Title: **Neurological Measures of Progression in Children with Friedreich Ataxia**

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Site Principal Investigator David R. Lynch, MD, PhD

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ABBREVIATIONS AND DEFINITIONS OF TERMS

9HPT	Nine Hole Peg Test
ADL	Activities of Daily Living
BBS	Berg Balance Scale
CCFS	Composite Functional Cerebellar
CHOP	Children's Hospital of Philadelphia
CrCEST	Creatine Chemical Exchange Saturation Transfer
CRF	Case Report Form
DM	Diabetes Mellitus
FRDA	Friedreich's Ataxia
FACT	Friedreich's Ataxia Composite Test
FARS	Friedreich's Ataxia Rating Scale
HRQOL	Health-related Quality of Life
LCSLC	Low Contrast Sloan Letter Charts
LiSN-S	Listening in Spatialized Noise – Sentences test
MEP	Motor Evoked Potentials
MLPA	Multiplex Ligation-dependent Probe Amplification
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MSQLI	Multiple Sclerosis Quality of Life Inventory
NAF	National Ataxia Foundation
OXPHOS	Oxidative Phosphorylation
PedsQL	Pediatric Quality of Life
T25W	Timed 25' Walk
TMS	Transcranial Magnetic Stimulation
TUG	Timed Up and Go
SDU	Standard Deviation Units
UCLA	University of California Los Angeles

ABSTRACT

Context: Friedreich's ataxia (FRDA) is a rare autosomal recessive degenerative disorder characterized by ataxia, dysarthria, sensory loss, diabetes and cardiomyopathy. The discovery of the abnormal gene in FRDA and its product (frataxin) has provided insight into possible pathophysiological mechanisms and novel approaches to treatments in this disease. New therapies based on prevention of oxidant damage from abnormal mitochondrial function improve surrogate markers of disease such as muscle spectroscopy and echocardiography. While such methods for assessing disease progression may be useful, evaluation in clinical trials will require specific clinical outcome measures. Until recently, quantitative tools for clinical assessment of FRDA have been unavailable. For the past 10 years this group of investigators has collaborated on development of clinical measures that can quantitatively assess FRDA, particularly in children (1). While a large amount of measure refinement remains to be performed, the data from their collaboration provide a framework for further investigation and for creating a network for performing further clinical and translational research including clinical trials.

Objectives: To advance clinical care, research and therapeutic approaches in FRDA through the development and validation of clinical outcome measures in FRDA, collection of quantitative serial clinical data on patients and expansion of our established research network. A secondary aim is to investigate frataxin dipsticks (buccal cells swabs and blood samples) designed to measure frataxin protein. A third objective is to define the progression of physiological dysfunction in affected tissues in FRDA. The study aims to quantify impaired skeletal muscle oxidative phosphorylation (OXPHOS) using a magnetic resonance imaging (MRI) protocol to assess post-exercise creatine chemical exchange saturation transfer (CrCEST) and to understand changes in motor evoked potentials (MEP) in subjects with FRDA.

Study Design/Setting/Participants: This study will primarily take place at the Children's Hospital of Philadelphia (CHOP), with a select number of subjects only participating in the clinical testing at the University of Florida and University of California Los Angeles (UCLA). We expect to recruit approximately 110 children across all three sites and study each of them over a 3 year period. Children with a genetically or clinically confirmed diagnosis of FRDA will be offered participation.

Study Measures: For all 110 participants there will be biyearly assessments of a core set of clinical measures and quality of life assessment measures. These include: Friedreich's Ataxia Rating Scale (FARS), timed 9-Hole Peg Test (9HPT), Timed 25 Foot Walk (T25FW), Pediatric Quality of Life questionnaire (PedsQL), Activities of Daily Living (ADL) scales, Ataxia disability score, and two extended walking tests for 1 minute and 6 minutes. Also included are the Timed up and go (TUG), Berg Balance scale (BBS), 2 tests of the Composite Functional Cerebellar (CCFS), electronic pegboard, and the click test. At each visit, whole blood and buccal swab samples will be obtained to monitor frataxin levels.

12 of the study participants at CHOP will undergo annual MRI scan testing for the CrCEST protocol in addition to the core set of tests listed above.

30 of the study participants at CHOP will have annual MEP measurements using transcranial magnetic stimulation (TMS) in addition to the core set of tests listed above.

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Friedreich's ataxia (FRDA) is a rare autosomal recessive degenerative disorder characterized by ataxia, dysarthria, sensory loss, diabetes and cardiomyopathy. FRDA is a progressive neurodegenerative disorder with a prevalence of about 1 in 50,000 persons in the United States. The discovery of the abnormal gene in FRDA and its product (frataxin) has provided insight into possible pathophysiological mechanisms in this disease (1-3). An expanded GAA triplet repeat is found in both alleles of the *FRDA* gene in 97% of people with FRDA. This triplet repeat is located within an intron, leading to decreased RNA transcription and decreased levels of frataxin. While the basic-science-related understanding of FRDA has advanced rapidly, translational and clinical research has moved much more slowly. This reflects two aspects of FRDA. First it is uncommon—even large academic medical centers may only follow 10- 20 patients with this disorder. This requires that, in any clinical or translational study, multiple centers collaborate and assess patients in a coordinated manner. Secondly, until recently, quantitative tools for clinical assessment of FRDA have been unavailable. This has limited the ability of translational approaches to move forward and the initiation of therapeutic trials. Using recent support from the MDA and FARA, a group of investigators collaborated on development of clinical measures that can quantitatively assess FRDA (4). While a large amount of measure refinement remains to be performed, the data from their collaboration provides a framework for further investigation and for creating a network for performing further clinical and translational research including clinical trials. Still a major deficiency is the relative lack of sensitivity of measures in children, and their correlation with potential biomarkers.

Therapeutic interventions are aimed primarily at either 1) up-regulating expression of the frataxin protein (patients all have residual levels of normal protein and experimental approaches have demonstrated proof of principle of this approach with various small drugable molecules), or 2) ameliorating the pathogenic effects of low frataxin levels, namely a) reduced activities of key mitochondrial enzymes involved in energy metabolism that are dependent on Frataxin-dependent mitochondrial iron metabolism (FeS cluster-containing enzymes such as aconitase and OXPHOS enzyme complexes I, II and III, and possibly heme-dependent enzymes such as OXPHOS enzyme complex IV), and b) generalized oxidative stress in mitochondria and its negative consequences on mtDNA, proteins and membrane lipids.

Efficacy of these therapies can be monitored both by scoring clinically-defined neurologic parameters, and also by biochemical measurement of key mitochondrial enzymes, functions or metabolites or by genetic modifiers. Currently, genetic modifier studies have been limited to the lack of DNA samples linked to detailed clinical information that allows for stratification of the disease group based on disease severity and phenotypic variability. Frataxin levels will be a primary biochemical endpoint in therapeutic interventions designed to up regulate the steady-state levels of this protein. Moreover, upregulated frataxin levels will of necessity precede any other markers of clinical efficacy, including neurological scores. Therefore, tests to monitor these levels should be able to use tissue samples that can be obtained repetitively and non-invasively. Examples of such samples are oral epithelial cells obtained by non-invasive gentle cheek swabs and whole blood obtained by standard blood draws or finger prick sampling. Additionally, physiological abnormalities from frataxin deficiency in affected tissues can be monitored using imaging techniques, including ^{31}P magnetic resonance spectroscopy (MRS) and MEP.

1.2 Compliance Statement

This study will be conducted in full accordance with all applicable Children's Hospital of Philadelphia, and participating sites (University of Florida and University of California Los Angeles) Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, and the HIPAA Privacy Rule. Any episode of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent (unless a waiver is granted), and will report unexpected problems in accordance with all participating sites' IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

1.3 Relevant Literature and Data

See references/ Appendix 1 of the attached grant.

2 STUDY OBJECTIVES

Overall Aim: To advance clinical care, research and therapeutic approaches in FRDA through the development of valid yet sensitive clinical and biochemical measures crucial to outcome assessment of children with Friedreich's Ataxia

2.1 Primary Objective (or Aim)

Aim 1: Identify markers of disease progression in children with FRDA and assess potential clinical measures of disease progression in the youngest subjects (n=110).

2.2 Secondary Objectives (or Aim)

Aim 2: Assess the biochemistry of frataxin deficiency in children with FRDA. Measure frataxin deficiency and downstream metabolic function. Understand how these biochemical markers change over time. Support genetic modifier studies and biomarker studies. Evaluate the clinical utility of measuring frataxin levels in easily-accessible tissue samples, cheek swabs, suitable for the repetitive testing needed to monitor efficacy of therapies designed to up-regulate levels of frataxin protein.

Aim 3: Define the progression of physiological dysfunction in affected tissues in children with FRDA. Use noninvasive testing to assess the physiological correlates of progression of FRDA.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

Pediatric Subjects will be recruited from each site's ongoing practice and the Friedriech Ataxia natural history study and invited to participate in this study. Dr. Lynch will recruit up to 90 children from his practice to participate at CHOP, and the remaining two sites, UCLA and University of Florida, will recruit up to 20 children in a similar manner to participate. Such subjects will return every 6 months for neurological measures, and biochemical measures (frataxin levels, platelet metabolic biomarkers). At each annual visit a select number of participants at CHOP will also complete tests assessing anatomical biomarkers. Data will be analyzed based on the change in measures over time to identify those that change serially in FA and the ability of disease features to predict such change.

3.1.1 Screening Phase

Subjects will be identified from natural history studies and clinical practices. Informed consent will be obtained by trained personnel at each site.

3.1.2 Assessment Phase

Visit 1 (0 months) Subjects will be assessed by medical history, neurological tests (FARS, Timed 25 foot walk (T25W), Timed 1 and 6 minute walks, Timed up and go (TUG), Berg Balance scale (BBS), 9 hole peg test (9HPT), PedsQL, ADL score and ataxia disability scale), biochemical tests (frataxin level, platelet metabolic biomarkers), and physiological testing (CrCEST, MEP). Data will be collected over a one day visit.

Visit 2. (6 months) Subjects will be assessed by medical history, neurological tests (FARS, Timed 25 foot walk (T25W), Timed 1 and 6 minute walks, TUG, BBS, 9HPT, PedsQL, ADL score, ataxia disability scale), and biochemical tests (frataxin level, platelet metabolic biomarkers). Data will be collected over a one day visit.

Visit 3 (12 months) Subjects will be assessed by medical history, neurological tests (FARS, Timed 25 foot walk (T25W), Timed 1 and 6 minute walks, Timed up and go (TUG), Berg Balance scale (BBS), 9 hole peg test (9HPT), PedsQL, ADL score and ataxia disability scale), biochemical tests (frataxin level, platelet metabolic biomarkers), and physiological testing (CrCEST, MEP). Data will be collected over a one day visit.

Visit 4. (18 months) Subjects will be assessed by medical history, neurological tests (FARS, Timed 25 foot walk (T25W), Timed 1 and 6 minute walks, TUG, BBS, 9HPT, PedsQL, ADL score, ataxia disability scale), and biochemical tests (frataxin level, platelet metabolic biomarkers). Data will be collected over a one day visit.

Visit 5 (24 months) Subjects will be assessed by medical history, neurological tests (FARS, Timed 25 foot walk (T25W), Timed 1 and 6 minute walks, Timed up and go (TUG), Berg Balance scale (BBS), 9 hole peg test (9HPT), PedsQL, ADL score and ataxia disability scale), biochemical tests (frataxin level, platelet metabolic biomarkers), and physiological testing (CrCEST, MEP). Data will be collected over a one day visit.

Visit 6 (36 months) Subjects will be assessed by medical history, neurological tests (FARS, Timed 25 foot walk (T25W), Timed 1 and 6 minute walks, Timed up and go (TUG), Berg Balance scale (BBS), 9 hole peg test (9HPT), PedsQL, ADL score and ataxia disability scale), biochemical tests (frataxin level, platelet metabolic biomarkers), and physiological testing (CrCEST, MEP). Data will be collected over a one day visit.

Visit 7 (subject dependent) Subjects will be assessed by medical history, neurological tests (FARS, Timed 25 foot walk (T25W), Timed 1 and 6 minute walks, Timed up and go (TUG), Berg Balance scale (BBS), 9 hole peg test (9HPT), PedsQL, ADL score and ataxia disability scale), biochemical tests (frataxin level, platelet metabolic biomarkers), and physiological testing (CrCEST, MEP). Data will be collected over a one day visit.

Data will be analyzed by Lynch and collaborators. No subject visits occur during this time.

3.1.3 Study Visit

All procedures will be conducted over a one day visit. For the 12 participants for the physiological tests (CrCEST evaluation) at CHOP, participants will be taken for the MRI scan in the morning and complete the neurological and biochemical assessments in the afternoon. For the 30 participants enrolled in the MEP protocol at CHOP, this procedure can be arranged either before or after the clinical assessment.

3.1.4 Case Ascertainment

Subjects recruited at the CHOP site are expected to be patients with Friedreich Ataxia followed by Dr. Lynch through the Neurogenetics clinics at the Children's Hospital of Philadelphia and the Hospital of the University of Pennsylvania. This study will also be posted on clinicaltrials.gov. Additional recruitment may take place by posting notices on the internet through FRDA support and advocacy groups such as Friedreich's Ataxia Research Alliance. Such advertisements would be forwarded to the IRB for approval.

3.1.5 Data sources

The Friedreich Ataxia Clinical Outcomes Study (FACOMS) (CHOP IRB study #01-002609) will serve as a supplemental data source for the study. For participants enrolled in both studies, and who have consented to the optional future use of their data as part of #01-002609, data will be shared from that study for those participants only.

3.2 Controls and Blinding

Not applicable

3.3 Study Duration, Enrollment and Number of Sites

3.3.1 Duration of Study

The total duration for each subject is three years, and initial recruitment will occur over a two year period.

3.3.2 Total Number of Study Sites/Total Number of Subjects Projected

The study will be conducted at 3 investigative sites in the United States. Clinical neurological (Aim 1) assessments and frataxin sample collections (Aim 2) will be carried out at all three sites. Physiological assessments (Aim 3) will only be conducted at CHOP.

The following is a list of sites included in the study:

Children's Hospital of Philadelphia

University of California Los Angeles

University of Florida

The University of Rochester will provide database support

Recruitment will stop when 110 children are enrolled (up to 90 at CHOP, up to 20 at other sites). The majority of subjects will be enrolled at the CHOP site and some will be included to participate in the MEP (n=30) and CrCEST (n=12) measures. A substantial number of participants will be recruited from the ongoing natural history study of all ages, FACOMS, in which some children are already enrolled. Dr. Lynch sees 30 new FRDA patients per year, with the majority being children. Study advertisements will be sent through the Friedreich Ataxia Research Alliance (FARA) registry, which is a database that includes 141 children living in the United States and over 2000 patients worldwide.

3.4 Study Population

3.4.1 Inclusion Criteria

1) Males or females age 2 to 18 years.

2) Genetically confirmed diagnosis of FRDA or clinically confirmed diagnosis of FRDA, pending confirmatory genetic testing through a commercial or research laboratory

3) Parental/guardian permission (informed consent) and if appropriate, child assent.

4) Weight ≥ 10 kg (for the MEP measures only)

3.4.2 Exclusion Criteria

1) Inability to complete study evaluations

2) Severe cardiomyopathy/congestive heart failure (for MEP)

3) A pacemaker, any metal-based medical or non-medical implants/devices, any non-removable metal-based object (e.g. body piercings, jewelry, etc.) (for the CrCEST measures only)

4) Any history of intraocular injury or fragment in or around the orbit that cannot be cleared through radiologic evaluation (for the CrCEST measures only)

5) Any history of bullet, shrapnel, or stabbing wounds that cannot be cleared through radiologic evaluation (for the CrCEST measures only)

6) Claustrophobia or any known medical conditions which can be exacerbated by stress, anxiety, or panic attacks triggered by enclosed spaces (for the CrCEST measures and MEP only)

7) Inability to lie flat on a patient bed in an MRI machine for up to 90 minutes (for the CrCEST measures only)

8) Pregnancy (for the CrCEST measures and MEP only)

4 STUDY PROCEDURES

4.1 Neurological Outcome Measures

a) Medical history: In the following description of study procedures, the term “patient” refers to individuals with Friedreich Ataxia. In order to obtain data and validate clinical outcome measures the following studies will be done. Quantitative and descriptive clinical data collection on all subjects, including medical history and medications. We will also try to obtain copies of subject’s most recent Echocardiograms and EKG reports, as found in their medical records.

b) Friedreich ataxia rating scale: The FARS is a rating scale based upon the clinical neurologic examination and will be administered by a neurologist who is masked to scores from the other measures. FARS ratings range from 0 to 125, based on summation of 25 different neurologic exam features.

c) The timed 25-foot walk (T25W): A timed measure of how fast a subject walks 25 feet.

d) The 9-hole peg test (9HPT): A timed test of how long it takes for a subject to place pegs in a standard pegboard. It also has an electronically measured version (the CCFS) and a “click test” in which patients click a button as fast as they can.

e) Extended timed walking tests: A 1-minute timed walk and a 6-minute timed walk (but not to exceed 6 minutes in length) will be conducted. The timed 1-minute and 6-minute walks are quantitative mobility and leg function performance tests based on distance

traveled in one and six minutes, respectively. The subject is directed to one end of a clearly marked large rectangular course and is instructed to walk around as quickly as possible for one or six minutes. The distance is calculated by measuring how far the subject travels along the marked course. Subjects may use assistive devices during this task. The 1 minute walk will be performed twice, separated by 5 minutes of rest.

f) Specific gait and balance tools utilized in other gait disorders of childhood, including the Timed Up and Go (TUG) and Berg balance scales (BBS). The TUG is a test of the time to rise from a chair, walk three meters (10 feet), turn around, walk balk to the chair, and sit down. It has previously been tested in older subjects with FRDA and has been used in both elderly populations and very young cerebral palsy cohorts. The BBS is a semi-quantitative rating scale based on ability to stand sit and balance in various positions (5).

g) The Pediatric Quality of Life (PedsQL) and the FARS ADL scores. Subjects aged 2-7 inclusive will not need to complete a PedsQL scale. The PedsQL scale is the Pediatric Quality of Life Inventory. This scale has two separate questionnaires: one is designed for subjects between the ages of 8-12, the other for subjects between 13-18. The subject will answer the questionnaire with the help of the study coordinator. The subject's parent or guardian will also answer a separate questionnaire on the same topic. The FARS ADL score is a similar questionnaire asking the participant how they view their performance on tasks such as dressing, bathing, etc. Subjects and their guardians will be asked to complete questionnaires annually.

In some situations, such as a global pandemic, travel restrictions, as well as poor health or an overall concern for safety, it may not be possible for subjects to have an in-person visit every year. Under these circumstances, for subjects who have previously consented for this study it is possible to conduct a visit remotely via phone or video/telehealth. A remote visit will not include the 9HPT or any sample collection. Remote visits can include the following: review of consent forms, medical history and medications, completion of questionnaires that can be mailed or emailed to subjects, certain assessments of gait and motor functioning including answers to the surveys which ask questions about gait and motor abilities, parts of the FARS exam (for example, the Bulbar facial nerve function exam which can be performed and viewed over video), and timed standing and/or walking tests that can be viewed over video (provided that it has been determined ahead of time to be a low fall risk to the subject - fall risk will be determined by taking a recent detailed fall history of each subject prior to any standing or walking test).

All procedures detailed above (except the BBS and TUG) have been included on other IRB approved studies at CHOP, including a currently open study (IRB 01-002609).

4.2 Blood Draw for Frataxin Protein Assay, and Platelet Metabolic Biomarkers

A standard venous blood draw is performed by a trained phlebotomist or equivalently trained study coordinator or nurse. No more than 40 ml whole blood will be collected from study subjects, which is a smaller amount than the NIH-recommended limit for all but the smallest children and below the NIH-recommended maximum limit for children. The maximum amount of blood collected from a participant will be determined based on either the above maximum volumes or based on the maximum amount of sample allowed based on weight (2cc/kg), whichever volume is lower, for safety purposes samples collected from subjects will not be sent to each laboratory involved in the study.

The sample will be used for the blood frataxin protein assay, and platelet metabolomics biomarkers. The samples are fractionated in Dr. Lynch's lab and are either frozen for frataxin analysis, or incubated with C13 metabolic precursors, then frozen for platelet metabolic assay. Both are performed in Dr. Blair's lab at the University of Pennsylvania.

5 ml of blood will be used for frataxin dipstick analysis. Immediately after the blood draw, the blood will be transported and stored at -80 degrees Celsius in the lab of Dr. Lynch. The sample remains in the freezer until it is ready to be analyzed. Specimen collections will function as exploratory measures and will be determined by lateral flow assay in samples from whole blood. These samples will be analyzed by a trained laboratory technician in the lab of Dr. David Lynch.

When samples are to be analyzed by outside laboratories, they will be shipped on dry ice according to proper guidelines. Samples will be sent with elements of dates labeled on tubes (date of collection). Each sample will be given a unique number.

4.3 Buccal Swab Collection

Sterile, disposable Epicentre MasterAmp Buccal Swab Brushes will be used to isolate buccal cells (cells lining the mouth) from the inner cheek as suggested by the manufacturer. The procedure is very gentle and causes no discomfort. The inner cheeks are first gently cleansed by light brushing with a new moist, soft toothbrush and then rinsed with a sip of water. An Epicentre MasterAmp Buccal Swab Brush is then gently but firmly used to brush both cheeks, collecting a light layer of easily sloughed-off epithelial cells on the surface of the brush. Exfoliated cells are then transferred to ice-cold extraction buffer preloaded in a small sample collection tube. The sample is then immediately coded and processed for frozen storage in Dr. Lynch's laboratory. The specimen is analyzed by lateral flow assay and performed by a trained laboratory technician.
As with the frataxin blood sample, the cheek swab will be processed in Dr. Lynch's lab. Samples from other sites will again be shipped on dry ice according to proper guidelines. Samples will not be sent with any PHI. Again, the purpose of sending the samples to Dr. Lynch's lab at CHOP is to ensure that similar results are obtained on this assay across multiple locations. This is required to validate the assay.

4.4 Magnetic Resonance Imaging (MRI) muscle phenotyping

At CHOP, 12 of the approximately 90 participants will be included for muscle phenotyping assessment as part of the study's aim to define the progression of physiological dysfunction in affected tissues. All participants will be offered the opportunity to participate, but 12 children will be selected on a first-come basis and must meet inclusion criteria. 12 children will participate in the protocol and be studied annually at 0, 12, 24, and 36 months, unless the subject wishes to complete their annual visit remotely. Creatine chemical exchange saturation transfer (CrCEST) is a technique that will be carried in the same session as ^{31}P Magnetic Resonance Spectroscopy (MRS) at CHOP using a 3-Tesla Magnet. ^{31}P MRS is an established method to measured oxidative phosphorylation (OXPHOS) in muscles. Briefly, the rate of creatine recovery (CrCEST) or phosphocreatine (PCr) resynthesis (PCrτ, ^{31}P MRS) was estimated, each after a 3-minute bout of plantar extension/flexion exercises. These studies showed that CrCEST produced similar results with respect to recovery kinetics, but offered superior anatomical resolution (6).

The participant will safely lie on an MRI bed and a coil will be placed around part of the body under investigation. The study participant may also wear ear-plugs to reduce the volume of MRI signal noise. They will then be moved directly into the magnet so that the body part under investigation is observable through the MRI program. The procedure will

proceed with the study team conducting communication with the participant via a two-way radio communication system. We will employ resting and post-exercise chemical exchange saturation transfer (CrCEST) to assess muscle OXPHOS capacity. During the session, we will ask subjects to perform several brief sub-maximal leg exercises (about 5 minutes in total). Subjects will be asked to press down on a pedal, similar to a car accelerator or piano foot pedal, approximately 90 times, or as many as they are able, over a 2 minute period. A member of the study team will remain in contact with the subject through a two-way intercom and will count aloud each repetition. A certified medical professional will continue to closely monitor the patient for safety and any sign of discomfort during the MRI procedures. Time in the MRI machine will not exceed 90 minutes. After completion of the imaging, they will be carefully and slowly removed from the MRI machine to prevent any lightheadedness caused by orthostatic hypotension.

Creatine CEST imaging sequence will be used to assess creatine distribution and concentrations in muscle (lower extremity), both at rest and after recovery from a brief foot pedal depression exercise (7,8). The CrCEST sequence is based on a standard single-slice FLASH pulse sequence with prep saturation pulses in front. The acquisition time for the CEST sequence is only about 30 seconds, but we need to acquire the images at varying saturation amplitude and pulse duration. Typical parameters used are: slice thickness = 8 mm, flip angle = 10°, TR = 5.6 ms, TE = 2.7 ms, field of view = 140 x 140 mm2, matrix size = 128 x 128.

If time permits, we will also perform mechanical and structural properties of bone (lower extremity) using MRI and finite element analysis. This test takes less than 10 minutes and will be added on at the end of the standard sequence in patients who have time

The CrCEST MRI sequences performed for this protocol are non-FDA approved and are therefore considered experimental. The bone strength MRI sequence is part of an FDA-approved sequence but its use is being re-purposed for the study, which requires special post-processing techniques. Results from the FDA-approved bone strength sequence will be entered into the medical record, but results from the non-FDA approved CrCEST research sequence will not. If we identify any incidental findings from non-investigational MRI sequences, we will evaluate them to determine if medical attention is needed. In addition, subjects have the option to have any findings forwarded to their primary care provider for review.

The procedure detailed above has been included on other IRB approved studies at CHOP, including an open study in mitochondrial disease by co-investigator Dr. Shana McCormack (IRB 15-012445).

4.5 MEP Measurements

At CHOP, 30 of approximately 90 participants will be included for muscle phenotyping assessment as part of the study's aim to define the progression of physiological dysfunction in affected tissues. All participants will be offered the opportunity to participate, but 30 children will be selected on a first-come basis and must meet inclusion criteria. MEPs are measured using transcranial magnetic stimulation (TMS), a device which produces a momentary electromagnetic pulse which stimulates the motor cortex of the brain. We will use a Magstim 200^2 machine with a 70 mm figure-of-eight coil (Magstim Co, Wales, UK). Motor evoked potentials will be obtained from adhesive surface electrodes placed on the first dorsal interosseous (FDI) of the dominant hand, and recorded using an amplifier (CED 1902 isolated amplifier, CED, Cambridge UK), an analog-to-digital converter (CED 1401), and Signal 3.13 for Windows software (CED, Cambridge, UK). In the unlikely event that a

participant is ambidextrous, the right side will be measured. The TMS machine emits a low volume click with each stimulus; for safety considerations (see below) each subject will wear foam earplugs during the session.

Resting motor threshold (RMT) will be measured as the minimum stimulus intensity needed to elicit MEPs of at least 50uV in 5 of 10 trials. The optimal site of stimulation for the FDI muscle will be determined as the site with the lowest RMT for eliciting MEPs. Finding the RMT and optimal site will take approximately 20-30 single stimuli. Stimulation will consist of 20 stimuli, each separated by at least 4 seconds, at a pulse intensity of 110% MT, with the option to deliver up to 30 additional stimuli if more than half of the trials are affected by movement. No sessions will exceed 1800 pulses of TMS, a safety standard we have specified in prior studies at CHOP (IRB 12-009124) and by the stimulation lab at the University of Pennsylvania (Penn IRB #806532). The expected time for MEP recording, including set up time is 60 minutes.

The procedure detailed above has been included on other IRB approved studies at CHOP, including an open study by principle-investigator Dr. Sudha Kessler (IRB 14-011627).

Useful References for Observational and Descriptive Research:

A copy of a recent publication describing the validation of these measures has been included.

4.6 Abstracted Data Elements

Friedreich Ataxia Clinical Outcome Measures Study (CHOP IRB# 01-002609): Below are the listed data elements that will be abstracted from this study. This includes data collected before and during the participant's enrollment.

- Functional Staging for Ataxia Score
- Friedreich Ataxia Rating Scale (FARS)
- Medical History Update- FA Symptoms Review
- Medical History Update - Medical Conditions Log
- Medical History Update – Medications
- Vital Signs- height, weight, BMI, blood pressure
- 9 Hole Peg Test
- Timed 25 Foot Walk
- Extended Timed Walks
- Activities of Daily Living
- Pediatric Quality of Life Questionnaires

4.7 Primary and Secondary Endpoints

The primary goal of this study is to gather long-term serial quantitative clinical data using the measures validated during the three years of the study.

Aims 1: The primary endpoint (s) are the change in each of the clinical measures over time. The rate of change of each of these will be compared for responsiveness to change (SD of change/mean change).

Aim 2: Biochemical measures: The rate of change of frataxin and each platelet biomarker will be determined as primary endpoints. The rate of change over time will be determined to define which measures are most sensitive to disease progression.

Aim 3: Anatomical measures: The primary endpoints are the post exercise recovery time, Tau, for CrCest and the MEP size for MEP testing. The rate of change over time will be determined to define which measures are most sensitive to disease progression.

The secondary analyses are:

The rate of change of each measure the (neurological measures, biochemical measures and anatomic measures) as a function of age, GAA repeat length and sex, as determined by linear regression models

4.8 Statistical Methods

Aim 1: Results will be summarized with standard descriptive statistics using parametric or non-parametric methods, as appropriate given the variable distributions. Each of the parameters tested here have been abnormal in FRDA patients previously, including children. However, the specific features of how they behave in large pediatric cohorts is unclear. Thus, the most important analyses will be how the tests behave over time and how they reflect disease severity. Data for test-retest for the first 5 subjects at the primary site will be evaluated by determining the coefficient of variation over the tests. This will be performed immediately after study initiation as a separate analysis to confirm that the initial protocols have sufficient reproducibility to be useful. Full data collection will then proceed. Data from measures that were repeated (timed walks, TUG, CCFS tests) will be analyzed as means of the repeated tests and test retest features between trials. CCFS values will be analyzed as each hand separately (dominant vs non dominant) and hands together. Each test will be assessed in regression or mixed linear models accounting for age, sex and GAA repeat length. In addition the change over time will be established as well as the reproducibility of that change.

Aim 2: Results will be summarized with standard descriptive statistics using parametric or non-parametric methods, as appropriate given the variable distributions. In this biochemical aim, the major analysis will examine the relationship of frataxin levels and downstream biomarkers with disease progression. This will be perfomed in 2 ways. First using cross sectional data, we will construct mixed linear models that allow the assessment of their relationship of marker values (frataxin, 13C metabolite levels, Apo A1) to age, GAA repeat length and sex. Secondly, we will assess the change in values over time both in absolute value and percent change to understand the overall meaning of results. In addition, the change over time will be examined in relation to age, sex, and GAA repeat length in linear models. We will also examine the levels of each marker at baseline with the change in clinical measures from aim 1. This will allow us to understand the degree to which clinical change reflects individual biochemical events and whether clinical and biochemical events correlate temporally. This analysis will be supplemented by analysis assessing whether the change in levels of the biomarkers predicts clinical change.

Aim 3. Each of the measures here (MEP, CrCEST) has differentiated FRDA subjects from controls in previous studies. For this proposal though, the crucial analyses should ascertain the relationship between physiological values from CrCEST and MEP and disease status from clinical measures. Results will be summarized with standard descriptive statistics using parametric or non-parametric methods, as appropriate given the variable distributions. In cross sectional analyses, results from each of the outcome measures (frataxin levels, platelet metabolic markers, ApoA-1, and recovery time from CrCEST) will

be used as dependent variables in multiple linear regression models or mixed models examining the independent effects of age, GAA repeat length and sex as dependent variables. This will allow us to define the degree to which each outcome marker is predicted by genetic severity (GAA repeat length), disease progression (age) or both. We will also directly correlate results between MEP and CrCEST to ascertain the degree to which they reflect the same construct (FRDA disease progression). Finally, we will examine correlations and regression modelling between physiological results and clinical measures (Aim 1) and frataxin levels (Aim 2)

4.9 Sample Size and Power

The sample size of main parts of the study is dictated by previous studies and by practicality. Previous power calculations suggest that even using even well-defined clinical measures, the sample size for therapeutic trials will be more than 100 subjects, even using selected cohorts (9-13). However, the tests performed in the present study generally show robust differences between FRDA subjects and controls, and should provide more sensitive measures of disease if they retain sufficient fidelity in children. A sample size of 110 subjects should allow us to assess the independent effects of age, sex and genetic severity (GAA length) on measure results, facilitating a true understanding of the degree they can be used in children. One hundred subjects is also practically the upper limit of intervention studies in rare disease, and thus is appropriate for this study. In the present, study subjects will return every year for MEP and CrCEST, and every six months for clinical testing and blood tests through 36 months. MEP and CrCEST will have smaller samples size (30 and 12 respectively) as they are more precise but more labor intensive tests. Total duration for each subject is approximately 3-4 years, and initial recruitment will occur over a 2 year period. In the present study the goal is to define how each of the tests change over time in children with FRDA, and the factors (age, GAA length) that predict such changes. Each marker has been differentiated from control values previously; however, here control subjects will be used only if needed to confirm the differences, to provide a context and to be sure that tests perform similarly in all sites as a quality control measure.

5 STUDY ADMINISTRATION

5.1 Data Collection and Management

The study will use a database constructed through the University of Rochester for the FACOMS study. The University of Rochester database is compatible with the federal regulations for clinical trials. The University of Rochester also acts as the data center for many other neurological diseases, such as Parkinson's and Huntington's.

We will use CRFs compatible with this database. The CRFs do not ask for subject name, social security number, address or telephone number. The CHOP PI and coordinator will maintain a password protected list of this information which does not leave CHOP. The University of Rochester or others will not have any access to subject's name, address or contact information.

In addition to the data collected at each study visit, study data will be supplemented by the FACOMS study (IRB #01-002609). This will include data created on, before, and after the date of initial IRB submission (8/31/2017). For participants enrolled in FACOMS and who have consented for that study's data sharing component, data collected from #01-002609 prior to enrollment in the current (#14390) study may be shared with the current study. Data from overlapping study procedures will be shared from study #01-002609 to the current study #14390. This specifically includes data from the following performance measures:

medical history review, FARS, T25FW, 9HPT, extended timed walks, PedsQL and FARS ADL questionnaires. Other sites (UCLA, University of Florida) will handle data in the same manner.

The CHOP site will continue to maintain paper copies of all CRFs for three years following completion of the study.

5.2 Regulatory and Ethical Considerations

5.2.1 Risk Assessment

Clinical outcome measures (ataxia scales, speech testing, auditory testing, vision testing, timed walks, and peg test), questionnaires are non-invasive and are very similar to standard clinical evaluation. The risks to all aspects of the study are minimal and include fatigue and boredom. There is a small risk a participant may fall and be injured when trying to complete the walking tests; however this risk is minimal. Assistive devices may be used to complete these tasks, and study staff will be present to supervise and assist.

A standard venous blood draw of up to 40 mls is of minimal risk for a person 40 lbs or more. There is a risk of pain at the insertion of the needle, this is only for a short time. There are low risks of fainting and infection. All venipunctures will be performed by a trained phlebotomist or equivalently trained study coordinator or nurse. All precautions will be taken to reduce the risks. No adverse events are expected.

The risks associated with the blood tests are directly related to confidentiality. All samples will be labeled with a unique identifier. Laboratory personnel handling the patient samples and performing the genetic studies will not have access to subject personal identifiers.

When any data is published all identifiers will be removed. When data or resources are shared with other study PIs or collaborators no personal identifiers will be shared.

For participants undergoing CrCEST and bone strength sequences, the main risk to study participation is related to MRI scanning. Risk is minimized with telephone screening for contraindications to MRI, screening in-person at the study visit via detailed medical record review, as well as testing for metal-based objects before any MRI procedures. In addition, all participants are screened for any condition that may affect their safety and wellbeing during the study via careful medical record review. The study team will also closely monitor patients before, during, and after study procedures (e.g., follow-up).

MRI is the least invasive of all imaging modalities, and there are no known risks from MRI examinations provided subjects are carefully screened for contraindications. Two potential sources of concern lie in the strength and duration of the radio frequency pulse and of gradient pulses. These effects are the subject of very close study by the FDA, and the Siemens Verio Avanto, Sonata & Trio, Siemens is carefully manufactured (with both software and hardware interlocks) so that it is not possible (even by accident) to exceed the FDA guidelines. MRI is listed by the FDA as approvable as a non-significant risk device, and the studies themselves are of minimal risk. The effects of MRI on an unborn baby are unknown. We will therefore exclude pregnant women. An MRI has the potential to identify incidental findings. During the study visit, if a participant is found to have any previously unknown medical conditions, they will be referred to their primary care physician or metabolic physician for care. The Children's Hospital of Philadelphia MRI protocols and safety measures will be observed.

In the MEP procedure the main risk is associated with the safety and tolerability of TMS application. There are more than 100 published studies with reports of TMS applied to more than 800 normal and more than 300 neurologically abnormal children (most receiving single

or paired-pulse TMS). No serious adverse events have ever been reported in this population, suggesting that single pulse TMS in children is a minimal risk procedure. A 2009 report from the Safety of TMS Consensus Group specifies single pulse studies as minimal risk and offers further discussion of the safety of TMS in pediatric populations (14). Single pulse TMS is used as a clinical procedure in children with epilepsy, brain tumors, or other neurologic disorders for mapping of the primary motor cortex. The main safety concerns regarding TMS are the potential for provoking seizures, the potential to effect hearing, and pain or discomfort.

5.2.2 Potential Benefits of Study Participation

This study will provide long-term data on quantitative measurements of disease progression in children that will provide natural history data for FRDA. This study will also test and validate a non-invasive approach to measuring frataxin protein which will be necessary for future clinical trials. It will also seek to identify metabolic and anatomical biomarkers required for clinical trials. Lastly, this proposal will further develop the infrastructure and support for evaluating new clinical outcome measures and biomarkers. Subjects will not benefit directly but their participation will contribute to the necessary clinical research required to support the advancement of therapeutics in FRDA.

5.2.3 Risk-Benefit Assessment

The risks are minimal and given the potential benefits, the risk/benefit ratio is therefore extremely small.

5.3 Recruitment Strategy

Subjects will be recruited from multiple sources. A substantial number will be recruited from the ongoing natural history study of all ages, FACOMS, in which some children are already enrolled, and from the new diagnoses presenting to the sites. The FACOMS study collects detailed data on phenotype, neurological testing, medical history, and genetic severity; this data is available for the present work, and the present study will extend it by adding new tests and addressing new age groups. Dr. Lynch alone sees 30 new FRDA patients per year, most of them children. The other two sites (University of Florida and UCLA) will also recruit through their practices' new patients and their FACOMS participants. In addition, study advertisements will be sent through the FARA registry. This database includes names of > 2000 FRDA pts worldwide, including 141 children living in the USA.

5.4 Informed Consent/Assent

The principal investigator and clinical coordinator will obtain informed consent or assent and parental consent from potential participants.

5.5 Payment to Subjects/Families

Participants will not receive financial rewards or inducements. There is also no cost to participation in the study. Participants who receive clinical care from any investigator or participating research sites, including the Children's Hospital of Philadelphia, will be responsible for those charges, as they are separate from the study.

Funds are available to reimburse some travel and hotel costs for all individuals traveling to the study sites. Our grant does provide funds to reimburse travel expenses up to $500 per visit for participants. This must be arranged with the study coordinator prior to your arrival, and will require an IRS Form W-9. Only full name and social security number will be

collected on the W-9 form. Participants will receive reimbursement checks within 8 weeks after each visit.

5.6 Confidentiality

Enrollment or non-enrollment in the study will not affect the patient's clinical care in any way. All participation is voluntary. Subjects who are non-ambulatory, more severely affected, or who have cardiomyopathy will not be asked to complete the timed walks. All study team will use a CHOP approved video platform to conduct these visits remotely to maintain subject confidentiality All data will be kept confidential except as required by law and kept in a secure data at the Children's Hospital of Philadelphia. All source documents will be stored in locked cabinets with access only for the principal investigator and co-investigators. Patients will be identified by identification numbers not by personal identifiers. Patient names will be anonymized in publications and only the personnel involved in the studies will be able to link results with patient names.

6 PUBLICATION

The identity of all subjects will be withheld from any publication, abstract, lecture or other oral presentation that derives from this investigation. Publications will state that the study had the approval of the Institutional Review Board at The Children's Hospital of Philadelphia.

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APPENDIX

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