The Children's Hospital of Philadelphia

Department of Radiology

Magnetic Resonance Spectroscopy (MRS) Estimates of Glutathione (GSH) and GABA as Biomarkers of Pathophysiology in FRDA

Short Title	GABA and GSH in FRDA
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ABBREVIATIONS AND DEFINITIONS OF TERMS

ATP	Adenosine Triphosphate
CHOP	Children's Hospital of Philadelphia
CNS	Central Nervous System
CRF	Case Report File
CST	Corticospinal Tract
CVs	Coefficients of variation
DOB	Date of birth
EEG	Electroencephalography
Ex Vivo	Takes place outside an organism
DTI	Diffusion Tensor Imaging
FARA	Friedreich's Ataxia Research Alliance
FARS	Friedreich Ataxia Rating Scale
FRDA	Friedreich Ataxia
GABA	Gamma amino-butyric acid
GSH	Glutathione
HERMES	Novel MRS protocol to measure GSH and GABA levels
In Vivo	Within an organism
LH	Left Hemisphere
mFARS	Modified Friedreich's Ataxia Rating Scale
mM	Millimolar
MOXIe	Omaveloxolone clinical trial
MRS	Magnetic Resonance Spectroscopy
NRF2	Nuclear factor erythroid 2-related factor 2
NT	Neurotypical
NAA	N-acetyl-aspartate
tNAA	N-acetyl-aspartate
RH	Right Hemisphere
T1w	T1-weighted
T2w	T2-weighted
tCho	Choline
tCr	Creatine
TD	Typically Developing

ABSTRACT

Context: (Background)

Friedreich Ataxia (FRDA) is a slowly progressive neurodegenerative resulting from relative deficiency of the protein frataxin. This leads to downstream, features of mitochondrial dysfunction and other events that mediate the clinical features of FRDA (ataxia, CNS degeneration, cardiomyopathy, scoliosis, diabetes, etc.). Still, future clinical trials of frataxin restoring therapies need novel biomarkers to assess outcomes in clinical studies, and to understand better the pathogenesis of the disease in patients with FRDA. In this proposal, we will use advanced, edited magnetic resonance spectroscopy (MRS) to assess brain levels of the reduction-oxidation (redox) compound glutathione and the inhibitory neurotransmitter γ-aminobutyric acid (GABA), two potential markers of the proximal aspects of the pathophysiology of FRDA. We will also acquire information on 2 markers of neuronal and glial integrity , NAA and MI.It is our hypothesis that the assessment of local motor cortex brain glutathione and GABA levels provide accurate biomarkers of disease status in FRDA.

Objectives:

Our prior pilot work has implemented a published MRS protocol (HERMES) for simultaneous assessment of GABA and glutathione (GSH) in a single scan using a 3T MR scanner, concentrating on the motor cortex, with repeated measure coefficient of variance (CV) values below 20% - indicating excellent measurement precision.

Specific Aim 1 is to assess *effect size* (metabolite depletion) in FRDA subjects versus age-matched control children ranging in age between 8 and 16 years (N= 17 controls, N=40 FRDA patients, evaluable). **Specific Aim 2** will attempt to follow the natural history longitudinally over time.

Study Design:

This is a **two-cohort descriptive observational** study of neurometabolites in a disease population (FRDA) vs controls.

Setting/Participants:

This study will be conducted in the Department of Radiology at the Children's Hospital of Philadelphia. The following cohort will be recruited and enrolled:

Aim 1, Children with Friedreich's Ataxia (FRDA): 17 evaluable children aged 8 <16 years old. Age matched controls: 17 evaluable neurotypical (NT) children aged 8 <16 years old.

Aim 2, Children with Friedreich's Ataxia (FRDA): 40 total evaluable children aged 8 <16 years old.Given the challenges of new methodology and MR requirements for maintaining motionless head position, we anticipate over-recruiting (N=17 controls, N=40 FRDA per cohort) to achieve these evaluable goals. We also expect the longitudinal analysis will require at least 20 subjects

Study Procedures, Interventions and Measures:

Subjects will undergo an MRI scan wherein we will use a published, but recently developed, MRS protocol (HERMES) for simultaneous assessment of GABA and glutathione (GSH) in a single scan using a 3T MR scanner. Some MRI/MRS sequences may need to be re-run if there is excess movement or artifact in the scan that interferes with the quality of the image.

Main study outcome measures

The main study outcome measures will be obtaining GABA and GSH assessment derived from MRS recording, expressed in absolute terms. Outcome measures will consist of estimates of GABA and GSH normalized to estimates of the stable metabolite creatine (Cr) from the same spectroscopy voxel: GABA/Cr and GSH/Cr.

Main study endpoints

We anticipate reduced GSH and GABA levels in our FRDA participants, relative to controls, and that this effect will increase longitudinally Group effect size of this decrease will be assessed using independent T-tests of GSH and GABA separately.

SCHEDULE OF STUDY PROCEDURES

Procedure	Screening	Visit 1, 2,3 MRI
Written or Verbal HIPAA Authorization	X	
Review Inclusion/Exclusion Criteria	X	
Review Previous FARS Score for Eligibility	X	
Written Informed Consent		X
Handedness Questionnaire		X
Medication Form		X
Mock brain MRI practice		X*
Females ≥ 11yrs of age and those younger who have begun menstruating: pregnancy status will be determined using approved CHOP Policies.		X
Brain MRI (anatomic) and MRS (HERMES)		X

*Optional. The mock scanner will be available on day of scan, if desired/deemed useful

1 BACKGROUND INFORMATION AND RATIONALE

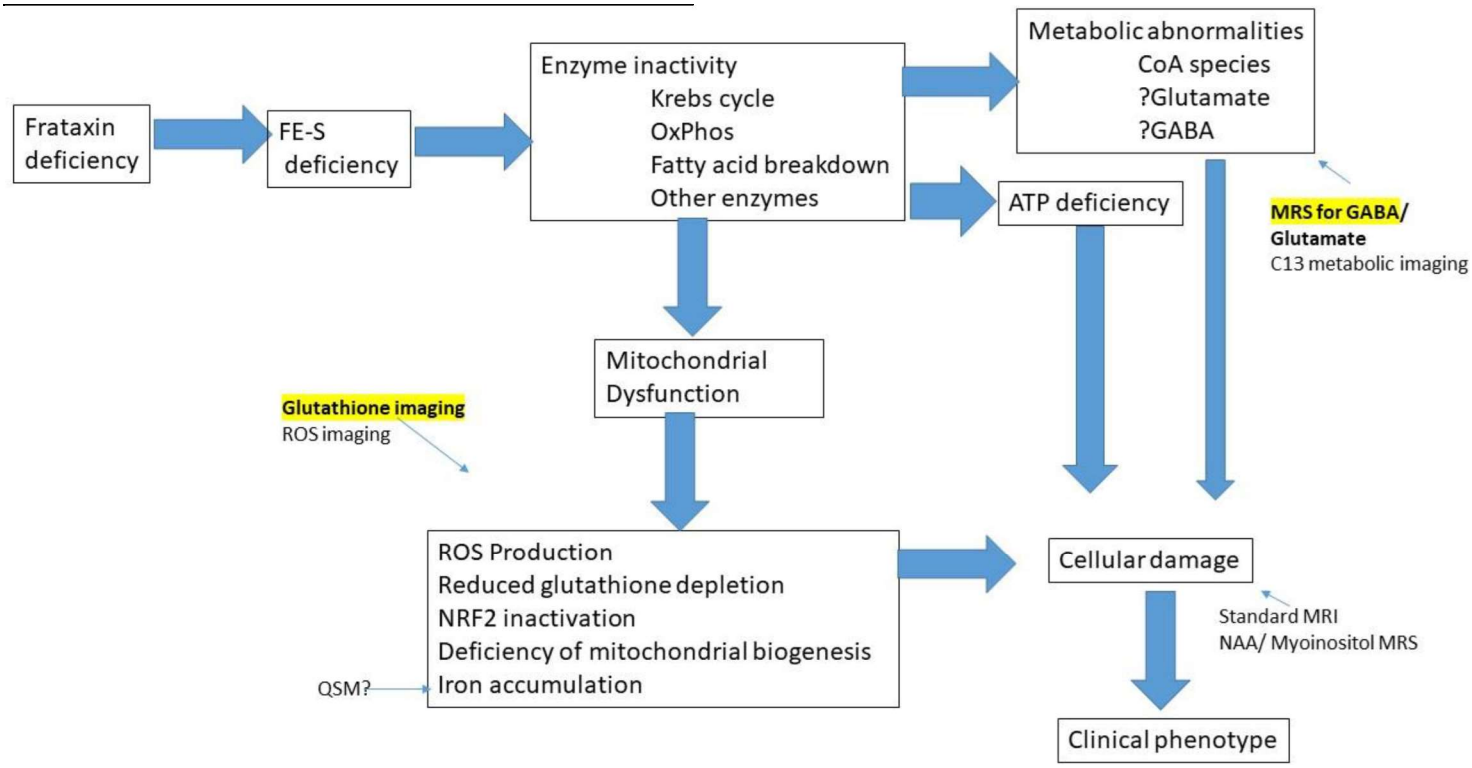
1.1 Introduction

Friedreich Ataxia (FRDA) is a slowly progressive neurodegenerative resulting from relative deficiency of the protein frataxin (12-14). Such deficiency results from mutations in the FXN gene, with 96% of all mutations being GAA expansions that lead to the silencing of the gene. 4% of all mutations are point mutations that lead to decreases in functional frataxin (15). Frataxin deficiency leads to decreases in synthesis of iron-sulfur clusters, which act as prosthetic groups in enzymes of oxidative phosphorylation, the Krebs cycle, and other metabolic pathways (16-17). This leads to downstream, features of mitochondrial dysfunction and other events that mediate the clinical features of FRDA (ataxia, CNS degeneration, cardiomyopathy, scoliosis, diabetes, etc.). At present, a series of biomarkers exist for FRDA, all of which are handicapped by specific issues (4-5). Sensitive assays of frataxin accurately assess protein levels, but the inaccessibility of the brain and heart limits their use. In contrast, brain imaging biomarkers provide utility in some situations, but ongoing studies of brain structure and chemicals such as N-acetyl-aspartate (NAA) reflect the late pathophysiology of FRDA, and thus are unlikely to be early markers of frataxin depletion or restoration (5-6). In this proposal, we will use advanced, edited magnetic resonance spectroscopy (MRS) to assess brain levels of the reduction-oxidation (redox) compound glutathione and the inhibitory neurotransmitter γ-aminobutyric acid (GABA), two potential markers of the proximal aspects of the pathophysiology of FRDA. Specific data from model systems and patients provide the rationale for examination of these specific markers (7-11). Depletion of glutathione and antioxidant defense mechanisms represents a crucial step in the pathophysiology of FRDA in cellular systems, along with paradoxical reduction in NRF2 (which controls glutathione homeostasis). However, measurement of glutathione in vitro is difficult because of its transient nature ex vivo and the inaccessibility of relevant tissue (brain, heart) (18). An in vivo magnetic resonance approach, if sufficiently sensitive, should be ideal for assessing glutathione as a selective marker of FRDA at the level of mitochondrial dysfunction/ROS production, upstream from cell death markers. In contrast, but complementary, the rationale for assessing GABA derives from new understanding of neurodegenerative diseases in general, the course of FRDA, and preliminary data. Initially, neurodegeneration likely reflects subtle dysfunction of metabolic pathways, inadequate maintenance of membrane potentials, and early changes in synaptic function (5). While these are difficult to assess in vivo, GABA levels reflect at least 2 of these processes in FRDA: its synthesis is partially governed by the Krebs cycle, and its presence marks the activity of inhibitory synapses.

A new treatment for FRDA has recently been introduced (omaveloxolone) that can be used to supplement over the counter vitamin therapies. A sensitive MRS based approach could be used to establish if such treatment alter the evolution of changes in the brain in FRDA.

Relevant Literature and Data

Specific data from model systems and patients provide the rationale for examination of these specific markers (7-11). Depletion of glutathione and antioxidant defense mechanisms represents a crucial step in the pathophysiology of FRDA in cellular systems, along with paradoxical reduction in NRF2 (which controls glutathione homeostasis). However, measurement of glutathione *in vitro* is difficult because of its transient nature *ex vivo* and the inaccessibility of relevant tissue (brain, heart) (18). An *in vivo* magnetic resonance approach, if sufficiently sensitive, should be ideal for assessing glutathione as a selective marker of FRDA at the level of mitochondrial dysfunction/ROS production, upstream from cell death markers. In contrast, but complementary, the rationale for assessing GABA derives from new understanding of neurodegenerative diseases in general, the course of FRDA, and preliminary data. Initially, neurodegeneration likely reflects subtle dysfunction of metabolic pathways, inadequate maintenance of membrane potentials, and early changes in synaptic function (5). While these are difficult to assess *in vivo*, GABA levels reflect at least 2 of these processes in FRDA: its synthesis is partially governed by the Krebs cycle, and its presence marks the activity of inhibitory synapses.



1.2 Compliance Statement

This study will be conducted in full accordance with all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable state laws and regulations including 45 CFR 46, 21 CFR Parts 50, 56, and 812, and HIPAA.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report unanticipated problems involving risks to subjects or others 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

Hypothesis: Assessment of local motor cortex brain glutathione and GABA levels provide accurate biomarkers of disease status in FRDA. As such we predict that both GSH and GABA will be reduced in FRDA and that such differences will increase over time and be responsive to therapies.

2.1 Primary Objective (or Aims)

In **Specific Aim 1**. to assess *effect size* (metabolite depletion) in FRDA subjects versus age-matched control children ranging in age between 8 and <16 years (N=40 per arm, evaluable).

In **Specific Aim 2**, 20 further FRDA subjects (from the above cohort), age between 8 and <16 years, will undergo longitudinal comparison of GABA/GSH assessment before and both 12 and 24 months after the initiation of Omaveloxolone therapy (as it becomes available clinically or via clinical trial participation). Overlap in recruitment between Aims 1 and 2 is likely, due to clinical trial participation in the BRAVE and

BOLD studies (which test the efficacy and safety of Omaveloxolone in FRDA participants <16 yrs). Note: the purpose of Aim 2 is to establish the sensitivity to change of proposed neurometabolite spectroscopy but not to determine signatures of treatment efficacy.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

FRDA patients will be selected from those children and adults seen clinically at CHOP (>400 visits per year) to include individuals of all stages and all GAA repeat lengths. Controls in Aim 1 will be matched overall by age and sex. FRDA participants age <16 yrs, will not be able to have Omaveloxolone in accordance with FDA labeling indication), and FRDA participants age > 16 yrs may have started to use Omaveloxolone medication. Thus, FRDA patients in Aim 1 will be naïve to Omaveloxolone, and depending on age and participation in clinical trial may initiate Omaveloxolone for Aim 2 (see details). The requirements for MRI (Absence of metal, need to remain still) will likely create a mild selection bias away from young children (less than age 10) and away from most affected individuals with FRDA. Controls will be matched overall by age and sex.

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

The study will be conducted at 1 investigative site in the United States, CHOP.

In study Aim 1, we plan to recruit 20 participants from each group and stop when 17 subjects with FRDA are evaluable. Non-evaluable participant data can be the result of head/body movement, artifacts, or technical data corruption. In study Aim 2 we plan to recruit 20 more participants with genetically confirmed FRDA. FRDA Subjects from Aim 1 and newly recruited participants recruited for Aim 2, will complete a total of three visits at CHOP. Aim 2 involves longitudinal comparison of GABA/GSH assessment in FRDA patients repeated before and after Omaveloxolone administration at 3 total time points. The first visit will be prior to the initiation of study drug treatment, Omaveloxolone. The next visit will be 12 months after Omaveloxolone initiation, and the final scan will be 24 months post Omaveloxolone initiation.

3.1.2 Study Population

3.1.3 Inclusion Criteria

FRDA Participants:

- Age ≥ 8 years; <16 years
- Written informed consent provided
- Balletic GAA repeat length > 55 in intron 1 of *FXN* and/or GAA repeat length > 55 in intron 1 of *FXN* in one allele and another type of mutation that is inferred to cause loss of function in the second *FXN* allele as documented in the medical record
- FARS Functional staging score of ≤ 5^ and total mFARS score of ≤ 65 on enrolment

Control Participants:

- Age ≥ 8 years; <16 years
- Written informed consent provided

3.1.4 Exclusion Criteria

FRDA Participants:

- Age < 8 years > 16 years
- Acute or ongoing medical or other conditions that is deemed to interfere with the conduct and assessments of the study

- Other psychiatric or neurologic conditions apart from FRDA that, in the opinion of the Site Investigator, would interfere with the conduct and assessments of the study
- MR contraindications (e.g., pacemaker or other metallic surgical implants)
- Presence of metallic dental braces
- Currently pregnant participants
- Confined to wheelchair or bed with total dependency for all activities of daily living. Total disability.
- Unable to understand English instruction

Control Participants:

1. Age < 8 years > 16 years
2. Diagnosed psychiatric or neurological condition
3. MR contraindications (e.g., pacemaker or other metallic surgical implants)
4. Presence of metallic dental braces
5. Acute or ongoing medical or other conditions that would interfere with the conduct and assessments of the study
6. Currently pregnant participants
7. Unable to understand English instruction

3.2 Study Duration, Enrollment and Number of Sites

For Aim 1, both control and FRDA patients will take place at CHOP and last about 2.5 hours. For Aim 2, FRDA participants will complete a total of 3 time point visits to CHOP (one may have taken place in Aim 1). Participants will have a baseline (T1) scan, 12-month scan (T2), and 24-month scan (T3). The total study duration will be approximately 24 months and correlate with a study visit for participation in a clinical trial involving either the efficacy or safety of pediatric Omaveloxolone. We expect that almost all if not all participants will be enrolled in the BRAVE or BOLD trials of omaveloxolone as such individuals represent the dominant majority (n=60-65) of Dr Lynchs pediatric FA cohort.

This will allow us 2 analyses of the data.

A. Comparison of values in controls with those of FRDA patients (Aim 1)

B. Longitudinal comparison of values in FRDA patients repeated after Omaveloxolone administration at 3 time points (minimum of 6 months) (Aim 2)

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4 STUDY PROCEDURES

4.1 Eligibility Screening

The following will be conducted over the phone, or in person, with the parent/guardian of the child participant to determine participant eligibility:

- Verbal HIPAA Authorization for screening
- Eligibility Screen (see attached form)
- Medical Chart Review as needed to confirm eligibility

The screener will be reviewed by PI-designated staff to ensure study eligibility criteria are met. Determination of eligibility may require review of CHOP electronic medical records to ensure that participant does not have any exclusionary conditions.

4.2 Study Visit

The study will involve up to three visits to The Children's Hospital of Philadelphia. During each visit the participant will undergo an MRI brain scan involving the use of MRS to measure in-vivo GABA and GSH levels. Sequences may be re-run during the MRI scan, if the scan data is affected by the participant moving, or any other artifact. This does not entail an additional study visit. We will not use sedation/general anesthesia, or contrast in this study.

Subjects will undergo the following procedures during their visit:

- Medication History form
- Medical Record review
- MRI Scan including MRS to assess in vivo GABA and GSH levels
- Optional mock scan for practice
- Pregnancy test, if applicable
- Completion of a Handedness Questionnaire

4.3 Subject Completion/Withdrawal

Subjects may withdraw from the study at any time without prejudice to their care. They may also be discontinued from the study at the discretion of the Investigator for lack of adherence to study treatment or visit schedules. The Investigator may also withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. Participants may be withdrawn from the study and not complete the imaging visit if their FRDA diagnosis progresses to a level 6. It will be documented whether or not each subject completes the clinical study. If the Investigator becomes aware of any serious, related adverse events after the subject completes or withdraws from the study, they will be recorded in the source documents.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1 Medical Record Review and Research Record Review

A medical record review may also be conducted to confirm medical history for study eligibility as needed. The variables that will be reviewed through the medical record are diagnosis, disease onset, metal implant make and model, confirmation of parent/guardianship, ongoing medical conditions, psychological conditions, and neurological conditions. The mFARS score will be obtained from each participant's clinical visits with CHOP Neurology in the past. These scores exist in the participants' medical record. We will not be performing the mFARS as a procedure in this research study. Additionally, research records from the UNIFAI Natural History Study (IRB # 01-002609) will also be accessed. UNIFAI study records will only be accessed for subjects who consented to future use of their data, and only subjects who consented to be contacted for future studies will be contacted for participation.

5.1.1 Phone Screen and Verbal Authorization for Screening

Initial determination of eligibility will be conducted by study staff over the phone or in person. The eligibility screen determines eligibility, and only subjects who meet study inclusion criteria and who do not meet exclusion criteria will be enrolled in this study. Verbal authorization will be obtained if screening procedures are conducted over the phone, and written authorization will be obtained if screening procedures are conducted in person.

5.1.2 Questionnaires, Surveys

Handedness Questionnaire - The Handedness Inventory is a measurement scale used to assess the dominance of a person's right or left hand in everyday activities.

Medication Form – The Medication Form is a parent completed questionnaire that asks subjects to list out all current medication use, dosage and reason for use.

5.1.3 MRS to Assess GABA and GSH

Magnetic resonance imaging (MRI) will be performed using a whole-body MR scanner in the Department of Radiology at Children's Hospital of Philadelphia. This state-of-the-art equipment is equipped with multi-channel RF capabilities (allowing parallel imaging). Contrast agent will not be used in this study.

Sequences will include:

1. Localizer scouts
2. Anatomical imaging
3. Magnetic resonance spectroscopy (MRS WIP and product sequence)
4. Susceptibility weighted imaging

The MRS WIP pulse sequences are not FDA-approved. These sequences were developed and provided by Siemens or collaborators developing sequences for use on Siemens MR systems. These sequences have additional options and capabilities as compared to their product sequences. These sequences are monitored and controlled by the MR safety systems in the same way as product sequences. These sequences are subject to the same safety limits as the product sequences. The WIP sequences are widely used for research purposes on an FDA-approved MRI device. This research study is not involved in any clinical decisions. The sequences are not purported or represented for use in supporting or sustaining human life, nor do they present a potential for serious risk to the health, safety, or welfare of the subject. In addition, they are not of substantial importance in diagnosing, curing, mitigating, or treating disease or otherwise preventing impairment of human health.

We will place an acquisition box in the brain (guided by the MRI scanner) and orient the box properly to include the brain tissue. We will acquire about a 10 min scan involving editing of GABA and Glutathione in the brain chemical spectrum. It will involve 4 steps: 1) editing both Glutathione and GABA; 2) Glutathione only; 3) GABA only; and 4) no editing. Hadamard combination of the two steps will result in the glutathione-edited or Gaba-edited spectrum with the Glutathione and GABA signal for analysis and quantification. This method is described in the study staff member's (Dr. Saleh) publication, citation 46.

Exported in vivo data will be processed in Osprey followed by linear combination modelling (LCM) of MRS spectra. We will calculate water-scaled metabolite estimates and correct for tissue composition and relaxation effects to generate metabolite concentrations, as prescribed in the study staff member's (Dr. Saleh) publication.

5.1.4 Scanning Safety Screen and Pregnancy test:

All participants will complete a metal screening questionnaire with MR imaging techs prior to their MRI. If they have metal in their body on the day of the visit that is not approved for the MR machine they will not be permitted to scan. As females who are pregnant are not able to undergo the MRI exam, we will conduct POC pregnancy testing compliant with CHOP Policies including CHOP IRB SOP 805 and Policy: Pregnancy Screening and Testing for Radiology Exams and Procedures in Female Patients of Childbearing Age. To determine pregnancy status in females, our protocol is as follows.

As detailed in Section 2 of the CHOP Radiology Pregnancy Protocol, pregnancy status will be one of three: (1) A post menarche female patient is considered pregnant if she states that she is pregnant or is confirmed to be pregnant via urine or serum pregnancy testing. (2) A post menarche female patient is considered to be "potentially pregnant" if the following apply: a. She states she is sexually active and unsure of pregnancy status, and b. The date of service is greater than 10 days from the onset of last menstrual period and the patient is known to be sexually active during that time. (3) A post menarche female patient is considered to be "not pregnant" if the following apply: a. The patient's last menstrual period is within a 35-day period, and she states she is not pregnant and/or sexually active, b Negative pregnancy test

results are on file (within 72 hours), or c. Patient is on oral, injectable, implantable, or patch contraceptive, on a regular schedule and using as directed and states she is not pregnant and/or sexually active.

If it is determined that the participant is pregnant, then the MRI exam will be cancelled. If the participant is considered potentially pregnant, then the participant will be asked if they would like to (1) have a urine pregnancy test to determine pregnancy status, or (2) cancel the MRI exam. If the participant elects to have a urine pregnancy test, a urine sample will be obtained and sent to the CHOP lab. If the lab report is negative, then MRI exam will be conducted. If the lab report is positive, the MRI exam will be cancelled. If the participant is considered not pregnant, the participant will undergo the MRI exam. If the participant is potentially pregnant and elects not to have a urine pregnancy test, the MRI will be cancelled. A MEG/MRI technician will complete the CHOP "Outpatient magnetic resonance imaging (MRI) procedure screening' form (see Section 12.02(2)) to document the results of the pregnancy screen (and when needed the results of the urine pregnancy test) This form (and on this form noting the results of their pregnancy screen) are uploaded to EPIC to document the metal screening and pregnancy results (and as required NOT placed in the participants "medical chart").

5.2 Safety Evaluation

Participant safety will be monitored by adverse events. It is possible that some participants may find the MRI uncomfortable. Blankets and cushions are available to make participants more comfortable. Since the MRI is a big magnet, we make sure that no metal is taken into the machine. On the day of the MRI scan, one of the MRI technologists (MRI experts) will go through the safety questionnaire with participants. Each of the MRI techs involved in this study are designated members of the investigative team and have considerable training and experience running MRS protocols for both clinical and research work. Additionally, female participants aged 11 and up will undergo a urine pregnancy test, administered in a CHOP CLIA Certified Lab, according to CHOP's most recent policies for pregnancy testing as a research procedure. Pregnancy tests results must be negative prior to a participant entering the MRI. The MRI can make knocking noises that may be bothersome to some participants. This noise is reduced by providing ear protection. Due to the unknown risk of the MRI on pregnancy, female participants who have begun menstruating or are 11 years of age or older will be excluded from participating if they have a positive pregnancy test or are considered "potentially pregnant" and elect not to have a urine pregnancy test prior to their MRI.

6 STATISTICAL CONSIDERATIONS

6.1 Primary Endpoint

We anticipate reduced NAA and increased mI to predict clinical impairment in FRDA. In addition, we predict reduced GSH and GABA levels in our FRDA participants, relative to controls, and that this effect will increase longitudinally. Group effect size of this decrease will be assessed using independent T-tests of GSH and GABA separately. At the end of the study, unmasking of medication use at the time of imaging will be used as a covariate in statistical models.

Control of Bias and Confounding

MRS data will be done blind to subject group.

6.2 Statistical Methods

6.2.1 Baseline Data

Baseline and demographic characteristics will be summarized by standard descriptive summaries (e.g., means and standard deviations for continuous variables such as age and percentages for categorical variables such as gender).

6.2.2 Analysis of Primary Outcome of Interest

Aim 1: Independent T-Tests of metabolites NAA, mI, GSH and GABA between Controls and FRDA participants.

Aim 2: Linear Mixed models for metabolites NAA, mI, GSH and GABA between \ FRDA participants at baseline and after 12 and 24 months. Medication use at the time of testing will serve as a covariate.

6.3 Sample Size and Power

With a sample size of N=20 recruited in each arm (FRDA vs TD) we are powered (at 1-b=80% and an a level of 0.05) to detect group differences in mean concentration values of either metabolite (GSH or GABA) of a magnitude comparable to the pooled standard deviation (N~17 required, allowing for a typical ~15% loss of evaluable data, due to motion and/or technical artifact) using a simple two-tailed Student t-test with unequal variance assumed (as proposed). Even if this proposed sample size does not reveal differences of statistical significance in the current study, it will provide the scientifically necessary estimates of population mean concentration values and standard deviations to inform statistical power calculations and sample size estimation for future, analogous studies in this domain.

7 SAFETY MANAGEMENT

7.1 Clinical Adverse Events

Clinical adverse events (AEs) will be monitored throughout the study.

7.2 Adverse Event Reporting

Since the study procedures are not greater than minimal risk, SAEs are not expected. If any unanticipated problems related to the research involving risks to subjects or others happen during the course of this study (including SAEs) these will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. AEs that do not meet prompt reporting requirements will be summarized in narrative or other format and will be tracked and documented internally by the study team but not submitted to the IRB.

7.3 Incidental Findings

Abnormal MRI: Investigators will follow CHOP Policy: Clinical Reading of Radiology and Cardiology Imaging Procedures and EKGs Performed in the Context of Research. All structural brain MRI scans will be interpreted by a clinical pediatric neuro radiologist at CHOP. If a clinically significant brain abnormality is observed, the study physician will provide the participant and their parents with the clinical information. If the participant has agreed to have their finding shared with their primary care physician, the study physician will contact their primary care physician to share the results. If the brain abnormality requires immediate further investigation, the study physician will directly contact the primary care provider or specialist caring for the participant to provide suggestions for the next steps to follow up the MRI scan.

Positive Pregnancy test: If a pregnancy test comes back positive, the PI or delegated staff member will inform the participant of the results and explain that this makes them ineligible to participate in the study due to unknown risks of MRI on an unborn fetus. In the case of a positive pregnancy test for a minor, Pennsylvania State law requires that only the minor is provided this information. However, we will ask the participant if they were aware and whether their parent/guardian may be present while discussing these results. If the participant is unwilling to inform their parent at that time, we will encourage them to find a time in the next few days to disclose this information to them and follow up with their primary care physician. In this case, the parent/guardian would be informed that their child is no longer interested in participating in the study and thus the study will be discontinued. If the subject does not have the capacity to comprehend the information regarding pregnancy, then the subject's parent/guardian will be involved in the discussion of the results.

8 STUDY ADMINISTRATION

8.1 Data Collection and Management

Hard copies of case report forms and source data will be stored in a locked cabinet in a locked study staff office space. Electronic source data will be stored on a CHOP network share drive with access controlled by the assigned study staff.

Hardcopy charts/paper copies will be stored in a locked filing cabinet in an approved study staff's office. Subjects will be assigned a unique identification number and the information will be password protected to insure confidentiality and security. Password- protected spread sheets with subject's PHI will be kept with restricted encrypted access only on the CHOP SAN drive. Backups of all data on the CHOP SAN drive is performed routinely. Members of the research team, including the funding partners, will have access to the de-identified data and limited PHI being the date of scan on the brain MRIs. The research team will have access to this limited PHI for data analysis purposes, but it will be kept separate from the master list which they will not have access to. The PI will monitor who is accessing the data. Participant information will be coded in a way that can be re-identified in the future, but only by members of the research team at this site. We will also remove the front part of the brain MRI image so that participants are not potentially identifiable.

The master list containing PHI and study data will be password protected on CHOP SAN drive. Backups on the CHOP SAN drive are performed daily. All links between a master list containing PHI and the study data will be destroyed six years after study completion and PHI will be removed from stored data unless the participant has agreed to be contacted about future studies and/or permitted us to use their data for future studies. The study will comply with CHOP policy A-3-9 for data retention.

In compliance with CHOP policy A-3-9, information from this study will be maintained until all analyses are complete and the study is closed.

In some cases, complete confidentiality of records cannot be guaranteed, because records may be examined by authorized personnel. In such cases, participants will be informed of this possibility prior to signing the consent form. Otherwise, records will be kept strictly confidential and will not be inspected by any other agency unless required by law.

8.2 Confidentiality

All data and records generated during the study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy and that the Investigator and other site personnel will not use such data and records for any purpose other than conducting the study.

In order to keep protected health information (PHI) from disclosure, all data will be coded and identified only by a code label assigned to the participant upon entry into the study. All information will be stored on a password protected server, password protected PCs, or in a locked filing cabinet in the lab's research space. If a need for further medical evaluation or treatment is identified during the research, participants will be appropriately referred and upon obtaining consent, information will be made available to the health-care provider.

The information collected as a part of this study will be retained until the study is completed, including full data acquisition, analysis and publication. All study data, including PHI and the master list, will be maintained until all analyses are complete and the study is closed.

For those participants who consented to be contacted about future studies we will keep their contact information indefinitely, on the lab's CHOP managed SAN drive in a password protected document. All links to their study Identifier will be removed from this document at study end.

8.3 Regulatory and Ethical Considerations

8.3.1 Data and Safety Monitoring Plan

The principal investigator will be responsible for monitoring data for accuracy and validity, and any unanticipated problems related to the research involving risks to participants or others will be reported to the IRB immediately. The CHOP PI will monitor and review the study progress, subject safety, and the accuracy and security of the emerging data

8.3.2 Risk Assessment

There are minimal risks associated with participation in this study.

Risk of Questionnaires: There are no physical risks, however participants will be informed in advance that responses to questionnaires are voluntary and not required for study participation.

Risk of Breach of Confidentiality: Protected Health Information (PHI): Protected Health Information will be collected as part of study records, and breaches of this information outside of the study team resulting in loss of confidentially is an unlikely but potential risk. We will follow CHOP policies to ensure compliance with HIPAA and IRB regulations for safeguarding participant information to protect against loss of confidentiality.

Risk of MRI Scan: There are no known long-term risks related to MRI scans as used in this research project. MRI is considered to be safe when performed at a center with appropriate procedures. However, the magnetic attraction for some metal objects can pose a safety risk, so it is important that metal objects are not taken into the scanner room. Metal detectors are in place at the MRI suite and an MRI safety questionnaire is completed by the trained MRI technician prior to scanning. Participant may find lying on the MRI bed slightly uncomfortable and/or feel mildly "closed-in" (or claustrophobic) with their head inside the imaging machine. We will make every effort to reduce any discomfort by including pillows, ear plugs, and blankets as needed. Each participant will be provided with a squeeze ball alarm as well as voice intercom to indicate that they would like to stop the MRI scan. Ear plugs will be provided to reduce the noise of the MRI scan.

8.3.3 Potential Benefits of Study Participation

Whilst there may be no direct benefit to you, this project will help establish if the use of brain MRI with edited magnetic resonance spectroscopy (MRS) to assess brain levels of the reduction-oxidation (redox) compound glutathione and the inhibitory neurotransmitter γ-aminobutyric acid (GABA) can be used as potential markers of the proximal aspects of the pathophysiology of FRDA. Should the proposed studies be successful (even simply the first parts of Aim 1), there is the potential for immediate extension of the techniques to other studies.

Risk-Benefit Assessment

Since risks associated with the procedures are negligible, the risk/benefit ratio is considered favorable.

8.4 Recruitment Strategy

FRDA patients will be selected from those seen clinically at CHOP (>400 visits per year) to include individuals of all stages and all GAA repeat lengths. The requirements for MRI (Absence of metal, need to remain still) will likely create a mild selection bias away from young children (less than age 10) and away from most affected individuals with FRDA. FRDA subjects who participated in Aim 1 and plan to or started taking Omaveloxolone will be asked to return for 2 more visits (3 total visits) in Aim 2. Regarding additional recruitment in Aim 2, we expect that most (if not all) of the

subjects recruited will be involved in the studies of Omaveloxolone, BOLD (IRB# 21274) and BRAVE (IRB#23192), as this represents a dominant majority patients followed by Dr. Lynch through the Neurogenetics clinics at the Children's Hospital of Philadelphia. In Aim 2, eligible subjects will be recruited via email and word of mouth. Controls will be matched overall by age, sex, and handedness. Control subjects will be identified and recruited through word of mouth, as well as CHOP's Research Discovery Finder Tool through the CHOP website. The study flyer will be used to recruit control participants, and the flyer contains information on compensation, hours of study participation, and eligibility requirements. Flyers will be posted within the CHOP Main Hospital campus, such as inside the Buerger Building and CHOP Main Hospital where other study flyers are often posted.

8.5 Informed Consent/Assent and HIPAA Authorization

8.5.1 Screening

Screening will be completed over the phone or in person. In instances where screening takes place over the phone, verbal HIPAA authorization will be obtained from the participant prior to screening process. Written HIPAA Authorization will be obtained when the screening takes place in person. The eligibility screen and pre-intake questionnaire (see attached forms) will also be completed over the phone to determine eligibility.

8.5.2 Main Study

Study staff will provide a copy of the study informed consent to all participants before they come in for their study visit. Subjects/families are encouraged to contact the study staff with any questions or concerns about the study if they arise. The study staff will obtain consent when the subject/family arrives for their first visit. Study staff will review the consent and remind subjects/parents they can ask any questions, can cease participating at any time, and do not have to take part in the study if they do not want to. Consent will be obtained prior to completing any other study procedures.

8.6 Payment to Subjects/Families

8.6.1 Payments to subject for time, effort and inconvenience (i.e., compensation)

Participants will receive $100.00 for participation, time, and effort, as well as a $10 meal stipend for themselves and their accompanying guardian.

9 PUBLICATION

It is anticipated that results from this study will be published in the peer-reviewed literature. No subject-identifying information will be included in such publications.

10 REFERENCES

1. Rodden LN, Lynch DR. Designing phase II clinical trials in Friedreich ataxia. Expert Opin Emerg Drugs. 2021 Dec;26(4):415-423.

2. Lynch DR, Schadt K, Kichula E, McCormack S, Lin KY. Friedreich Ataxia: Multidisciplinary Clinical Care. J Multidiscip Healthc. 2021 Jun 28;14:1645-1658.

3. Lynch DR, Chin MP, Delatycki MB, Subramony SH, Corti M, Hoyle JC, Boesch S, Nachbauer W, Mariotti C, Mathews KD, Giunti P, Wilmot G, Zesiewicz T, Perlman S, Goldsberry A, O'Grady M, Meyer CJ. Safety and Efficacy of Omaveloxolone in Friedreich Ataxia (MOXIe Study). Ann Neurol. 2021 Feb;89(2):212-225.

4. Lynch DR, Farmer G. Mitochondrial and metabolic dysfunction in Friedreich ataxia: update on pathophysiological relevance and clinical interventions. Neuronal Signal. 2021 May 17;5 (2):NS20200093.

5. Harding IH, Lynch DR, Koeppen AH, Pandolfo M. Central Nervous System Therapeutic Targets in Friedreich Ataxia. Hum Gene Ther. 2020 Dec;31(23-24):1226-1236.

6. Saleh MG, Oeltzschner G, Chan KL, Puts NAJ, Mikkelsen M, Schär M, Harris AD, Edden RAE. Simultaneous edited MRS of GABA and glutathione. Neuroimage. 2016 Nov 15;142:576-582

7. Abeti R, Baccaro A, Esteras N, Giunti P. Novel Nrf2-Inducer Prevents Mitochondrial Defects and Oxidative Stress in Friedreich's Ataxia Models. Front Cell Neurosci. 2018 Jul 17;12:188.

8. Abeti R, Parkinson MH, Hargreaves IP, Angelova PR, Sandi C, Pook MA, Giunti P, Abramov AY. 'Mitochondrial energy imbalance and lipid peroxidation cause cell death in Friedreich's ataxia'. Cell Death Dis. 2016 May 26;7(5):e2237.

9. Bulteau AL, Planamente S, Jornea L, Dur A, Lesuisse E, Camadro JM, Auchère F.Changes in mitochondrial glutathione levels and protein thiol oxidation in Δyfh1 yeast cells and the lymphoblasts of patients with Friedreich's ataxia. Biochim Biophys Acta. 2012 Feb;1822(2):212-25.

10. Calabrese V, Lodi R, Tonon C, D'Agata V, Sapienza M, Scapagnini G, Mangiameli A, Pennisi G, Stella AM, Butterfield DA. Oxidative stress, mitochondrial dysfunction and cellular stress response in Friedreich's ataxia. J Neurol Sci. 2005 Jun 15;233(1-2):145-62.

11. 11.Shoichet SA, Bäumer AT, Stamenkovic D, Sauer H, Pfeiffer AF, Kahn CR, Müller-Wieland D, Richter C, Ristow M. Frataxin promotes antioxidant defense in a thiol-dependent manner resulting in diminished malignant transformation in vitro. Hum Mol Genet. 2002 Apr 1;11(7):815-21.

12. Campuzano V, Montermini L, Moltò MD, Pianese L, Cossée M, Cavalcanti F, Monros E, Rodius F, Duclos F, Monticelli A, Zara F, Cañizares J, Koutnikova H, Bidichandani SI, Gellera C, Brice A, Trouillas P, De Michele G, Filla A, De Frutos R, Palau F, Patel PI, Di Donato S, Mandel JL, Cocozza S, Koenig M, Pandolfo M. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science. 1996 Mar 8;271(5254):1423-7.

13. Campuzano V, Montermini L, Lutz Y, Cova L, Hindelang C, Jiralerspong S, Trottier Y, Kish SJ, Faucheux B, Trouillas P, Authier FJ, Dürr A, Mandel JL, Vescovi A, Pandolfo M, Koenig M. Frataxin is reduced in Friedreich ataxia patients and is associated with mitochondrial membranes. Hum Mol Genet. 1997 Oct;6(11):1771-80

14. Babady NE, Carelle N, Wells RD, Rouault TA, Hirano M, Lynch DR, Delatycki MB, Wilson RB, Isaya G, Puccio H. Advancements in the pathophysiology of Friedreich's Ataxia and new prospects for treatments. Mol Genet Metab. 2007 Sep-Oct;92(1-2):23-35

15. Cossée M, Dürr A, Schmitt M, Dahl N, Trouillas P, Allinson P, Kostrzewa M, Nivelon-Chevallier A, Gustavson KH, Kohlschütter A, Müller U, Mandel JL, Brice A, Koenig M, Cavalcanti F, Tammaro A, De Michele G, Filla A, Cocozza S, Labuda M, Montermini L, Poirier J, Pandolfo M. Friedreich's ataxia: point mutations and clinical presentation of compound heterozygotes.Ann Neurol. 1999 Feb;45(2):200-6

16. Schmucker S, Puccio H. Understanding the molecular mechanisms of Friedreich's ataxia to develop therapeutic approaches. Hum Mol Genet. 2010 Apr 15;19(R1): R103-10.

17. Martelli A, Puccio H. Dysregulation of cellular iron metabolism in Friedreich ataxia: from primary iron-sulfur cluster deficit to mitochondrial iron accumulation.- Front Pharmacol. 2014 Jun 3;5:130.

18. Piemonte F, Pastore A, Tozzi G, Tagliacozzi D, Santorelli FM, Carrozzo R, Casali C, Damiano M, Federici G, Bertini E. Glutathione in blood of patients with Friedreich's ataxia. Eur J Clin Invest. 2001 Nov;31(11):1007-11.; Tozzi G, Nuccetelli M, Lo Bello M, Bernardini S, Bellincampi L, Ballerini S, Gaeta LM, Casali C, Pastore A, Federici G, Bertini E, Piemonte F. Antioxidant enzymes in blood of patients with Friedreich's ataxia. Arch Dis

Child. 2002 May;86(5):376-9.

19. Worth AJ, Basu SS, Deutsch EC, Hwang WT, Snyder NW, Lynch DR, Blair IA. Stable isotopes and LC-MS for monitoring metabolic disturbances in Friedreich's ataxia platelets. Bioanalysis. 2015;7(15):1843-55.

20. Chantrel-Groussard K, Geromel V, Puccio H, Koenig M, Munnich A, Rötig A, Rustin P. Disabled early recruitment of antioxidant defenses in Friedreich's ataxia. Hum Mol Genet. 2001 Sep 15;10(19):2061-7.

21. Sparaco M, Gaeta LM, Santorelli FM, Passarelli C, Tozzi G, Bertini E, Simonati A, Scaravilli F, Taroni F, Duyckaerts C, Feleppa M, Piemonte F Friedreich's ataxia: oxidative stress and cytoskeletal abnormalities. J Neurol Sci. 2009 Dec 15;287(1-2):111-8.

22. Carletti B, Piermarini E, Tozzi G, Travaglini L, Torraco A, Pastore A, Sparaco M, Petrillo S, Carrozzo R, Bertini E, Piemonte F. Frataxin silencing inactivates mitochondrial Complex I in NSC34 motoneuronal cells and alters glutathione homeostasis. Int J Mol Sci. 2014 Apr 4;15(4):5789-806.

23. Iskusnykh IY, Zakharova AA, Pathak D. Glutathione in Brain Disorders and Aging. Molecules. 2022 Jan 5;27(1):324.

24. Asanuma M, Miyazaki I. Glutathione and Related Molecules in Parkinsonism. Int J Mol Sci. 2021 Aug 13;22(16):8689;. Matuz-Mares D, Riveros-Rosas H, Vilchis-Landeros MM, Vázquez-Meza H. Glutathione Participation in the Prevention of Cardiovascular Diseases. Antioxidants (Basel). 2021 Jul 29;10(8):1220. 25.

25. Richard W Olsen and Timothy M DeLorey. GABA Synthesis, Uptake and Release, in Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th edition, accessd on line 3/12/2022

26. Hertz L, Chen Y Integration between Glycolysis and Glutamate-Glutamine Cycle Flux May Explain Preferential Glycolytic Increase during Brain Activation, Requiring Glutamate.. Front Integr Neurosci. 2017 Aug 25;11:18;.

27. Kann O. The interneuron energy hypothesis: Implications for brain disease. Neurobiol Dis. 2016 Jun;90:75-85;. Buddhala C, Hsu CC, Wu JY. A novel mechanism for GABA synthesis and packaging into synaptic vesicles. Neurochem Int. 2009 Jul-Aug;55(1-3):9-12.

28. Naeije G, Coquelet N, Wens V, Goldman S, Pandolfo M, De Tiège X. Age of onset modulates resting-state brain network dynamics in Friedreich Ataxia.Hum Brain Mapp. 2021 Nov;42(16):5334-5344.

29. Naeije G, Wens V, Coquelet N, Sjøgård M, Goldman S, Pandolfo M, De Tiège XP. Age of onset determines intrinsic functional brain architecture in Friedreich ataxia. Ann Clin Transl Neurol. 2020 Jan;7(1):94-104

30. Marty B, Naeije G, Bourguignon M, Wens V, Jousmäki V, Lynch DR, Gaetz W, Goldman S, Hari R, Pandolfo M, De Tiège X. Evidence for genetically determined degeneration of proprioceptive tracts in Friedreich ataxia. Neurology. 2019 Jul 9;93(2):e116-e124.; Naeije G, Bourguignon M, Wens V, Marty B, Goldman S, Hari R, Jousmäki V, Pandolfo M, De Tiège X. Electrophysiological evidence for limited progression of the proprioceptive impairment in Friedreich ataxia.Clin Neurophysiol. 2020 Feb;131(2):574-576.

31. Rummey C, Zesiewicz TA, Perez-Lloret S, Farmer JM, Pandolfo M, Lynch DR. Test-retest reliability of the Friedreich's ataxia rating scale.Ann Clin Transl Neurol. 2020 Sep;7(9):1708-1712.

32. Rummey C, Corben LA, Delatycki MB, Subramony SH, Bushara K, Gomez CM, Hoyle JC, Yoon G, Ravina B, Mathews KD, Wilmot G, Zesiewicz T, Perlman S, Farmer JM, Lynch DR. Psychometric properties of the Friedreich Ataxia Rating Scale. Neurol Genet. 2019 Oct 29;5(6):371.

33. Rummey C, Farmer JM, Lynch DR. Predictors of loss of ambulation in Friedreich's ataxia. EClinicalMedicine. 2020 Jan 8;18:100213.

34. Saleh MG, Oeltzschner G, Chan KL, Puts NAJ, Mikkelsen M, Schär M, Harris AD, Edden RAE Simultaneous edited MRS of GABA and glutathione..Neuroimage. 2016 Nov 15;142:576- 582.

35. Near J, Edden R, Evans CJ, Paquin R, Harris A, Jezzard P. Frequency and phase drift correction of magnetic resonance spectroscopy data by spectral registration in the time domain. Magn Reson Med. 2015 Jan;73(1):44-50.

36. Edden RA, Puts NA, Harris AD, Barker PB, Evans CJ Gannet: A batch-processing tool for the quantitative analysis of gamma-aminobutyric acid–edited MR spectroscopy spectra. .J Magn Reson Imaging. 2014 Dec;40(6):1445-52.

37. Weng L, Laboureur L, Wang Q, Guo L, Xu P, Gottlieb L, Lynch DR, Mesaros C, Blair IA. Extra-mitochondrial mouse frataxin and its implications for mouse models of Friedreich's ataxia. Sci Rep. 2020 Sep 25;10(1):15788.

38. Guo L, Wang Q, Weng L, Hauser LA, Strawser CJ, Rocha AG, Dancis A, Mesaros C, Lynch DR, Blair IA. Liquid Chromatography-High Resolution Mass Spectrometry Analysis of Platelet Frataxin as a Protein Biomarker for the Rare Disease Friedreich's Ataxia. Anal Chem. 2018 Feb 6;90(3):2216-2223.

39. Guo L, Wang Q, Weng L, Hauser LA, Strawser CJ, Mesaros C, Lynch DR, Blair IA. Characterization of a new N-terminally acetylated extra-mitochondrial isoform of frataxin in human erythrocytes. Sci Rep. 2018 Nov 19;8(1):17043.

40. Wang Q, Guo L, Strawser CJ, Hauser LA, Hwang WT, Snyder NW, Lynch DR, Mesaros C, Blair IA. Low apolipoprotein A-I levels in Friedreich's ataxia and in frataxin-deficient cells: Implications for therapy. PLoS One. 2018 Feb 15;13(2):e0192779

41. La Rosa P, Petrillo S, Turchi R, Berardinelli F, Schirinzi T, Vasco G, Lettieri-Barbato D, Fiorenza MT, Bertini ES, Aquilano K, Piemonte F. The Nrf2 induction prevents ferroptosis in Friedreich's Ataxia. Redox Biol. 2021 Jan;38:101791.

42. Piguet F, de Montigny C, Vaucamps N, Reutenauer L, Eisenmann A, Puccio H. Rapid and Complete Reversal of Sensory Ataxia by Gene Therapy in a Novel Model of Friedreich Ataxia. Mol Ther. 2018 Aug 1;26(8):1940-1952.

43. Perdomini M, Belbellaa B, Monassier L, Reutenauer L, Messaddeq N, Cartier N, Crystal RG, Aubourg P, Puccio H. Prevention and reversal of severe mitochondrial cardiomyopathy by gene therapy in a mouse model of Friedreich's ataxia. Nat Med. 2014 May;20(5):542-7.

44. Oeltzschner G, Zöllner HJ, Hui SC, Mikkelsen M, Saleh MG, Tapper S, Edden RA. Osprey: Open-Source Processing, Reconstruction & Estimation of Magnetic Resonance Spectroscopy Data. Journal of Neuroscience Methods. 2020;343.

45. Saleh MG, Papantoni A, Mikkelsen M, Hui S, Oeltzschner G, Puts NA, et al. Effect of Age on GABA+ and Glutathione in a Pediatric Sample. American Journal of Neuroradiology. 2020;41:1099-104.

46. Muhammad G. Saleh, Georg Oeltzschner, Kimberly L. Chan, Nicolaas A.J. Puts, Mark Mikkelsen, Michael Schär, Ashley D. Harris, Richard A.E. Edden, Simultaneous edited MRS of GABA and glutathione, NeuroImage, Volume 142, 2016, Pages 576-582, ISSN 1053-8119, https://doi.org/10.1016/j.neuroimage.2016.07.056.

(https://www.sciencedirect.com/science/article/pii/S1053811916303706)