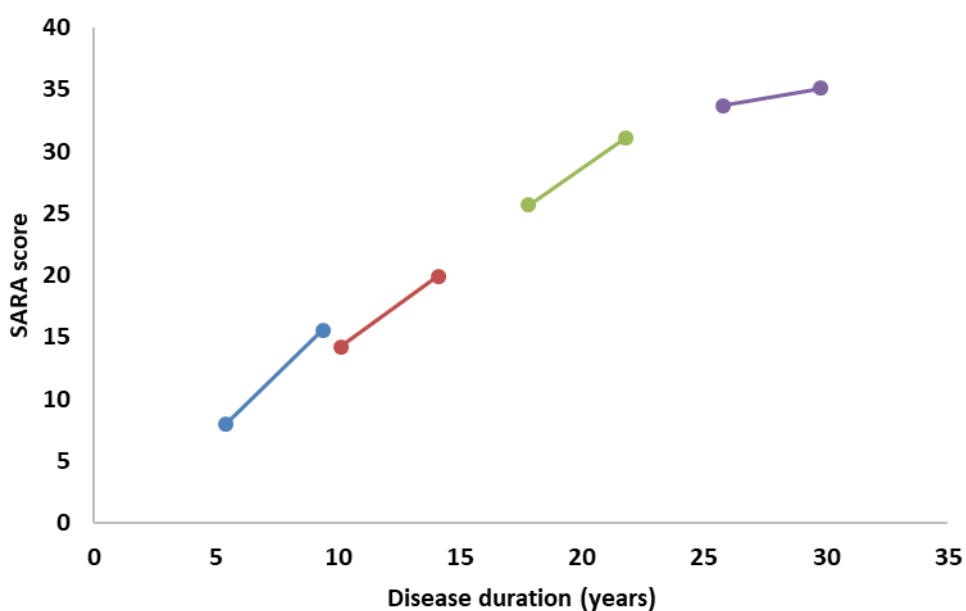
Baseline SARA score	Estimate slope (SE)	95% CI	P-value
0-10	1.90 (0.41)	1.07-2.74	<0.0001
10.5-20	1.42 (0.29)	0.83-2.00	<0.0001
20.5-30	1.36 (0.21)	0.94-1.78	<0.0001
30.5-40	0.36 (0.17)	0.03-0.69	0.031

Figure A- 5. Annual increase of SARA score according to baseline score.

Table A- 1. Standardized Response Mean (SRM) estimates for total SARA score and for each item at 1 to 4 year follow-up in subjects divided according to baseline SARA score.

Baseline SARA	1 year follow-up				2 years follow-up				3 years follow-up				4 years follow-up			
	0-10	10.5-20	20.5-30	30.5-40	0-10	10.5-20	20.5-30	30.5-40	0-10	10.5-20	20.5-30	30.5-40	0-10	10.5-20	20.5-30	30.5-40
SARA score	0.04	0.74	0.25	0.02	0.69	0.99	0.59	0.17	1.04	1.35	0.72	0.05	1.42	1.62	1.27	0.61
Gait	0.18	0.78	0.09	0.14	0.62	1.09	0.39	0.21	0.93	1.79	0.56	0.15	1.90	2.20	0.83	0.16
Stance	0.00	0.59	0.34	-0.14	0.64	0.59	0.38	0.00	0.61	1.09	0.68	0.00	1.20	0.93	0.68	0.00
Sitting	-0.19	-0.21	0.10	0.14	-0.19	0.28	0.44	0.59	0.25	0.64	0.57	0.44	0.48	0.92	1.09	0.89
Speech	0.19	0.46	0.06	0.13	0.34	0.17	0.07	0.42	0.77	0.47	-0.14	0.42	0.55	0.30	0.48	0.71
Nose-finger	-0.29	0.36	-0.29	0.03	-0.19	0.33	-0.39	-0.08	0.28	0.34	0.00	-0.14	0.66	0.22	0.58	0.55
Finger chase	-0.15	0.26	-0.03	-0.14	0.15	0.24	0.29	-0.06	0.29	0.45	0.32	-0.12	0.31	0.78	0.84	0.03
Alternating hand movements	-0.12	-0.06	0.10	-0.29	0.00	-0.05	0.08	-0.25	0.29	0.39	0.06	-0.32	0.32	0.06	0.56	-0.16
Heel-to-shin slide	0.31	0.48	0.42	0.0	0.88	1.00	0.51	0.00	1.24	1.13	0.65	0.00	0.91	0.82	0.57	0.00

(Porcu L, Fichera M et al., in preparation)

B) SARA-FARS comparison

Ninety-nine consecutive FRDA subjects (53M/46F) were evaluated with both SARA and FARS scale. Mean age at examination was 31.8±14.0 years (range 12–69 years), mean age at instability onset was 16.4±10.8 years (range 4-60, 16 LOFA) and mean disease duration at the time of the evaluation was 15.4±8.4 years (1–36 years); 48/99 subjects were non-ambulatory at evaluation. Mean SARA, FARS III and mFARS total scores were 21.6±8.8 (range 5.5–39), 72.7±23.2 (range 13.5–119), and 57.9±17.7 (range 7.5–93). As previously found, SARA score displayed a non-normal distribution in our sample (Shapiro-Wilk test p=0.025), while FARS and mFARS scales were normally distributed (p=0.652 and p=0.486, respectively). Internal consistency, as evaluated by Cronbach alpha on single items raw scores, was 0.917 for SARA scale, 0.960 for FARS scale and 0.951 for mFARS scale. When considering FARS and mFARS subscales Cronbach's alpha was 0.811 and 0.745, respectively. Internal consistency was lower for FARS subscale A (bulbar, 0.591) compared to part B (upper limbs, 0.929), part C (lower limbs, 0.947), part D (peripheral, 0.864) and part E (stance and gait, 0.874).

For SARA scale item-total score correlation coefficients were all above 0.6 (p<0.001 in all cases), with the lowest correlation being identified for Speech (ρ=0.705) and Nose-finger (ρ=0.610) items. In FARS scale a non-significant correlation with total score was identified for Tongue atrophy and fasciculation item (p<0.05 in all other cases). All the correlation coefficients were above 0.6, except facial atrophy and fasciculation (ρ=0.412), finger to finger test right (ρ=0.399) and left (ρ=0.364), vibratory sense right (ρ=0.268) and left (ρ=0.308), deep tendon reflexes right and left (ρ=0.352), tandem stance (ρ=0.447), stance on dominant foot (ρ=0.234) and tandem walk (ρ=0.287). SARA and FARS items demonstrated good convergent and divergent validity, meaning that the correlation was higher between items evaluating similar function compared to items dissimilar in content; item tongue atrophy and fasciculation of FARS scale was not correlated with any of SARA scale items. SARA total score was highly correlated with FARS (r=0.968) and mFARS (r=0.959) total scores. Both SARA and FARS/mFARS scales were significantly correlated with ADL scores, GAA1, disease duration and age at onset (Table B-1).

Table B- 1. Correlations between clinical scales for ataxia rating and demographical characteristics.

	SARA	FARS	mFARS
Age at onset	-0.400**	-0.483**	-0.440**
GAA1	0.348**	0.394**	0.372**
Baseline age	0.218*	0.128	0.168
Disease duration	0.660**	0.607**	0.626**
ADL	0.896**	0.862**	0.859**

* p<0.05; ** p<0.005

FARS scale displayed no floor effect and a negligible ceiling effect (4% of subjects scoring 90% or more of the possible total score). Similarly, mFARS displayed no relevant floor (1%) and ceiling effects (4%). When considering separately each item composing the scale a marked floor effect was identified for the following items of FARS scale: cough, tongue atrophy and fasciculation, facial atrophy and fasciculation. Relevant ceiling effect was present in items E2-E6, that also displayed a clear bimodal distribution of scores (i.e. most subjects either scored 0 or 4) (Figure B-1). Total SARA scores displayed no floor effect and, similarly to FARS/mFARS scales, only 4% of subjects scored more than 36 points (ceiling effect). Floor and ceiling effect of SARA subitems has been already discussed in the previous section. Principal component analysis showed that the rating results of SARA items were loaded on a single factor (eigenvalue = 5.663), which explained 70.79% of the variance. All other factors had eigenvalues lower than 1. For FARS scale, 7 factors with eigenvalue >1 were identified, explaining 78.0% of the variance, while for mFARS scale 4 factors, explaining 73.33% of variance, were identified.

The ADL part of FARS scale displayed a normal distribution of values in this population. Mean ADL score was 15.8±7.3 points (range 2–33). Internal consistency, as evaluated with Cronbach' alpha, was 0.914. Item-total score correlation coefficients were all above 0.6 (p<0.001 in all cases), except for Bladder item (ρ=0.290). All the items also correlated significantly between each other, except Bladder item that did not correlate with any other item in the scale. Floor and ceiling effect were not relevant for total ADL score (2.5 and 0%, respectively). Relevant floor effect was present for Bladder item, while relevant ceiling effect was present for Falls and Walking items. Principal component analysis identified only 2 factors with eigenvalue >1, explaining 74.2% of total variability. All the items, except Bladder item, weighted more on the first factor.
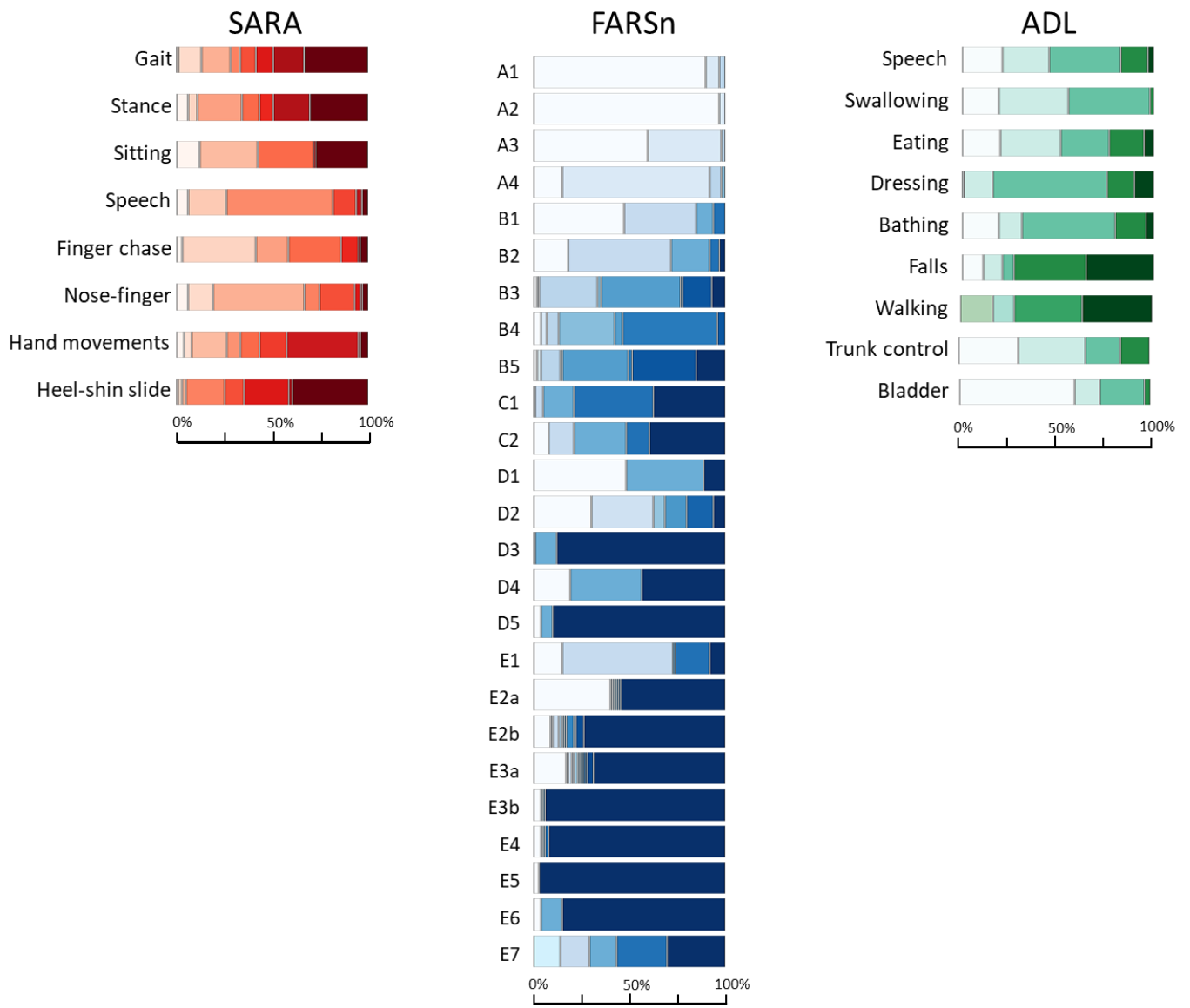
Figure B- 1. Heatmap distribution of scores for SARA, FARSn and ADL scales. Darker colors indicate worse, higher scores.

Effect of exercise on scale scores

Eighteen subjects (11M/7F) of the previous cohort repeated SARA and FARS part E (stance and gait) following maximal exercise test. All patients were typical-onset FRDA, mean age at onset was 16.3±4.34 years (range 8-23), mean age at evaluation was 25.9±6.8 years (18-43) and mean disease duration was 9.6±5.7 years (1-23). Only 2 patients (11%) were non-ambulatory. Mean SARA and FARS part E scores before exercise were 15.6±5.8 (median 13.8) and 23.7±6.2 (median 21.9) points, respectively.

Following exercise SARA score increased to 16.3±6.1 points, though the increase was not statistically significant (p=0.09, two-tailed test). Single items scores did not change significantly, only a trend for a significant increase in sitting score was identified (p=0.06). Conversely, a non-significant decrease to

22.9±6.9 points was identified for FARS part E score (p=0.11). None of the items displayed significant changes following exercise (Figure B-2).
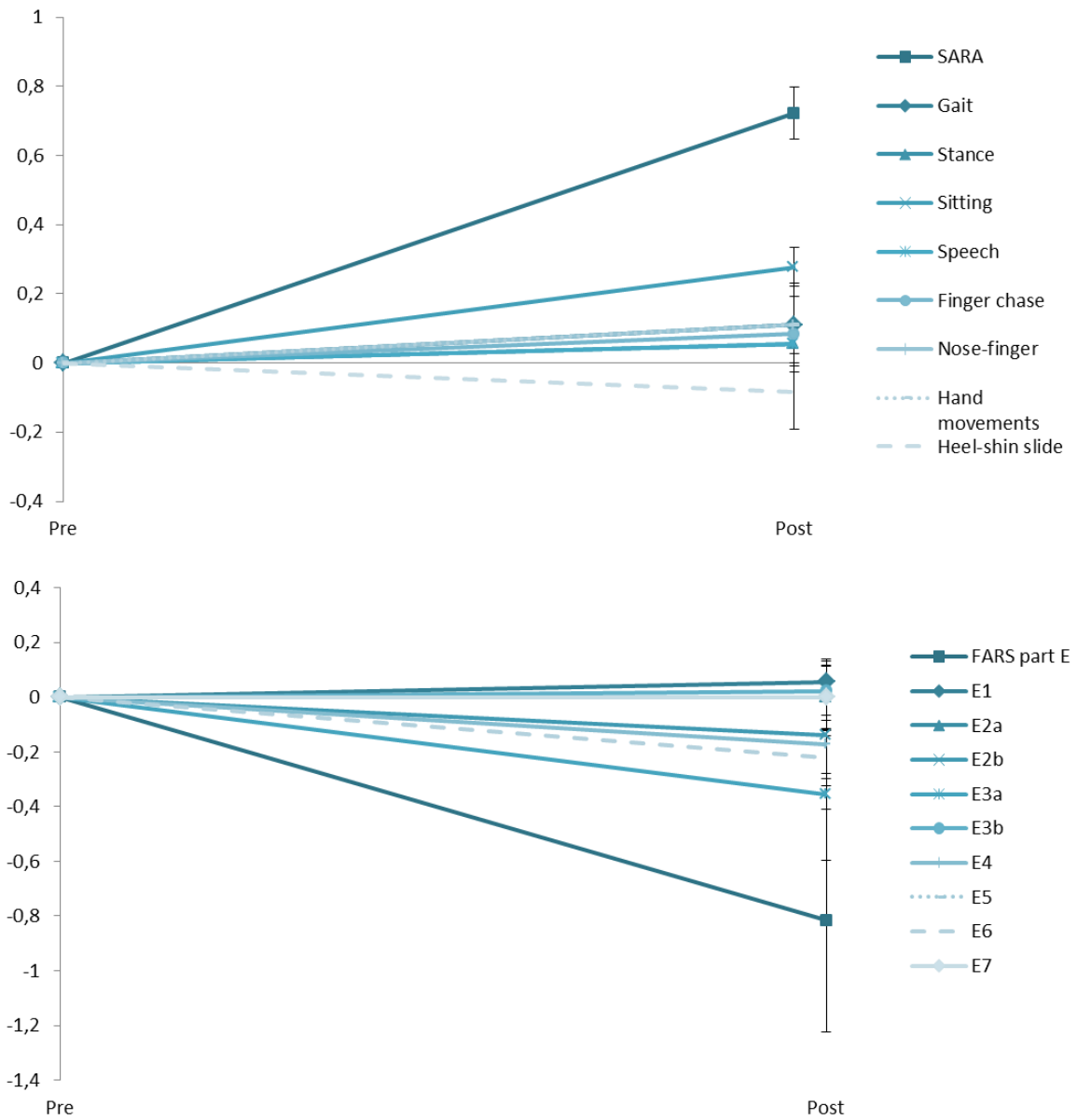

Figure B- 2. Absolute change following intense physical exercise (Post) for SARA scale (upper part) and for FARS part E (lower part) total score and for the items composing the scales.

C) Posturography

Ninety-nine subjects with FRDA were enrolled in this study. Twenty subjects unable to complete correctly any of the task provided and 3 subjects with age at onset >50 years were excluded. Seventy-six subjects with FRDA (43F/33M) that completed baseline clinical evaluation and posturography assessment with DELOS system were included in the analyses. Mean age at baseline was 33.3±15.3 years (range 10-69), mean age at FRDA onset was 18.9±11.0 years (range 3-44) and mean disease duration was 14.3±8.7 years (range 0-39). Typical onset patients accounted for 75% of the sample (N=57). Mean baseline SARA score was 16.6±8.0 points, 10 subjects were wheelchair bound at baseline.

At baseline Seated task was completed by 76/76 subjects (100%), Natural stance by 55 subjects (72%), Feet-together stance by 40 subjects (52%), while only 10 subjects (13%) completed the Tandem stance task. Deviation from median x-y axis, for both the *x* (medio-lateral) and *y* (antero-posterior) axis during Seated, Natural stance, Feet-together stance and Tandem stance were neither correlated with baseline disease duration and total SARA scores, nor with Gait, Stance and Sitting items of the SARA scale. Better performances were associated with older age at baseline and with older age at instability onset. Age at baseline was significantly correlated with oscillation during Seated task in both x (r=-0.337; p=0.003) and y (r=-0.308; r=0.007) axes, in Natural stance on the x axis (r=-0.350; p=0.011) and on Feet-together stance on x (r=-0.614; p<0.001) and y (r=-0.482; p=0.025) axes. Age at onset of instability was correlated with the deviation on the *x* and *y* axes during Seated task (r=-0.503 and r=-0.426 for x and y, respectively; p=0.001 in both cases), during Natural stance (r=-0.442 and r=-0.372 for x and y, respectively; p<0.01 in both cases) and Feet-together stance (r=-0.614 and r=-0.482 for x and y, respectively; p<0.01 in both cases) (Figure C-1). GAA1 size was also correlated with deviation on the *y* axis during Seated task (r=0.300; p=0.039), and with sway on both x and y axes during Feet-together stance (r=-0.535, p=0.015 and r=-0.468, p=0.038, respectively).
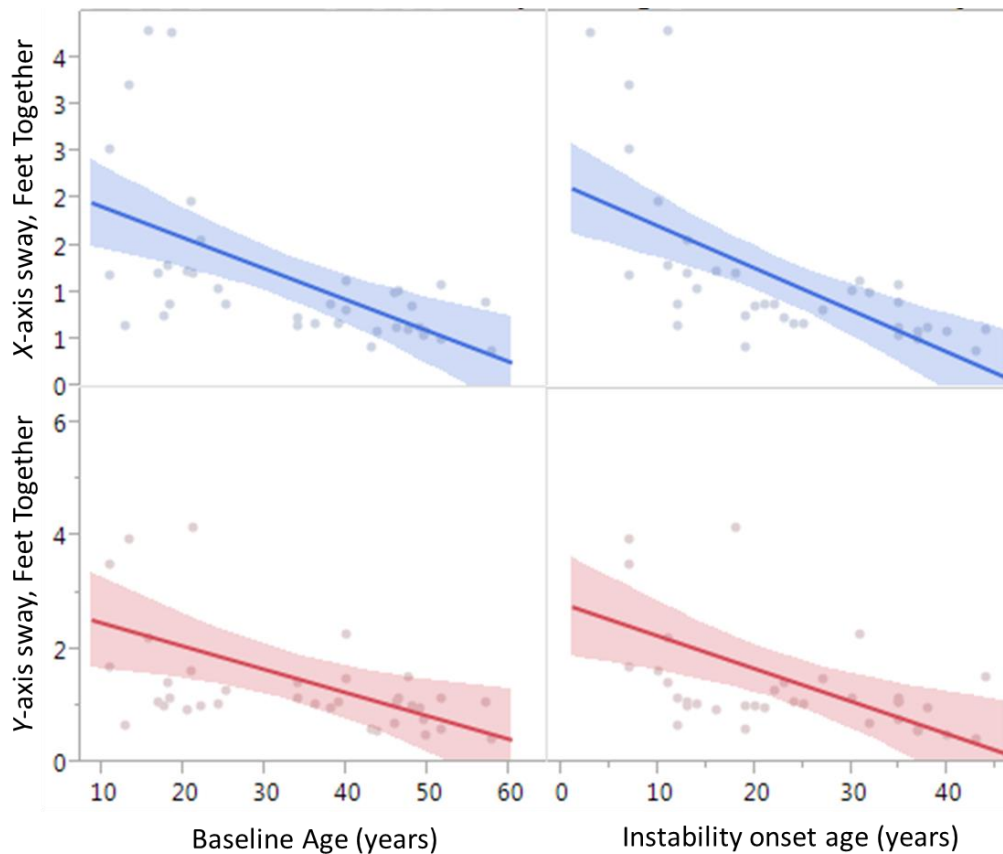
Figure C- 1. Correlation of baseline age and age at onset of instability with *X* and *Y* axes sway during Feet together stance task.

Fifty-eight (76%) subjects completed the 1-year follow-up assessment. At this timepoint Seated task was completed by all subjects; 37 (63%), 21 (36%) and 7 (12%) subjects completed Natural stance, Feet-together stance and Tandem stance tasks, respectively. Two-years assessment was completed by 37 (48%) subjects: of them 100% completed Seated task, 61% completed Natural stance and 36% completed Feet together tasks, only 1 subject completed the Tandem stance task. Due to significant drop-out rate at follow-up 2, we restricted statistical analyses to 1-year follow-up. SARA total score did not change significantly at follow-up 1 (mean score 17.1 vs. 17.4 at baseline). Gait item score showed a trend towards worse performances (from 4.9 to 5.1 points, p=0.06), that reached statistical significance in the typical onset group (from 5.2 to 5.5 points, p=0.02). A significantly worse performance was identified for both *x* and *y* sway (p=0.027 and p=0.010, respectively) at follow-up, compared to baseline, during Feet together stance. The significance was driven by typical onset patients (N.13; *x*-axis sway p=0.027; *y*-axis sway p=0.016) and was not present in LOFA subjects (Figure C-2).
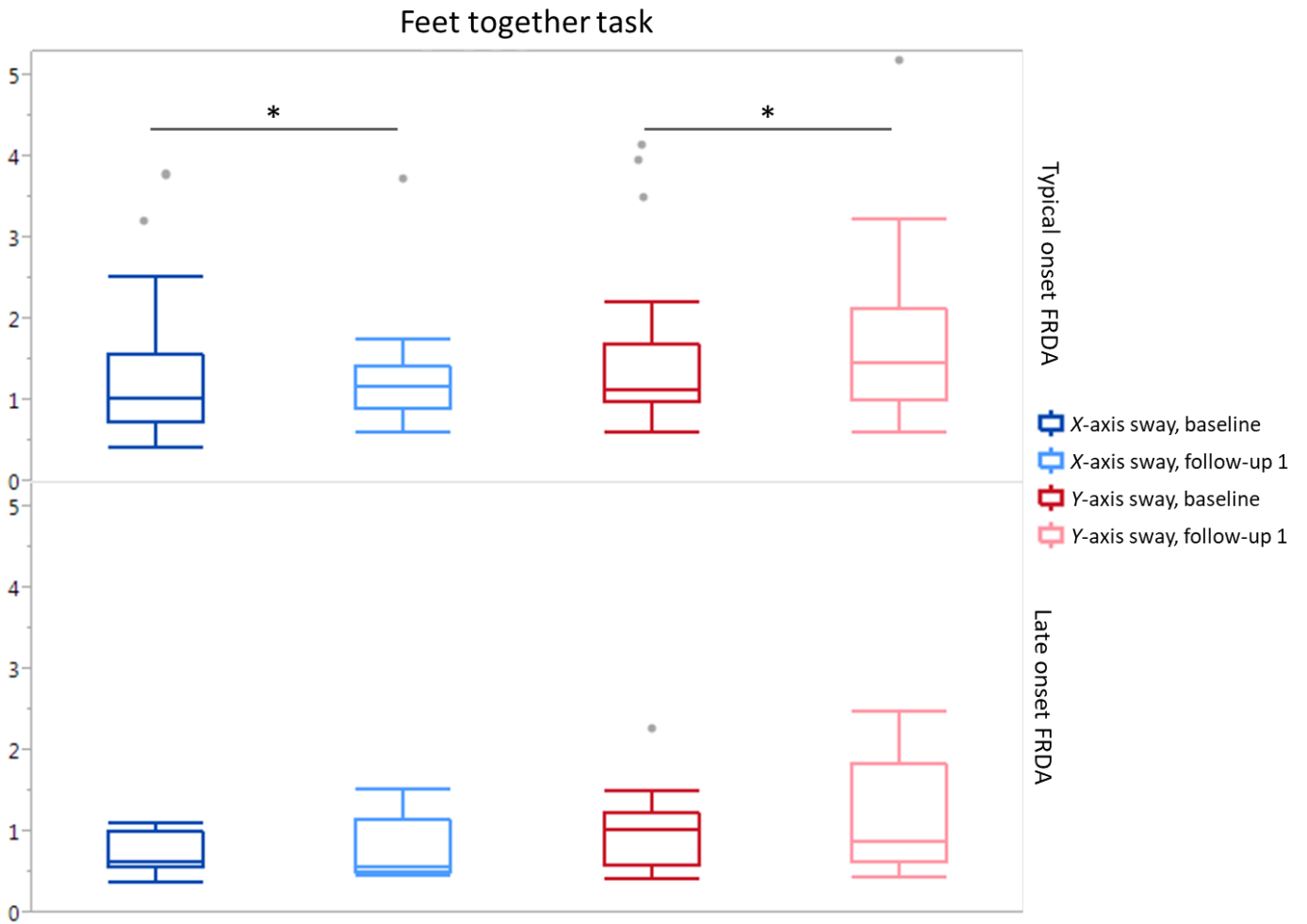
Figure C- 2. . *X* and *Y* axes sway during Feet together task at baseline and follow-up 1 for typical onset FRDA (upper panel) and late onset FRDA (lower panel). Vertical axis indicates degree of sway; * indicates significantly (p<0.05) higher sway at follow-up 1 compared to baseline.

D) Wet Biomarkers

Fifty-three subjects with FRDA (27F/26M) were included in the first part of the study. Mean age at evaluation was 39±12 years (range 20-72), 20/53 subjects (48%) had evidence of hypertrophic cardiomyopathy. In 22 patients (44%) onset of ataxia was before 14 years of age, 16 subjects (32%) had ataxia onset between 15 and 24 years of age and 12 (24%) subjects were late-onset FRDA. Data for mRNA expression were available for 39 patients (74%, mean age 42±13 years) and 23 non-related healthy controls (mean age 41±13 years). Data for protein expression were available for 41 patients (77%, mean age 39±13 years) and 27 non-related healthy controls (mean age 41±12 years). FXN and HAX-1 expression appeared to be co-regulated, both at mRNA and protein levels, regression analysis showed a statistically significant association between FXN and HAX-1 both at mRNA ($R^2 = 0.55$, $p<0.05$, Pearson $r = 0.54$) and protein ($R^2 = 0.6$, $p<0.001$, Pearson $r = 0.81$) levels (Figure D-1). The levels of expression of FXN and HAX-1 between subjects with and without hypertrophic cardiomyopathy was not significantly different, though the HAX-1 mRNA expression was lower in subjects with hypertrophic cardiomyopathy.
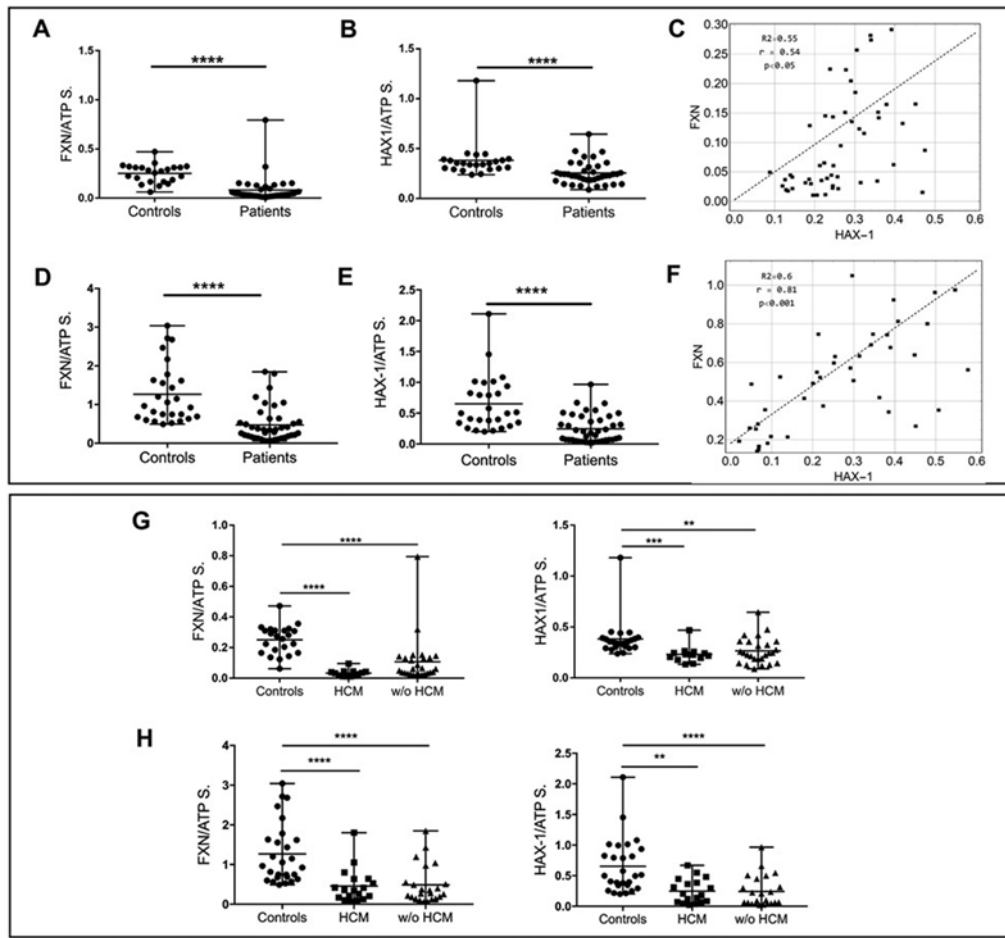
Figure D- 1. FRDA PBMCs express lower levels of HAX-1. FXN and HAX-1 expression were analyzed in PBMCs derived from FRDA patients (Patients) and healthy individuals who did not exhibit any type of heart problems (Controls). mRNA expression was normalized with ATP SYNTHASE (A, B) and ATP Synthase in (D, E). Scatter plot showing Frataxin (FXN) versus HAX-1 mRNA (C) and protein (F) values across all subjects. R2: R squared value of the regression model. FXN and HAX-1 mRNA (G) and protein (H) levels in 13 FRDA subjects with hypertrophic cardiomyopathy (HCM), 26 without hypertrophic cardiomyopathy (w/o HCM) and 23 healthy individuals (Controls). ($**P < 0.01$, $***P < 0.001$, $****P < 0.0001$).

miRNA expression, evaluated in 37 FRDA (mean age 42±14 years) compared to 22 non-related healthy controls (mean age 40±13 years) showed a significant increase in hsa-miR223-3p in FRDA patients (Figure D-2); the result remained statistically significant when dividing FRDA patients according to onset age in EOG, IOG and LOFA groups. hsa-miR223-3p expression levels were not associated with age at the time of analysis neither to gender. Using ROC analysis we found that hsa-miR223-3p differentiated FRDA patients from controls with a total accuracy of 0.835 (95% CI: 0.736, 0.933; P < 0.0001), AUC for EOG, IOG and LOFA compared with matched controls was 0.951 (P < 0.001), 0.774 (P = 0.005) and 0.784 (P = 0.008), respectively. In typical onset FRDA a positive trend (p=0.06) was identified between hsa-miR223-3p expression and SARA score (r=0.37). We also analyzed hsa-miR223-3p expression in regard to

echocardiographic parameters such as IVS (interventricular septal wall thickness) and LPW (left ventricular posterior wall thickness) in 31 FRDA subjects with complete data.
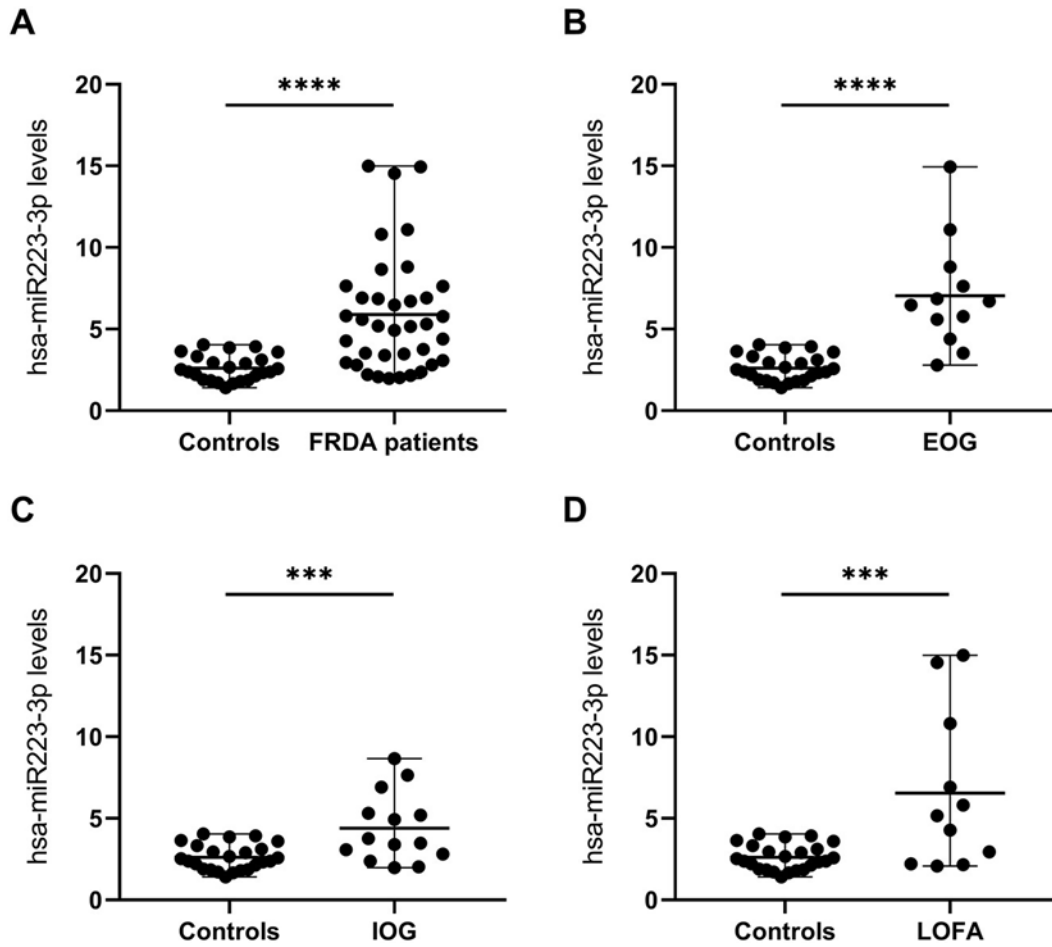
Figure D- 2. Validation of hsa-miR223-3p plasma expression level in FRDA patients. Hsa-miR223-3p expression (2−△Ct) in: (A) FRDA patients (n = 37); (B) EOG group (n = 12); (C) IOG group (n = 14) and (D) LOFA group (n = 11) compared with control subjects (n = 22). Values are means of two independent experiments. Mean and data range are illustrated. (***P < 0.001, ****P < 0.0001).

A significant positive correlation between hsa-miR223-3p level and both IVS and LPW was found in typical-onset FRDA (onset < 25 years) (r=0.47, p<0.05 for IVS; r=0.54, p=0.01 for LPW; Figure D-3). A significant negative correlation between hsa-miR223-3p and HAX-1 mRNA and protein levels was identified only in FRDA patients (r =-0.40, p<0.05 and r=-0.53, p<0.005, respectively), suggesting that HAX-1 gene could be a functional target of hsa-miR223-3p.
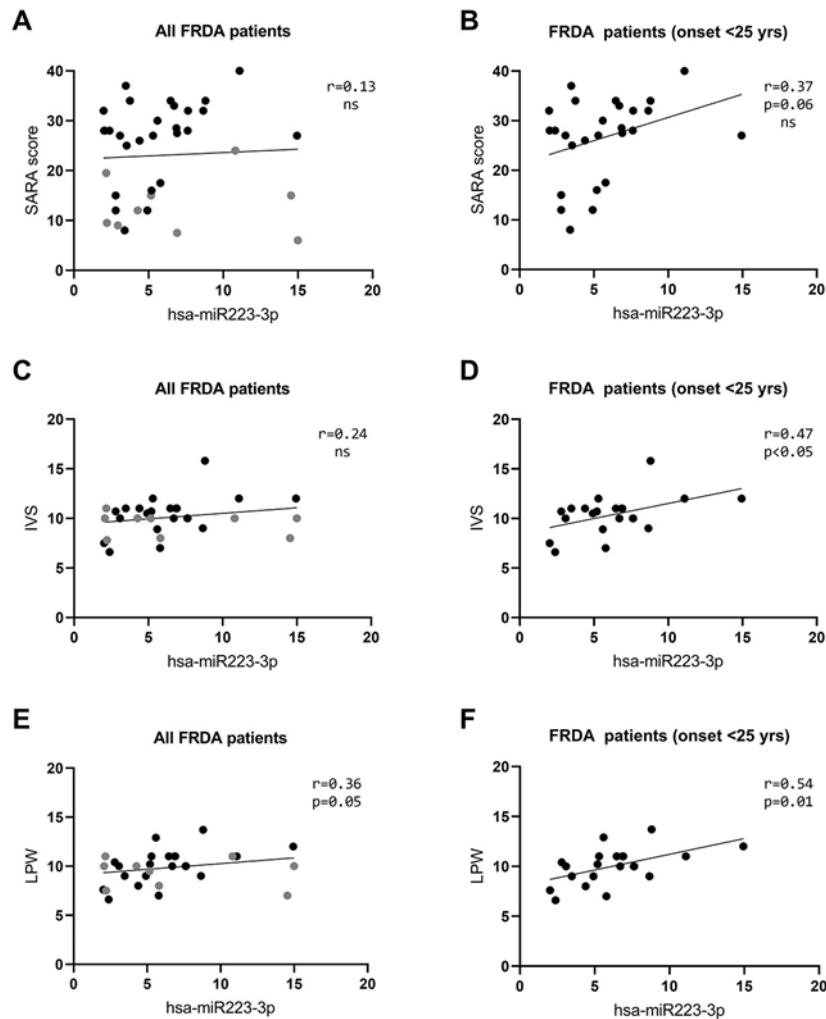
Figure D- 3. Correlation analysis of Hsa-miR223-3p with clinical parameters in FRDA. Scatter plots showing hsa-miR223-3p expression versus: (A, B) the SARA (Scale for the Assessment and Rating of Ataxia) score; (C, D) the interventricular septal wall thickness (IVS) values and (E, F) the left ventricular posterior wall thickness (LPW) values. The panels on the same row, referred to the correlation analysis of hsa-miR223-3p versus the same clinical parameter, have been carried out on: (left) all available FRDA patients [n = 37 (A), 31 (C), 31 (E)] and (right) FRDA patients with typical age at onset < 25 years [n = 26 (B), 21 (D), 21 (F)]. FRDA patients with an age of onset of clinical symptoms ≥25 years (LOFA patients) are presented in A, C, E as gray dots.

Data from this study have been published in:

Tiano F, Amati F, Cherubini F, Morini E, Vancheri C, Maletta S, Fortuni S, Serio D, Quatrana A, Luffarelli R, Benini M, Alfedi G, Panarello L, Rufini A, Toschi N, Frontali M, Romano S, Marcotulli C, Casali C, Gioiosa S, Mariotti C, Mongelli A, Fichera M, Condò I, Novelli G, Testi R, Malisan F. 2020. 'Frataxin Deficiency in Friedreich's Ataxia Is Associated with Reduced Levels of HAX-1, a Regulator of Cardiomyocyte Death and Survival'. Human Molecular Genetics 29 (3): 471–82. https://doi.org/10.1093/hmg/ddz306.

Quatrana A, Morini E, Tiano F, Vancheri C, Panarello L, Romano S, Marcotulli C, Casali C, Mariotti C, Mongelli A, Fichera M, Rufini A, Condò I, Novelli G, Testi R, Amati F, Malisan F 2022. 'Hsa-MiR223-3p

Circulating Level Is Upregulated in Friedreich's Ataxia and Inversely Associated with HCLS1 Associated Protein X-1, HAX-1'. Human Molecular Genetics 31 (12): 2010–22. https://doi.org/10.1093/hmg/ddac005

Moreover, as part of the EFACTS study group, sample and data from our cohort have been used for the study published in:

Hayer SN, Liepelt I, Barro C, Wilke C, Kuhle J, Martus P, Schöls L; EFACTS study group (Fichera M). NfL and pNfH are increased in Friedreich's ataxia. J Neurol. 2020 May;267(5):1420-1430. doi: 10.1007/s00415-020-09722-6. Epub 2020 Jan 30. PMID: 32002649; PMCID: PMC7184046.

E) Actigraph

Twenty-six subjects with FRDA and 13 healthy controls were enrolled for the ActiGraph study and wore the devices as planned. All FRDA subjects (16M/10F) were still ambulatory (i.e. SARAgait <8) at the time of the evaluation, mean age at baseline was 27.1±7.8 years (range 18-43), mean age at onset was 15.4±4.9 years (range 7-24) and mean disease duration was 11.7±6.9 years (range 1-28). Healthy controls were age (25.9±3.1 years) and sex (6M/7F) matched (p>0.05 for both comparisons). Mean SARA score in FRDA subjects was 17.2±6.0 (range 5.5-30).

All subjects except one in FRDA group returned both Actigraphs for data collection and analyses; one subject with FRDA lost the Actigraph worn on wrist. Subjects with and without FRDA completed the one-week assessment with high adherence, mean number of days with viable data was 6.92 in FRDA and 6.84 in controls, mean number of analyzed epochs was 38984±12137 and 39327±11760 in controls and 40246±13400 and 39367±13531 in subjects with FRDA for wrist and waist Actigraphs, respectively.

Table E-1 summarize the results obtained for both wrist and waist worn Actigraph. Subjects with FRDA consumed significantly less active Kcals, spent more time in sedentary activities compared to healthy controls and took less steps during the recorded period. A significant difference was also identified for all variables for "Location" factor, due to higher activity recorded by wrist-worn Actigraph compared to waist-worn Actigraph. The effect was present both in healthy controls and in subjects with FRDA without significant differences: a significant interaction between Group and Location factors was identified only for % of time spent in MVPA (p=0.0078), where wrist-worn Actigraph estimated relatively more activity in healthy subjects than in FRDA subjects compared to waist-worn Actigraph.

Table E- 1. Comparison between subjects with FRDA and healthy controls (CTR) for the analyzed variables.

	FRDA	CTR	p-value
Waist			
Activity Kcals	180.88±228.78	1382.84±821.41	<0.001
Average Kcals per day	22.69±28.43	173.36±102.01	<0.001
MET rate	1.02±0.02	1.17±0.10	<0.001
% time in Sedentary	91.98±4.01	81.67±5.27	<0.001
% time in Light activity	7.48±3.72	13.82±4.00	<0.001
% time in MVPA	0.54±0.48	4.50±2.39	<0.001
% in very Vigorous activity	0.00±0.00	0.00±0.00	0.143
Step count	9885.46±8538.48	45340.69±13918.16	<0.001
Average steps count per minute	0.25±0.18	1.23±0.52	<0.001

Wrist			
Activity Kcals	2306.01±2244.26	4263.79±1766.46	0.006
Average Kcals per day	290.36±281.36	542.14±219.32	0.005
MET rate	1.28±0.19	1.54±0.19	0.007
% time in Sedentary	67.10±11.01	53.00±11.90	0.002
% time in Light activity	23.83±7.18	29.08±6.41	0.029
% time in MVPA	9.07±5.30	17.91±5.67	<0.001
% in very Vigorous activity	0.00±0.00	0.00±0.00	n.e.
Step count	36220.64±19969.13	65795.77±11967.61	<0.001
Average steps count per minute	0.90±0.41	1.82±0.52	<0.001

No significant correlations were identified between demographical, clinical and activity variables in healthy controls. In subjects with FRDA we identified a number of significant correlations between activity and clinical variables. For waist-worn Actigraph the most relevant correlations were found with Step count, that was highly correlated with disease duration (ρ=-0.568, p=0.002); SARA gait (ρ=-0.823, p<0.001) and stance (ρ=-0.751, p<0.001), with total SARA score (ρ=-0.807, p<0.001), with ADL falls (ρ=-0.618, p=0.001) and walking (ρ=-0.689, p<0.001), with FARS part B (ρ=-0.518, p=0.008), part C (ρ=-0.684, p<0.001), part E (ρ=-0.786, p<0.001) ant total mFARS score (ρ=-0.755, p<0.001). Significant correlations were also found with functional tests, such as CCFS derived H-index (ρ=-0.719, p<0.001), 9HPT (ρ=-0.690, p<0.001) and 8MWT (ρ=-0.783, p=0.002). The second most correlated activity variable was % spent as sedentary, that was also correlated with GAA1 expansion (ρ=0.418, p=0.038), but not with age of instability onset. Data from wrist-worn Actigraph confirmed that step count was still the most correlated variable overall, though with lower correlation coefficients compared to waist-worn Actigraph. Differently from waist-worn Actigraph data, time spent in sedentary activities was not correlated with any clinical variable, while % spent in MVPA showed significant correlation with SARA score (ρ=-0.570, p=0.003), ADL score (ρ=-0.530, p=0.007), mFARS score (ρ=-0.577, p=0.003), as well as with CCFS H-index (ρ=-0.543, p=0.008), 9HPT (ρ=-0.608, p=0.002) and 8MWT (ρ=-0.572, p=0.041) (Figure E-1).
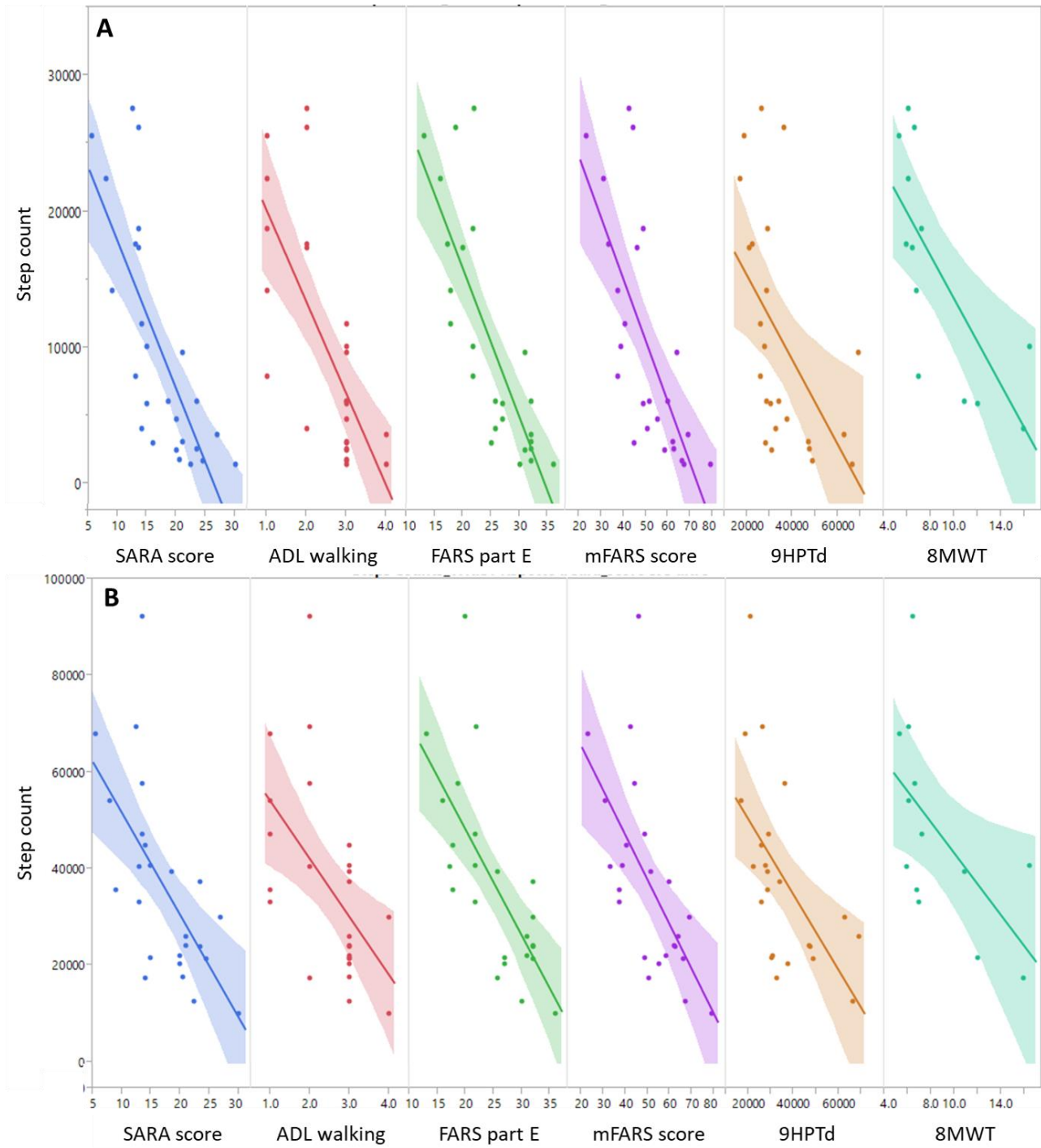
Table E-1. Correlations between step count derived from waist (A) and wrist (B) worn Actigraph and selected variables in subjects with FRDA.

(Fichera et al., in preparation)

Discussion

We aimed to test existing outcome measures (parts A and B) and to identify alternative and complementary outcome measures to rate disease severity and progression (parts C, D and E). So far, no single outcome measure can be considered the "gold-standard" to assess disease severity in FRDA. When designing interventional trials, especially in rare diseases, it is essential to maximize trial power, while keeping the number of participants required low. This goal can be achieved only if the proper outcome measure is adopted. In past years many trials have tried to identify disease-slowing drugs (Appendix Table 1), with unsatisfactory results. It is possible that some of these negative results are due to poor patient selection and/or the use of inadequate outcome measures. The results presented here increase our knowledge of available outcome measures, and propose new tools to assess FRDA.

A) Properties of SARA scale

Many factors that have been implied as predictors of more rapid progression of ataxia severity in subjects with FRDA. Most of these factors are demographical and genetic characteristics, such as age at examination, age at onset, GAA1 repeat length, ambulatory status and disease duration (Patel et al., 2016; Reetz et al., 2021; Rummey et al., 2022). The identification of the specific population of subjects with FRDA where disease progression is most evident with the available tools is complementary to the identification of other, more robust and sensitive outcome measures. SARA scale is applied to assess ataxia severity and progression in the ongoing European natural history study on FRDA, and has also been applied in some clinical trials to assess efficacy of interventions (Appendix Table 1). Statistical properties of SARA scale applied in subjects with FRDA have been reported only in a single study, as previously stated (Bürk et al., 2009). We sought to identify intrinsic properties of SARA scale that impact rating of disease severity with this instrument. For this reason we focused on typical onset FRDA, to reduce variability of measurement due to different progression rate in LOFA (Reetz et al., 2021, 2015). Our data show that SARA scores follow a bi-modal rather than normal distribution. Non-normal distribution of scores was present at baseline and was confirmed at subsequent follow-ups. We are not able to clearly discern if the bimodal distribution of data is a characteristic of FRDA disease progression or if it is related to SARA scale properties. The latter option appears more plausible, since as shown in part B, FARS scale seems to have a normal distribution of scores. We also cannot exclude bi-modal distribution of SARA scores as a characteristics of ataxia rating at our site, though no site effect was detected in the overall analysis of the EFACTS cohort (Reetz et al., 2016, 2015). If confirmed, this finding may have relevant implications. Due to non-normal distribution, mean and standard deviation cannot be considered appropriate indicators to summarize data and data itself need to be analyzed with different statistical tools. Moreover, power calculation relying on normal data distribution, such as those provided previously (Reetz et al., 2021, 2016), are likely to provide incorrect

results. Results of the present study also confirms that items composing SARA scale contribute differently to the total score according to disease stage (Pandolfo, 2020). SARA scale showed low ceiling and floor effect, though the same was not true for the single items composing the scale. Gait, stance and lower limb coordination are affected early on and contribute to SARA score increase up to 20-25 points, when they reach a plateau. This score corresponds roughly to the loss of ambulation. After this point SARA score progression is mainly driven by sitting, upper limb, and speech items. Notably, items assessing upper limb tremor and dysmetria showed a highly non-linear progression, remaining stable up to the advanced phase, when scores increase rapidly. Non-linear progression of items composing ataxia rating scales has been observed also for ICARS scale, where patients the posture/gait subscale contributed by >50% to the rate of change in (Metz et al., 2013) until reaching a plateau around 20 years of disease duration.

Longitudinal analyses were carried on considering the non-linear increase of SARA score with disease duration and the non-normal distribution of scores. Steeper slope was identified for subjects with the lowest baseline SARA score (0-10), and a progressive lowering of the slope coefficient was observed with increasing baseline score. This confirms the non-linear evolution of SARA scores throughout disease progression in FRDA. Similar results have been observed in FACOMS cohort with FARS scale (Patel et al., 2016). While this could reflect a slowing of disease progression with time, it likely reflects ceiling effects of some items composing the scales. To assess signal-to-noise ratio we calculated the standardized response mean for each group of subjects at all timepoints. Interestingly, highest SRMs were identified, at each follow-up, for subjects with baseline SARA between 10.5 and 20, despite higher mean change in the SARA 0-10 group. Our data is in agreement with a previous study (Tanguy Melac et al., 2018) that identified a non-linear relationship of SARA score with CCFS, a measure linearly correlated with disease duration, below 10 points and above 24 points of total SARA score. Taken together these data suggest a reduced variability between 10 and 20 point of total SARA score. This finding may serve as a clear inclusion criterion for clinical interventional trials. Our analysis also shows that items assessing upper limb function show poor SRM and increase the "noise" in the measurement. Removing or modifying these items may improve overall performances of the SARA scale. As a matter of facts, SARA scale has been recently reviewed by the U.S. Food and Drug Administration, resulting in a modified version to be used in clinical interventional trials. This latter version has not yet been tested in FRDA and its psychometric properties in this population are unknown.

B) SARA-FARS comparison

In our population of subjects with FRDA SARA was confirmed to have a non-normal distribution of values, while FARS, and mFARS, scores were normally distributed. This finding may indicate that presence of bi-modal distribution of scores is specific for SARA scale measurement of ataxia in FRDA subjects, rather than a characteristic of FRDA itself. SARA scores, FARS scores and ADL scores were highly inter-correlated, and

were correlated with demographical and genetic characteristics of subjects, in agreement with previous findings (Bürk et al., 2009); we also show that mFARS scores are similarly highly correlated with SARA scores. Though no relevant floor or bottom effects were identified for FARS/mFARS scores, relevant floor effect was present for items in section A (bulbar), while ceiling effect was most apparent for items in section E (gait and stance), a finding that has been reported only recently in literature (Rummey et al., 2019b). All tested items in SARA and FARS/mFARS scales demonstrated high construct validity, as well as good convergent and divergent validity as demonstrated by the high correlation with items assessing similar functions. Our factorial analysis confirms that SARA total scores in FRDA are determined by one single factor, namely ataxia, while for 7 and 4 main factors were identified for FARS and mFARS scales, respectively, not clearly coinciding with subscales construct. This result is in accordance with data provided by Burk et al. and by Rummey et al., that identified more than one factor weighing on FARS and mFARS scores (Bürk et al., 2009; Rummey et al., 2019b). As previously reported (Rummey et al., 2019b) removal of items A1 and A2 and of part D from FARS (i.e. mFARS) improves overall psychometric properties of the scale. No data are available in current literature regarding ADL scale psychometric properties. In our sample ADL sum scores were normally distributed, similarly to FARS/mFARS scores. No relevant ceiling or floor effect was identified for total score, though present at single items level. ADL scale displayed high internal consistency and good item-total score correlation coefficients were found for all items except Bladder item, that was also not correlated with the other items of the scale. Principal component analysis confirmed that Bladder item indeed weighted on a different factor than all the other items. Our data suggest that this item may not be suitable to assess functional capacity in subjects with FRDA, and may therefore be omitted without impairing scale capacity to rate functional impairment due to ataxia. Further data from longitudinal analyses are needed to confirm this finding.

Effect of exercise on scale scores

We tested the effect of strenuous physical exercise on clinical rating of ataxia in a subset of FRDA subjects. Non-statistically significant changes in SARA (+0.7 points) and FARS part E (-0.8 points) were identified following physical exercise in our sample. Of note, one-tailed Wilcoxon test reached statistical significance for worsening of total SARA score and of Sitting item (+0.28 points). The effect of chronic physical exercise in FRDA has been assessed in trials evaluating its therapeutic potential (Milne et al., 2018), but the acute effect of such interventions is unknown. On the other hand, test-retest reliability under experimental conditions has been assessed for FARS scale (Rummey et al., 2020b), using data derived from repeated assessments in interventional trials, where the same standardized conditions for assessment are expected. The small sample size is of course a factor limiting statistical significance in our study and may also explain the opposite trends of SARA and FARS part E score following exercise. Nevertheless, the observed changes are of a magnitude similar to changes reported in interventional trials. Most interventional trials do not provide a precise order of assessments, while in a recent trial (Lynch et al., 2021) clinical assessment was

performed after physical exercise with cycloergometer, as in our case. Our finding highlight the importance of confounding factors on clinical assessment, and suggest that physical exhaustion can be a relevant factor for ataxia assessment. Future interventional trials should include a precise schedule of assessment during each in-clinic visit, to limit such confounding factor, and should also consider physical activity in a similar manner to other interventions, such as pharmacological therapies.

C) Posturography

Classical posturography adopts force plates or optoelectronic systems, providing accurate and reliable measurements for balance. However, these tools are expensive, encumbering, and require dedicated equipment and personnel, thus limiting their use for long-term monitoring in clinical practice. As a response, research on posturography is focusing on wearable technologies, which should provide objective and real-life monitoring of postural ability at a cheaper cost. Inertial sensors are the most used solution and have been tested in various neurological conditions, including ataxia (Ilg et al., 2016; Shirai et al., 2019). We aimed to measure the progression of balance deficits in subjects with FRDA using static posturography with inertial sensors, placed over trunk. The task completed by the patients were chosen in order to reflect assessment of balance included in routine clinical assessment of SARA scale and included a Seated task and increasing difficulty stance task: natural stance, feet together stance and tandem stance. We did not find any significant correlation between sway on x and y axes and demographical and clinical variables at baseline, in contrast with previous findings (Milne et al., 2021; Mueller et al., 2021; Schwabova et al., 2012). Surprisingly, we did not detect any significant change in total SARA score at one-year follow-up in this cohort of subjects, with only a trend toward deterioration of Gait score. Posturographic measures showed a significant worsening in balance performances at 1 year follow-up when considering the feet-together stance task, for both x and y axis, no other significant changes were identified. When dividing subjects according to age at onset into typical vs. late the significativity remained only for the typical onset group. As of today only one paper has assessed the use of posturographic assessment in FRDA in a longitudinal study (Milne et al., 2021), using a postural test on a force platform. In this study, the authors tested 61 subjects with FRDA at baseline, 6 and 12 months and identified a number of measures that significantly changed over time, and identified the medial-lateral index as the most sensitive measure at 12 months. This measure was superior to clinical evaluation with FARS in detecting change. Posturographic assessment has been tested more extensively in other hereditary cerebellar ataxias, where superiority to clinical assessment has been found by multiple studies (Ilg et al., 2016; Shirai et al., 2019; Zhou et al., 2021). Quantitative assessment of balance was also used successfully to detect subtle, sub-clinical abnormalities in subjects with hereditary ataxia in the "premanifest" stage (Ilg et al., 2016; Nanetti et al., 2017; Velázquez-Pérez et al., 2021).

Our results do not support the use of DELOS system to evaluate balance impairment in Friedreich ataxia, though some limitations must be taken into account. In our study we enrolled patients regardless of their ambulatory status, reflected in the high number of subjects unable to complete all the tasks provided already at baseline. Moreover, our population of subjects with FRDA included both typical onset and late onset subjects, whose disease progression is notably slower. Inclusion of a more homogeneous population of subjects could have reduced inter-subjects variability. Wearable devices may also suffer a reduced test-retest reliability compared to other tools, such as clinical scales (Mueller et al., 2021).

Finally, though posturographic parameters were not correlated with disease duration or ataxia severity, a significant correlation was identified with baseline age and age of ataxia onset. Subjects with onset of ataxia at a younger age displayed worse balance performances, and younger subjects performed worse than older patients. This is particularly interesting since an opposite pattern is present in the healthy population, with older subjects displaying more postural sway than younger subjects (Roman-Liu D, 2018). A similar correlation with some posturographic parameters was present for GAA1 length. These data may underlie compensatory strategies (Milne et al., 2014) that develop early in the disease course or may reflect a neurodevelopmental alteration in posture and sensory control (Marty et al., 2019).

D) Wet Biomarkers

Our study showed a positive correlation between FXN and HAX-1 expression both at protein and mRNA levels in FRDA, suggesting that HAX-1 downregulation is secondary to FXN deficit. HAX-1 comprises a ubiquitously expressed family of proteins with antiapoptotic properties, especially relevant for the survival of different cell types including cerebellar neurons (Lu et al., 2018). HAX-1 is highly expressed in the heart and is involved in protection of cardiomyocytes from injury (Lam et al., 2013). Both HAX-1 and FXN are upregulated in hypoxic environment (Jiang et al., 2013), and HAX-1 downregulation in different types of cells results in increased apoptosis due to oxidative stress (Bidwell et al., 2018). Linked expression of FXN, HAX-1 and antioxidant proteins such as Nrf2 was observed in peripheral blood mononuclear cells from FRDA patients and healthy controls, as well as in human cardiomyocytes (Tiano et al., 2020). Considering that apoptosis is one of the mechanisms driving the progression of cardiomyopathy and neurodegeneration, reduced levels of HAX-1 could contribute to the development of hypertrophic cardiomyopathy and neuronal deficit. We also identified a significant increase in expression levels of a particular miRNA, hsa-miR223-3p, in subjects with FRDA compared to controls, in agreement with a previous study (Dantham et al., 2018). Hsa-miR223-3p showed a good discrimination power between patients and healthy controls (AUC value of 0.835). A significant negative association between hsa-miR223-3p and HAX-1 mRNA and protein expression in FRDA was found, suggesting a functional role of hsa-miR-233-3p on HAX-1 expression. This association was not present in healthy controls, possibly reflecting a potential hsa-miR223-3p modulation on HAX-1 expression linked to pathological mechanisms. Indeed, hsa-

miR223-3p overexpression in cardiac hypertrophy and dysfunction is supported by other studies in other cardiovascular diseases (Rizzacasa et al., 2019). In our sample of typical onset FRDA subjects hsa-miR223-3p expression was positively correlated with cardiac parameters (ISV and LPW), while it was not associated with subjects age or gender (Quatrana et al., 2022). The correlation of hsa-miR223-3p expression was absent in LOFA patients, in which cardiac disease is milder and less frequent compared to typical onset FRDA. This finding may indicate that increased hsa-miR223-3p expression is associated with a more severe cardiac phenotype. Cardiac dysfunction is a well-recognized feature of FRDA, usually developing early in the course of the disease and a leading cause of death in FRDA (Tsou et al., 2011). Recently another molecule, C-Terminal Cross-linked Telopeptide of Type I Collagen, has been proposed as a biomarker for fibrosis in subjects with FRDA (Pane et al., 2022). The identification of new disease biomarkers associated to cardiac alteration could be important both in clinical practice to predict morbidity and mortality and as outcome measure of therapeutic response in future clinical trials. Investigation of HAX-1 and hsa-miR223-3p expression in a larger FRDA population and in longitudinal studies might provide insights into FRDA-related cardiomyopathy, as well as probing its role as a cardiac biomarker in this population.

E) Actigraph

Digital biomarkers are increasingly considered as outcome measures in neurological diseases, and have already been included as secondary outcome measures in a number of interventional trials, providing good quality and informative data (Lipsmeier et al., 2022). Here we tested real-life activity monitoring with Actigraph in a cross-sectional study, to evaluate the usability and discriminative power of activity monitoring. Actigraph monitoring was well tolerated by subjects and generated high amount of good quality data. We found that subjects with FRDA were inactive most of the day, significantly more than healthy controls, and were more likely to engage in low intensity movements and less likely to perform high intensity movements compared to controls. In particular, step count showed a potential role as a biomarker, as it separated clearly subjects with and without FRDA, was highly correlated with clinical measures of ataxia severity and was not influenced by other demographical characteristics, such as age. Our data are in agreement with another study that employed activity monitoring with a different tool, and identified step count as a possible sensitive digital biomarkers in FRDA in a longitudinal study (Milne et al., 2021), though results over 6 and 12 months follow-up were partially discordant. Free-living activity monitoring in subjects with other hereditary ataxias with a similar phenotype, such as ataxia-teleangectasia (Khan et al., 2022), has yielded similar positive results. In our study greater activity levels were measured for the wrist sensor compared to waist sensor. We would have expected a higher overestimation of activity for wrist sensors in subjects with FRDA compared to healthy controls, possibly related to upper limb dysmetria, and as it has been reported by other authors (Mueller et al., 2021). The effect was indeed shared by both FRDA and controls, without significant differences between the two groups. In previous

studies on healthy subjects step count derived from wrist-worn accelerometers has been found to be less precise than waist-worn accelerometers, especially during free-living conditions (Tudor-Locke et al., 2015). This may partially explain the lack of correlation of most activity data from wrist sensors with clinical measures.

Our study has some limitations. We provided subjects with a daily diary, in order to ensure that analyzed wear time was correct, however since the data were collected in a free-living context, the precise nature of the activities and behaviors being recorded remains largely unknown. Subjects with FRDA could either have been engaged in the same activities as healthy controls, but less vigorously, or they could have been engaged more often in less vigorous activities. Moreover, the analysis of activity was based on proprietary software (Actilife) that lacks an algorithm specific for the ataxic population, thus over- or under-estimating performance and introducing other possible bias.

In conclusion, digital assessment of FRDA, using wearable equipment, has the potential to provide robust and sensitive biomarkers. Simple parameters, such as step count, are easy to interpret and are likely to be of use in clinical practice. More complex assessment may reduce compliance of subjects, generate difficulties in dealing with the equipment or with the task provided, and ultimately lead to low-quality data (Mueller et al., 2021). Present findings need to be confirmed in longitudinal studies (ongoing at our Centre), and in larger cohorts.

Conclusions

Clinical scales, namely SARA and FARS, remain essential to monitor disease progression, and modified version have been created to increase their sensitivity.

Biomarkers and activity measurements, can be used to assess specific features of the ataxia diseases, and provide a more objective, real-life information. The final aim of reliable measures in rare diseases is to identify outcome measure/s that can potentially lower the number of participants in upcoming interventional trials.

The combined use of clinical scales and functional measures may be ideal for heterogeneous diseases, such as ataxia.

Bibiography

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Appendix

Table 1. Comparison of study design, duration, and patient characteristics in pharmacological interventional trials in FRDA.

Drug	Study design	Subjects N. Drug/ Placebo	Duration (weeks)	Center N.	Clinical Outcome measure	Mean change End of study Drug	Mean change End of study Placebo	Reference
(+)-Epicatechin	OL	10/0	24	1	FARS mFARS	-2.9 -2.0		Quareshi MY et al., 2020
A0001	OL	31/0	4	1	FARS	-4.9		Lynch DR et al., 2012
Carbamylated Erythropoietin	RCT	23/13	2	6	FARS SARA	0 -0.3	-1.0 -0.1	Boesch S et al., 2014
Deferiprone	RCT	21/17	26	6	FARS ICARS	-0.5* -0.8*	-0.8 -1.4	Pandolfo M et al., 2014
EPI-743	OLE	42	78	3	FARS	+1.8		Zesiewicz T et al., 2018
EPI-743	RCT	40/20	26	3	FARS	-5.3*	-2.6	Zesiewicz T et al., 2018
Epoetin alpha	RCT	27/26	48	3	SARA	-0.3	+1.0	Saccà F et al., 2016
Erythropoietin	OL	8/0	26	1	FARS SARA	-8.4 -5.3		Boesch S et al., 2008
Erythropoietin	OL	5/0	12	1	SARA	-2.3		Nachbauer W et al., 2011
Erythropoietin	RCT	11/5	24	1	SARA	+0.5	+0.5	Mariotti C et al., 2012
Exenatide	OL	7/0	5	1	SARA	-0.9		Igoillo-Esteve M et al., 2020
Gamma-IFN	OL	11/0	26	1	SARA	-0.23		Vavla M et al., 2020
Gamma-IFN	OL	10/0	12	1	FARS	-5.0		Seyer L et al., 2015
Gamma-IFN	OL	9/0	5	1	SARA	0		Marcotulli C et al., 2016
Gamma-IFN	OLE	42	52	4	mFARS	+0.2		Lynch DR et al., 2019
Gamma-IFN	RCT	46/45	26	4	FARS mFARS	-0.2 -0.6	-0.6 -1.0	Lynch DR et al., 2019

Intervention	Design	Patients	Duration	Centers	Scale	Mean change	Placebo	Reference
Idebenone	OL	68/0	52	2	FARS ICARS	+2.2 +1.1		Meier T et al., 2012
Idebenone	OL	24/0	Up to 270	2	ICARS	+9.0		Pineda M et al., 2008
Idebenone	OL	9/0	52	1	ICARS	-11.3		Artuch R et al., 2002
Idebenone	RCT	46/24	24	2	FARS ICARS	-1.4 -2.5	+0.5 -1.3	Lynch DR et al., 2010
Idebenone	RCT	37/11	26	1	FARS ICARS	-5.8* -4.1*	-2.5 -0.1	Di Prospero NA et al., 2007
Idebenone	RCT	14/14	52	1	ICARS	0	0	Mariotti C et al., 2003
Idebenone	RCT	9/9	6	1	ICARS	-1.0	-2.0	Schols M et al., 2001
Idebenone + darbepoetin alpha + riboflavin	OL	9/0	Up to 60	1	SARA	-1.6		Arpa J et al., 2013
Idebenone + deferiprone	OL	19/0	48	1	ICARS	-0.9		Velasco-Sanchez D et al., 2011
Idebenone + deferiprone + riboflavin	OL	13/0	60-180	1	SARA	+1.0		Arpa J et al., 2013
IGF-1	OL	5/0	52	1	SARA	-0.4		Sanz-Gallego I et al., 2014
Liraglutide	OL	9/0	5	1	SARA	+0.6		Igoillo-Esteve M et al., 2020
Luvadiglustat	RCT	37/26	12	6	mFARS	-1.4	-2.9	Wang H et al., 2021
Methylprednisolone	OL	11/0	26	1	FARS mFARS	+0.6 +0.8		Patel M et al., 2019
Nicotinamide	OL	10/0	8	2	SARA	-0.4		Libri V et al., 2014
Omaveloxolone	RCT	52/17	12	9	mFARS	-3.3	-1.6	Lynch DR et al., 2018
Omaveloxolone	RCT	40/42	48	11	mFARS	-1.6	+0.9	Lynch DR et al., 2020
Resveratrol	OL	24/0	12	1	FARS ICARS	-2.8* -2.1*		Yiu EM et al., 2015
RT001	RCT	13/6	4	1	FARS	-4.8	-2.8	Zesiewicz T et al., 2018
Thiamine	OL	14/0	52	3	SARA	-1.8		Costantini A et al., 2016

Appendix Table 1. Pharmacological interventional trials in FRDA performed between 2001 and 2022. Study characteristics (intervention, design, duration, included patients and number of centers involved) as well as main results are reported. Mean change refers to the mean difference in clinical scores between baseline and end-of-treatment. Negative figures indicate improvement in total clinical score. OL: open-label; RCT: Randomized Controlled Trial; OLE: open-label extension; FARS: Friedreich Ataxia Rating Scale; ICARS: International Cooperative Ataxia Rating Scale; SARA: Scale for the Assessment and Rating of Ataxia; IFN: interferon; IGF-1: insulin growth factor-1; wk: weeks; n.a.: not available; * indicates change for the best dosage.