Title: **Clinical Outcome Measures in Friedreich's Ataxia**

Study Key Name **Key Name** (assigned by CTO)

Protocol No: **2001-11-2609**

Protocol Date: May 2019

Site Principal Investigator

David R. Lynch, MD, PhD

TABLE OF CONTENTS

ABBREVIATIONS AND DEFINITIONS OF TERMS

FA	Friedreich's Ataxia
FARS	Friedreich's Ataxia Rating Scale
FACT	Friedreich's Ataxia Composite Test
HRQOL	Health-related Quality of Life
ADL	Activities of Daily Living
T25W	Timed 25' Walk
9HPT	Nine Hole Peg Test
LCSLC	Low Contrast Sloan Letter Charts
LiSN-S	Listening in Spatialized Noise – Sentences test
MSQLI	Multiple Sclerosis Quality of Life Inventory
PedsQL	Pediatric Quality of Life
CRF	Case Report Form
CTRC	Clinical and Translational Research Center
VFQ	Visual Functioning Questionnaire

ABSTRACT

Context: Friedreich's ataxia (FA) is a rare autosomal recessive degenerative disorder characterized by ataxia, dysarthria, sensory loss, diabetes and cardiomyopathy. The discovery of the abnormal gene in FA 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 FA have been unavailable. For the past four years this group of investigators has collaborated on development of clinical measures that can quantitatively assess FA (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 FA through the development and validation of clinical outcome measures in FA, collection of quantitative serial clinical data on patients and expansion of the established research network. A secondary objective is to build a parallel DNA and RNA repository for use in large-scale translational research including modifier gene studies. Lastly, frataxin dipsticks (cheek swabs) will be investigated on their ability to measure frataxin protein.

Study Design/Setting/Participants: This is a multicenter natural history and clinical measure study. The recruitment goal is for approximately 2000 patients with Friedreich ataxia worldwide (750 subjects at the CHOP site and 1250 patients at other sites) Participants will be studied annually until the end of the study. All individuals with a genetic or clinical diagnosis of FA will be offered participation. We also plan to enroll up to 2000 carriers and 1000 controls to collect samples for comparison.

Study Measures: Yearly assessments of a core set of clinical measures and quality of life assessment measures. A onetime collection of blood or saliva for DNA repository. A onetime collection of a cheek swab sample for validation of protein/biomarker analysis.

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Friedreich's ataxia (FA) is a rare autosomal recessive degenerative disorder characterized by ataxia, dysarthria, sensory loss, diabetes and cardiomyopathy. FA 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 FA 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 FA. 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 FA has advanced rapidly, translational and clinical research has moved much more slowly. This reflects two aspects of FA. 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 FA 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 FA (4). 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.

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.

1.2 Compliance Statement

This study will be conducted in full accordance all applicable Children's Hospital of Philadelphia 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 The Children's Hospital of Philadelphia 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.

2 STUDY OBJECTIVES

Overall Aim: To advance clinical care, research and therapeutic approaches in FA through the development of valid yet sensitive clinical measures crucial to outcome assessment of patients with Friedreich's Ataxia and the creation of a clinical research network that can facilitate the advancement of clinical research and therapeutic interventions.

2.1 Primary Objective (or Aim)

Aim 1: Expand the network of clinical research centers in FA that provide quantitative serial clinical and functional data on patients, including HRQOL, and design improved clinical measures. This network will also provide the framework for facilitating therapeutic interventions, including a planned partnership with a clinical data coordinating center.

2.2 Secondary Objectives (or Aim)

Aim 2: .Support genetic modifier studies and biomarker studies. Build in parallel a DNA repository for use in large scale translational research. Extract and evaluate RNA from blood or saliva samples to study its characteristics and better understand the disease. 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: Create a mechanism for sharing data and resources (DNA) with basic and clinical investigators.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

Aim 1: Collection of serial clinical and function data, Clinical Outcome Measures (FARS, timed walking tests, 9HPT, speech evaluations, LCSLC) and health-related quality of life.

The FARS, a rating scale based upon the clinical neurologic examination, will be administered by a neurologist who is masked to scores from the other clinical outcome measures. FARS ratings range from 0 to 125, based on summation of 25 different neurologic exam features. Studies from the MS Functional Composite include: 1) the timed

25-foot walk (T25W); and 2) the 9-hole peg test (9HPT). In addition, other timed walking tests such as a 1-minute timed walk and a 6-minute timed walk (but not to exceed 6 minutes in length) will be performed. Non-ambulatory patients will be assigned a set score for the timed walking tests. Contrast letter acuity testing for vision will be performed using the low-contrast Sloan letter charts (LCSLC – Precision Vision, La Salle, IL). Binocular LCSLC measurements will be obtained at 2 meters for each of three contrast levels (100% - equivalent to high-contrast visual acuity, 2.5%, and 1.25% contrast levels). As a summary measure for use in the statistical analyses, the sum of the three chart scores (100% score + 2.5% score + 1.25% score), expressed as number of letters identified correctly, will be calculated. Scores for each chart will also be recorded so that patterns across contrast levels may be noted.

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 course and is instructed to walk back and forth 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. Participants with Friedreich ataxia will be asked to complete both of these tasks.

Scores from the timed walking tests, 9HPT, and LCSLC will converted to z-scores, based on the numbers of standard deviation units (SDU's) that each patient's score is from the mean score for an age-matched control group. A composite z-score (similar to that for the MSFC) will be calculated based on the average of component z-scores from the timed walking tests, 9HPT, and LCSLC.

Speech testing using the Redenlab software will include a battery of evaluations performed by each subject with Friedreich ataxia. Evaluations included in this battery are several speech tasks that are recorded on a laptop for analysis. Subjects will be asked to read a pre-determined "grandfather passage," which is a phonetically-balanced paragraph. Subjects will next be asked to make a sustained vowel sound, such as "ah" for as long as possible. They will also be asked to list the days of the week twice, beginning with Monday, and perform sequential motion rate tasks. For example, subjects will be asked to say "PATA" or "PA-TA-KA" as many times as possible as a 10-second timed exercise. Finally, subjects will be asked to complete a free speech task, in which they perform a monologue for one minute on an unprepared topic. Subjects will be asked to read or perform all of these tasks using their typical pitch and voice volume.

A Listening in Spatialized Noise – Sentences test (LiSN-S) assessment will also be performed yearly on all subjects who participate in the study. This test is a speech perception measure which evaluates auditory processing of sounds coming from different directions by measuring the listener's ability to separate target sentences from competing background noise. This test is administered using headphones and computer software which generates several different conditions. These conditions differ based on location of the noise source (0^0 to 90^0 degrees) and on the vocal quality of the speaker (different speakers produce the target and background signals). The test takes approximately 15 minutes to administer, and will be done by a member of the study staff. Carriers and controls will be asked to complete the auditory testing once only.

In addition, all adult subjects who participate in the clinical outcome measure assessments (Aim 1) will be asked to complete the MSQLI and Activities of Daily Living (ADL) scale. Questionnaires will be self-administered, with assistance provided when necessary, if for example an adult subject is physically unable to write due to disease progression. The questionnaires take about 45-60 minutes to complete. The MSQLI is a modular disease-specific HRQOL measure which will ask adult subjects to complete. It consists of a widely-used generic core, the SF-36, supplemented by symptom-specific scales that capture fatigue, pain, sexual function, bladder/bowel function, vision, perceived deficits, mental health, and social support. The SF-36, the generic core for the MSQLI, consists of 8 HRQOL domains and two summary measures, the Physical Component Summary (PCS) and Mental Component Summary (MCS) (203). Since psychometric properties for the MSQLI are based upon administration of the entire measure (137 items), each adult patient will complete this scale in its entirety. The Activities of Daily Living scale is a nine item instrument that focuses on physical activities. This questionnaire can be administered to participant or caregiver. The Activities of Daily Living scale will be administered to all adult study subjects. For subjects under age 18, parents will be asked to complete the ADL scale for their child with FA.

The Pediatric Quality of Life (PedsQL) Scale will be administered to subjects between 8 and 18, inclusive, and their parents. Subjects under age 8 will not need to complete a PedsQL scale. This scale has two separate questionnaires: one is designed for subjects between the ages of 8-12, the other for subjects between 13-18 years. The subject will answer the questionnaire with the help of the study coordinator if needed. The subjects' parent or guardian will also answer a separate questionnaire on the same topic. Subjects and their guardians will be asked to complete the questionnaires annually. The Visual Functioning Questionnaire (VFQ) will also be used in order to assess vision difficulties in FA. For the VFQ, subjects will be asked to complete a 25-item survey. Adults will fill this out on their own, and parents of subjects under age 18 will be asked to fill this out for their child.

Aim 2: Support genetic modifier studies and biomarker studies.

Specific targeted modifier studies are ongoing as well as unbiased approaches. These will include the presence of interrupted GAA repeats, expanded mitochondrial haplotype studies, testing of glutathione-S-transferase and dihydrolipoamide dehydrogenase polymorphisms, HDAC inhibitor status, and OPA1 and PGC1 a status. All of these have been directly implicated in FA and Dr. Lynch has experience performing directed modifier gene studies. In addition, other investigators will be able to use the repository of clinical data and DNA for similar studies. All data and resources shared will be de-identified to ensure the confidentiality of subjects.

MitoSciences' Frataxin-specific dipstick tests will be used to measure Frataxin levels. This will be done in order to determine the diagnostic clinical utility of the Frataxin dipstick tests. Based on previous results, it is expected that FA carriers will comprise a discrete group with have Frataxin levels ~50-60% of normal, while FA-affected individuals will comprise another discrete group having Frataxin levels of less than 50% of normal.

Another goal is to determine if Frataxin levels in FA-affected individuals correlates:

a) inversely with the number of GAA repeats (measured in either the small allele, or the large allele, and/or the mean of both alleles)

b) inversely with penetration of the FA phenotype (severity at time of sampling or rapidity of previously observed clinical course)

or

c) positively with the age of onset.

Additionally, if a sufficiently large population of FA-affected individuals can be enrolled the study may also provide an initial determination of whether or not Frataxin levels progressively degrade over time in affected individuals. This analysis could be done by grouping FA-affected individuals into cohorts based on genomic diagnosis, e.g., # of GAA repeats on the smaller allele (and/or the mean # of GAA repeats on both alleles) in individuals homozygous for extended GAA repeats and then determining if frataxin levels correlate with the length of time between onset of an individual's symptoms and the time at which tissue samples were obtained. Although such a cohort-based study should be able to provide an initial answer to this question, it is likely that longitudinal studies of individual affected patients will be required to precisely define the relationship if it exists.

As the pathophysiology of FA is becoming better defined, there appears to be heterogeneity in the disease in progression and biochemistry. For example, mitochondrial DNA haplotype may influence rate of progression. This is important not only for providing prognosis, but also for devising new approaches to disease therapy. However, the ability to conduct large scale modifier gene studies is limited by the presence or absence of high quality clinical data. Indeed, the study on mitochondrial haplotypes had sufficient power only by grouping such haplotypes, a suboptimal statistical approach. The clinical research data provided from the 250 patients currently enrolled in this study provides such as basis for the high-quality database needed for modifier studies. In conjunction with the clinical measures performed in Aim 1, a DNA repository will be established and housed at the University of California-Los Angeles in the laboratory of Dr. Giovanni Coppola).

Following this, specific targeted modifier studies can be initiated. These will include the presence of interrupted GAA repeats (for which Dr. Lynch has specific evidence), expanded mitochondrial haplotype studies, and testing of glutathione-S-transferase and dihydrolipoamide dehydrogenase polymorphisms. All of these have been directly implicated in FA and Dr. Lynch has experience performing directed modifier gene studies. In addition, other investigators will be able to use the repository of clinical data and DNA for similar studies. The internal advisory board of the clinical network will review all requests for data and resource sharing. If the study is found meritorious then the coordinating site (CHOP) will proceed with the standard data and usage agreements. All data and resources shared will be de-identified to ensure the confidentiality of subjects.

In addition to collecting frataxin dipsticks from FA subjects, frataxin dipsticks will be collected from carriers and controls. In order to validate that the frataxin dipstick machine works, samples are needed from non-affected individuals as well as those who are carriers. People who are carriers of the FA gene make less frataxin than the non-affected individual. As such, it will be important to know the "normal" level of frataxin in the general population, as well as the "normal" range for carriers. Future treatments that aim to increase frataxin levels will try to raise the frataxin levels to that of the carrier. In order to make sure the assay works, it is crucial to know average levels of FA frataxin, as well as that of carriers and controls. Therefore, approximately 1500 carrier and 750 control samples will be recruited at the Children's Hospital of Philadelphia, with a total of 2000 carrier and 1000 control samples study-wide in order to preserve the fidelity of the results from the frataxin protein analysis performed thus far and to further characterize biomarkers and genetic modifiers in Friedreich ataxia. This information is extremely useful for the identification of biomarkers for use in clinical trials. Carriers/controls will be asked to sign a separate consent form.

No private health information from the carrier/control will be collected except their name and age, which they will provide when signing the consent.

3.1.1 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 an FA support and advocacy group websites such as Friedreich's Ataxia Research Alliance (FARA). Such advertisements would be forwarded to the IRB for approval.

Carriers and controls will be identified during the FA subject's normal clinic visit. Control subjects may also be identified through the CHOP Intranet Research Finder tool. The study is listed on this website and control subjects may approach the study team to participate. They will be asked to complete study procedures if they are eligible for the study. It is expected that many/most of the carrier and control subjects will be family members or friends of affected individuals. Carriers and controls will be matched for age. For example, if a parent or sibling accompanies the subject, informed consent will be obtained prior to collection of a cheek swab them as well. No swab samples will be collected on anyone prior to obtaining informed consent.

3.1.2 Data sources

Not applicable

3.2 Controls and Blinding

Not applicable

3.3 Study Duration, Enrollment and Number of Sites

Since on the primary aims of the study are to collect detailed natural history data on subjects and establish a clinical research network that can support multiple studies simultaneously, this study will be ongoing as determined by available funding. FARA has made a long-term commitment to funding this study, therefore contracts and funding are updated and renewed on an annual basis to reflect number of subjects enrolled at each site.

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

The study is currently conducted at 9 investigative sites in the United States, 2 sites in Canada, 1 site in Brazil, and 1 site in Australia.

The following is a list of sites included in the study:
Children's Hospital of Philadelphia/University of Pennsylvania – Coordinating Center
University of California Los Angeles
University of Colorado-Denver
Emory University
University of Iowa
University of Rochester
University of South Florida
University of Florida

Ohio State University Medical Center
University of Campinas in Sao Paulo, Brazil
Toronto SickKids
Murdoch's Children's Hospital/University of Melbourne, Australia
University of Montreal

Recruitment will stop when approximately 2000 subjects are enrolled. It is expected that approximately 2000 subjects will be enrolled (identified for further review) to produce 2000 evaluable subjects. Approximately 750 subjects will be recruited at the CHOP site and studied annually. In addition, 1500 carrier and 750 control samples will be collected for a cheek swab assay and blood sampling at CHOP. Overall, the goal is to recruit 2000 carriers and 1000 controls study-wide.

3.4 Study Population

3.4.1 Inclusion Criteria

1) Both male and female children and adults of any age.

2) Genetically confirmed diagnosis of FA (for carrier/control cheek swab and blood samples this is not required).

3.) Clinically confirmed diagnosis of FA, pending confirmatory genetic testing through a commercial or research laboratory (for carrier/control cheek swab and blood samples this is not required).

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

4 STUDY PROCEDURES

Quantitative Clinical Data and Clinical Outcome Measures (FARS, Timed walking tests, 9HPT, LCSLC, speech tests, LiSN-S)

In the following description of study procedures, the term "patient" refers to individuals with Friedreich ataxia. In order to obtain natural history 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. Ideally, copies of subject's most recent Echocardiograms and EKG reports will be obtained from their medical records. The FARS, a rating scale based upon the clinical neurologic examination, will be administered by a neurologist who is masked to scores from the other clinical outcome measures. FARS ratings range from 0 to 125, based on summation of 25 different neurologic exam features. Studies from the Multiple Sclerosis Functional Composite have been validated in FA including: 1) the timed 25-foot walk (T25W); and 2) the 9-hole peg test (9HPT). In addition, other timed walking tests such as a 1-minute timed walk and a 6-minute timed walk (but not to exceed 6 minutes in length) will be performed. Non-ambulatory patients will be assigned a set score for the timed walks. Contrast letter acuity testing for vision will be performed using the low-contrast Sloan letter charts (LCSLC – Precision Vision, La Salle, IL). Binocular LCSLC measurements will be obtained at 2 meters for each of three contrast levels (100% - equivalent to high-contrast visual acuity, 2.5%, and 1.25% contrast levels). As a summary measure for use in the statistical analyses, the sum of the three chart scores (100% score + 2.5% score + 1.25% score), expressed as number of letters identified correctly, will be calculated. Scores for each chart will also be recorded so that patterns across contrast levels may be noted.

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 course and is instructed to walk back and forth 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. Participants with Friedreich ataxia will be asked to complete both of these tasks.

Speech testing will include a battery of evaluations performed by each subject with Friedreich ataxia. Evaluations included in this battery are several speech tasks that are recorded on a laptop for analysis. Subjects will be asked to read a pre-determined "grandfather passage," which is a phonetically-balanced paragraph. Subjects will next be asked to make a sustained vowel sound, such as "ah" for as long as possible. They will also be asked to list the days of the week twice, beginning with Monday, and perform sequential motion rate tasks. For example, subjects will be asked to say "PATA" or "PATAKA" as many times as possible as a 10-second timed exercise. Finally, subjects will be asked to complete a free speech task, in which they perform a monologue for one minute on an unprepared topic. Subjects will be asked to read or perform all of these tasks using their typical pitch and voice volume.

In addition, subjects will be asked to complete the Listening in Spatialized Noise – Sentences test (LiSN-S), which will be administered by a member of the study team each year. This test will evaluate auditory processing of sounds coming from different directions with the use of headphones and LiSN-S computer software. This evaluation will allow us to gain a better understanding of the underlying auditory processing abnormalities observed in Friedreich ataxia. This testing will also be performed on carriers and controls once only.

Questionnaires will also be self-administered, unless assistance is required due to disease progression. The questionnaires take about 45-60 minutes to complete. The MSQLI is a modular disease-specific HRQOL measure. It consists of a widely-used generic core, the SF-36, supplemented by 9 symptom-specific scales that capture fatigue, pain, sexual function, bladder/bowel function, vision, perceived deficits, mental health, and social support. The SF-36, the generic core for the MSQLI, consists of 8 HRQOL domains and two summary measures, the Physical Component Summary (PCS) and Mental Component Summary (MCS) (203). Since psychometric properties for the MSQLI are based upon administration of the entire measure (137 items), each patient will complete this scale in its entirety. The exception to this will be questions on sexual function, which will not be completed by subjects under the age of 18 years. The next questionnaire is an activities of daily living scale, a nine item instrument that focuses on physical activities.

The Pediatric Quality of Life (PedsQL) Scale will be administered to subjects between ages 8 and 18, inclusive. Subjects under age 8 will not be asked to complete the PedsQL modules. The first PedsQL scale is the Multidimensional Fatigue Scale (see attached for all PedsQL scales). 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 second PedsQL scale is the Pediatric Quality of Life Inventory. This scale also 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 third PedsQL scale is the Pediatric Quality of Life Cardiac Module. This scale also 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. Subjects and their guardians will be asked to complete all three questionnaires annually.

In order to assess vision difficulties in FA subjects will be asked to complete a 25-item Visual Functioning Questionnaire (VFQ) survey. Adults will fill this out on their own, and parents of subjects under age 18 will be asked to fill this out for their child.

Blood Draw for DNA Collection, RNA analysis, Frataxin Protein Assay, and Biomarkers; Saliva sample for DNA collection

A standard venous blood draw is performed by a trained phlebotomist or equivalently trained study coordinator or nurse. Some blood draws may be performed by the CHOP Clinical and Translational Research Center (CTRC), at the discretion of the Principal Investigator and with prior CTRC approval. The CHOP CTRC will only be drawing blood for CHOP subjects. Procedures: No more than 80 mL whole blood will be collected from adult study subjects, which is a smaller amount than the NIH-recommended limit for adults. No more than 50 mL whole blood will be collected from child study subjects, which is 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 (2mL/kg), whichever volume is lower, for safety purposes. As this protocol involves several different laboratories which will receive samples from CHOP, with varying volumes of blood required for their individual analyses (described below), samples collected from subjects will not be sent to each laboratory involved in the study. The laboratories receiving blood samples from a particular subject will be determined at the PI's discretion, and the total blood volume collected from each participant will not exceed the volumes outlined above. As different laboratories have different sample requirements (i.e. different populations needed and varying number of samples requested), the study PI will determine which laboratories will receive samples from each participant, based on the needs of the laboratories and on the needs of the study at large.

A portion of the sample will potentially be used for the DNA bank; the remaining sample will be used for blood frataxin protein, metabolic, biomarker, and RNA analysis. Serum and plasma will be obtained from a blood sample as well and maintained in Dr. Lynch's lab at -80 degrees Celsius. These blood draws are routine procedures with minimal risk and discomfort. Samples will be labeled with a unique study ID or initials, and the date.

DNA samples will be stored in Dr. Lynch's lab at 4 degrees Celsius. The DNA samples will be shipped to the laboratory of Giovanni Coppola, MD at The University of California Los Angeles. At this laboratory, DNA will be extracted and aliquots will periodically be shipped back to the laboratory of David Lynch at CHOP for storage. DNA will be tested to evaluate for unknown genotypes leading to the diagnosis of Friedreich ataxia. These DNA analyses regarding alternative, unknown genotypes causing symptoms of Friedreich ataxia are research analyses; reports will not be generated, and results will not be disclosed to patients.

For patients who are unwilling or unable to provide a blood sample, DNA will be collected from saliva samples instead. These will be collected using saliva kits, which are non-invasive and simple to use.

Approximately 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. Approximately 2.5 mL of blood will be used for RNA studies in the laboratory of

Giovanni Coppola, MD at The University of California Los Angeles. RNA will be extracted from the blood sample and characterized to better understand the progression of FA.

Any additional sample requests to outside sites will be managed via a CHOP materials transfer agreement (MTA) as needed which will be set up in advance of shipment of any samples.

When samples are to be analyzed by outside laboratories, they will be shipped on dry ice according to proper guidelines. Samples are coded with, and labeled with a unique ID number or initials, and elements of dates (date of collection). Types of samples collected at a single study visit will be determined by the Principal Investigator based on the current needs of collaborators. The amounts of blood collected from child and adult study subjects will not exceed the total blood volumes specified above.

Cheek swab for frataxin assay

Sterile, disposable Puritan Cytology 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. A Puritan Cytology 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 with a unique ID or initials and the date of collection, and processed for frozen storage in Dr. Lynch's laboratory.

This same procedure will be administered to patients or carrier/control subjects.

As with the frataxin blood sample, the cheek swab will be processed in the lab of Dr. Lynch. When samples are sent to outside laboratories, they will again be shipped on dry ice according to proper guidelines. Samples are coded and labeled with a unique ID number or initials, and elements of dates (date of collection). Again, the purpose of sending the samples to outside laboratories is to ensure that similar results are obtained on this assay across multiple locations. This is required to validate the assay.

Useful References for Observational and Descriptive Research:

Off-Site Study Procedures

Blood draws, saliva samples, and cheek swabs will be occasionally obtained at home visits in nearby states. Because FA is a rare disease and some patients cannot travel to the CHOP site, it is important that study members be able to make visits to these patients. Members of the study team who are certified to draw blood will visit patients who express interest in donating blood and cheek swab samples. All patients and carriers/controls involved in home visits will sign the informed consent form prior to study procedures. Home visits will generate a greater diversity of samples and increase study recruitment, and thus accelerate research that holds potential benefit for FA patients.

STATISTICAL CONSIDERATIONS

4.1 Primary and Secondary Endpoints

The primary endpoint of this study is to gather long-term serial quantitative clinical data using the measures validated during the first three years of the study. This data could ultimately be used for natural history studies and possibly because of the rare nature of the disease be used as a natural history control cohort in future therapeutic trials.

The secondary endpoints are:

- to further refine the validated measures and identify and validate new measures
- to create a DNA repository with correlative clinical data to support genetic modifier studies
    - This endpoint will be evaluated by monitoring the amount of data and number of samples contributed towards the database and repository.
- to validate biomarkers for use in future clinical trials
    - To validate the clinical utility of measuring RNA in blood and saliva samples as a marker for disease progression, and to study the frataxin levels in buccal cell samples, suitable for the repetitive testing needed to monitor efficacy of therapies designed to up-regulate levels of Frataxin protein. This endpoint will be evaluated by collecting tissue samples for measurement and utilizing the data to evaluate its clinical utility.

4.2 Statistical Methods

Scores from the timed walking tests, 9HPT, and LCSLC will converted to z-scores, based on the numbers of standard deviation units (SDU's) that each patient's score is from the mean score for an age-matched control group. A composite z-score (similar to that for the MSFC) will be calculated based on the average of component z-scores from the timed walking tests, 9HPT, and LCSLC.

For comparison of new measures and improvement of measures, including the speech and auditory testing, assessment will include the ability of such measures (and their annual change) to be predicted by age and GAA repeat length in linear regression. Correlation with disease duration and other clinical markers of disease activity will also be assessed. Superior measures will have higher adjusted R2 values in such models.

The results from the auditory testing will also be compared with those of carriers and controls. The two independent sample t-test or the Wilcoxon Rank Sum test (a nonparametric test) will be used for comparing FRDA to the carrier group and the control group. The carrier group will also be compared with the control group using these methods.

For establishment of a DNA repository individual studies will be evaluated by the ability of genetic markers to predict disease status as defined by multiple clinical markers (exam scores, age of onset etc). Again, the main analysis method will use multivariate linear regression analysis accounting for GAA repeat length (and age when appropriate) using other genetic markers a dummy or indicator variables. Each analysis will be tailored to the specific genetic marker being addressed. An example of this type of analysis is found in Lynch, D. R., Mozley, P. D., Sokol, S., Maas, N. M. C., Balcer, L. J., and Siderowf, A. D. Lack of effect of polymorphisms in dopamine metabolism related genes on imaging of

TRODAT-1 in striatum of asymptomatic volunteers and patients with Parkinson's Disease. *Movement Disorders* 18:804-12, 2003.

For assessment of the utility of frataxin levels and other biomarkers, their correlation with disease measures and duration will be assessed using linear and nonlinear correlation analyses.

4.3 Sample Size and Power

Sample sizes (>2000 patients) for this part of the study are based on the idea that ongoing expansion of the database will be necessary as the group expands beyond clinical measurement into providing data for translational approaches such as biomarker studies and studies of potential modifier genes (Aim 2). This large sample size allows us to account for more confounding variables than smaller sizes.

5 STUDY ADMINISTRATION

5.1 Data Collection and Management

Currently, the natural history data resides on the University Of Rochester Medical Center for Health and Technology eClinical database, while the sample database resides on the CHOP IT network and requires password entry. The University of Rochester is now a part of the collaborative clinical network in FA. The current CHOP database can only be accessed through the CHOP network, but the Rochester database is an internet-based electronic data capture system. This allows for access the study information from any location. The University of Rochester database is compatible with the federal regulations for clinical trials. Each PI and study coordinator is able to log in and view their own site's data.

The University of Rochester also acts as the data center for many other neurological diseases, such as Parkinson's and Huntington's.

The University of Rochester also provides the case report forms (CRFs) which are compatible with the new database. These CRFs do not ask for subject name, social security number, address or telephone number. This information will not be entered into the database. Instead, CHOP PI and coordinator will maintain a password protected list of this information which does not leave CHOP. The University of Rochester does not have any access to subject's name, address or contact information.

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

The Redenlab speech testing includes a battery of evaluations performed by each subject with Friedreich ataxia which are recorded on a laptop for analysis. Data will be sent via encrypted message to Dr. Adam Vogel in Australia. He will receive data coded by subject ID number, along with the date of the test and the subject's date of birth and sex.

CTCC Unique ID number (optional for study participants)

At the study visit, a patient with Friedreich ataxia (or his/her parent if under 18) can opt to have the study team generate a unique ID number through the University of Rochester database system. This ID number is 9 digits and will be used to connect subjects' research data to other studies of Friedreich ataxia in which he/she may participate. This applies to all Friedreich ataxia-related studies run through the University of Rochester database only if the subject provides consent for the use of data across studies in this way. To receive this unique ID number, subjects with FA will need to provide his/her last name at birth, first name at birth, gender at birth, full date of birth, city and country of birth, and mother's maiden name. The information will be entered by study staff into a secure website, which will generate the 9-digit unique ID number. The subject will be given the unique ID number that can be printed for them for use in other studies run through the University of Rochester CTCC database.

5.2 Regulatory and Ethical Considerations

5.2.1 Risk Assessment

Clinical outcome measures including the ataxia scales, speech testing, auditory testing, vision testing, timed walks, peg test and 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 test; however this risk is minimal. Subjects who are non-ambulatory, more severely affected, or who have cardiomyopathy will not be asked to complete the timed walks. Assistive devices may be used to complete these tasks, and study staff will be present to supervise and assist.

Cheek swab sampling is of minimal risk as the lining of the mouth is left intact. No adverse events are expected. Cheek swabs are gentle procedures akin to brushing one's inner cheeks with a soft toothbrush. Buccal swab collection is gentle and does not cause wounding or bleeding.

A standard venous blood draw of up to 50 mL 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. All precautions will be taken to reduce the risks. No adverse events are expected.

The risks associated with the DNA repository and genetic modifier studies are directly related to confidentiality. All samples will be labeled with a unique identifier. Samples are coded and inclusive of date of sample collection. 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.

5.2.2 Potential Benefits of Trial Participation

This study will provide long-term data on quantitative measurements of disease progression that will provide natural history data for FA. This study will provide a much needed DNA repository to support genetic modifier studies that could lead to the identification of novel therapeutic approaches. This study will also test and validate a non-invasive approach to measuring frataxin protein which will be necessary for future clinical trials. Lastly, this network will provide 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 FA.

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

Through the multicenter study, the recruitment goal is for 2000 patients with Friedreich ataxia worldwide (up to 750 subjects at the CHOP site) to be studied annually. All individuals with a confirmed genetic or clinical diagnosis FRDA will be offered participation. This study will also be posted on clinicaltrials.gov. In addition, approximately 1500 carriers and 750 controls will be recruited for sample collection at CHOP, with a total of 2000 carriers and 1000 controls study-wide. Most of the subjects recruited at the CHOP site are expected to be patients followed by Dr. Lynch through the Neurogenetics clinics at the Children's Hospital of Philadelphia and the Hospital of the University of Pennsylvania. If the subject is accompanied by family members at their clinic visit, we will ask permission to obtain cheek swabs and blood samples from the family members as well to obtain carrier/control samples.

Control subjects may also be identified through the CHOP Clinical Research Finder website: https://www.research.chop.edu/research/clinical-research, which is a public website. The study is listed on this website and control subjects may approach the study team to participate. They will be asked to complete study procedures if they are eligible for the study. All study advertisements would be forwarded to the IRB for approval.

5.4 Informed Consent/Assent

An investigator or clinical coordinator will obtain informed consent or assent and parental consent from potential participants. Each participant will be re-consented each year for his or her participation. Each participant will also be allowed to select specific studies for participation. For example, a subject can choose to participate in the clinical outcome measures but not the blood draw for genetic studies. At the end of the consent form the subject can select the specific studies in which he/she wants to participate. Control/carrier subjects will only consent to participate in the cheek swab, blood sample, and the LiSN-S hearing test.

Study consent will be done in person if possible, and will otherwise be performed over the phone or via email. The subject will mail back a signed copy of the consent form. This mail-back consent process will specifically be done on subjects who have previously consented but who have not provided a DNA sample. As part of this mail-back consent process,

subjects will also be asked to collect a DNA sample using a saliva kit that can be mailed back to the study team along with the signed consent form.

5.5 Payment to Subjects/Families

Participants will not receive financial rewards or inducements, however this study's grant does provide funds to reimburse travel expenses up to $300 per participant, as many participants travel from outside the greater Philadelphia area. There is also no cost to participation in the study. Participants who receive clinical care from the investigator or the Children's Hospital of Philadelphia will be responsible for those charges as they are separate from the study.

There will be no reimbursement for carrier/control subjects.

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. 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 and 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.

From carriers/controls for cheek swabs and blood samples, only age and gender will be collected as these are necessary for RNA metabolomics, and frataxin analysis. The subjects' name will be collected as this is required to sign the informed consent. Thee cheek swab samples will be labeled with only the subject initials and the date of collection. The blood samples sent to the laboratories specified in the informed consent will be labeled with study ID number and the date the sample is drawn. The informed consents will be kept locked in a filing cabinet.

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.

7 REFERENCES

See references in grant application.