1. Abstract

Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

There is a recognized need in the muscular dystrophy community for novel biomarkers of muscle health and disease. The submitted IRB protocol will utilize magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) to develop non-invasive, quantitative imaging biomarkers for facioscapulohumeral muscular dystrophy (FSHD), a genetic disorder that causes progressive skeletal muscle atrophy. The primary objective of this protocol will be to perform muscle MRI and MRS on groups of subjects with FSHD and control subjects. The imaging protocols employed in this study will be developed in a collaborative effort between researchers at the Center for Genetic Muscle Disorders at the Kennedy Krieger Institute and the Department of Radiology and Radiological Sciences at Johns Hopkins Hospital. Imaging and spectroscopic measurements will be correlated with muscle strength testing measurements to identify biomarkers that accurately reflect disease severity. In order to confirm that these biomarkers are specific for FSHD, both healthy and diseased controls will be enrolled. The successful characterization of imaging and spectroscopy biomarkers in FSHD will provide a powerful, non-invasive tool for future therapeutic trials in FSHD.

2. Objectives (include all primary and secondary objectives)

1) To establish quantitative magnetic resonance imaging (MRI) and proton spectroscopy (MRS) patterns in affected and unaffected muscles of patients with FSHD.
2) To compare MRI/MRS profiles of subjects with FSHD to groups of healthy and diseased controls in order to identify imaging profiles that are unique to FSHD.
3) To perform correlation analysis between MRI/MRS measurements and strength testing in subjects with FSHD to identify potential disease biomarkers.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

There is an identified need in muscle disease research for biomarkers and surrogate outcome measures of disease severity. Multiple investigators have identified the advantages of developing magnetic resonance imaging (MRI) as a biomarker for muscle disease. Inflammation, fatty infiltration, and fibrosis are common findings in muscular dystrophy, and MRI distinguishes these
Several studies have used MRI to qualitatively characterize muscle disease severity. A recent trial of the myostatin inhibitor MYO-029 conducted at our institution included volumetric analysis of muscle in subjects with multiple types of muscular dystrophy using MRI. While these measurements are clearly related to the longitudinal progression of disease, volumetric analysis may not sufficiently reflect the metabolic changes that occur in diseased muscle. Animal models of muscular dystrophy demonstrate a complex pattern of atrophy and compensatory hypertrophy in different muscle groups that may confound volumetric measures alone.

Magnetic resonance spectroscopy (MRS) is an imaging technique that also shows promise in the characterization and quantification of muscle disease. Studies in both mice and humans have identified metabolic profiles on spectroscopy that are indicative of muscle degeneration and regeneration in DMD. In 2009, Kan et al. imaged the lower extremities of 8 patients with FSHD and were able to differentiate between affected and unaffected muscles using MRS, a finding which further supports the use of MRS in the monitoring of disease progression.

In recent years, investigators at Johns Hopkins Hospital have developed techniques for performing magnetic resonance spectroscopy of skeletal muscle using a 3T MR scanner. The improved signal to noise ratio at 3T (compared to the standard 1.5T magnet) provides for greater spatial resolution and more accurate discernment of subtle metabolite concentration differences. These protocols have been used to differentiate benign and malignant musculoskeletal lesions and to characterize metabolic changes in muscles in subjects with inflammatory myopathies. We will therefore use MRS to evaluate FSHD with the working hypothesis that affected muscles in subjects with FSHD will have a unique metabolic profile on proton spectroscopy that can serve as an indicator of muscle degeneration.

Four FSHD subjects participating in a separate IRB-approved protocol (NA_00019985) were imaged using MRI and MRS. No adverse events occurred in the context of the imaging protocol.

4. Study Procedures
   a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

   Recruitment: Subjects will be recruited from the patient population at the Kennedy Krieger Outpatient clinic, the Johns Hopkins Muscular Dystrophy Association clinic and via IRB-approved advertisements with the FSH Society and National Registry for FSHD. FSHD patients with pre-existing confirmatory genetic testing and unaffected family members will be asked to participate and give informed consent. We aim to recruit 30 subjects with FSHD for this initial study with an equal number of unaffected controls. Within the control group, we plan to include 20 healthy volunteers with no evidence of neuromuscular disease and 10 diseased controls. The diseased controls will include patients with other muscle diseases (such as Becker, myotonic dystrophy, or inflammatory myositis) and muscle weakness in the extremities comparable to those subjects with FSHD. The inclusion and exclusion criteria for the diseased controls will be...
the same as the healthy controls. Assuming a 10% screen failure rate, we plan to screen 66 potential subjects in anticipation of screen failures.

Study procedure: Study subjects who contact the study team will undergo a telephone screening to determine eligibility for the study. The telephone screening will inquire about the potential participant’s diagnosis, assess ability and willingness of complete all elements of the study, and screen for contraindications to MRI (as defined by the screening form used by the Radiology Department at Johns Hopkins Hospital). Study subjects who are recruited through the Kennedy Krieger Institute will undergo the same screening in person after their routine clinic visits. If subjects have had genetic testing for FSHD in the past, they will be asked to provide copies of these testing results to confirm their diagnosis. If they do not have copies of these results, we will ask them to sign a release of information form so that these results can be obtained from their physicians.

Subjects that meet all screening criteria for this study will be asked come to the Kennedy Krieger Outpatient Clinic for an outpatient visit. This visit will include informed consent, a general history and physical examination, manual muscle strength testing (MMT), quantitative muscle strength testing (with dynamometry), timed function testing (such as time to climb 4 steps, timed to stand, time to walk 30 feet, and 6-minute walk testing), and an outpatient MRI scan at Johns Hopkins Hospital or the Kennedy Krieger Institute. If a subject has been screened over the telephone, we can arrange for all of these elements to be completed in a single outpatient visit or be divided over 2 visits at the participant’s request. If a subject is recruited and screened through clinic, he or she will be asked to return for their MRI scan at a later date. This scan will include MRI studies of the upper and lower extremities and MR spectroscopy of different muscle groups in the upper and lower extremities using a 3T MRI scanner. Each scan will take approximately 2 hours and will be supervised by members of the research team and radiologic technicians from the Division of MR Research. Although it is anticipated that some research participants will undergo screening and imaging on the same day, it is expected that their participation will be divided into 2 separate outpatient visits. Participation in the study will not require interruption or discontinuation of the participant’s ongoing therapy or routine medical care.

The MRI protocol will include sequences that are routinely performed in musculoskeletal imaging studies (such as T1-weighted, T2 weighted, short T1 inversion recovery, and diffusion-weighted imaging sequences) as well as additional MR sequences that will be evaluated as potential imaging biomarkers. Muscle fat content will be determined using the Dixon technique that has been validated in DMD. Transverse relaxation times (T2) of muscle tissue will be calculated using a monoexponential decay model. The anatomic imaging sequences will be used to select regions for proton MR spectroscopy (Figure 1). While single-voxel MRS was used to obtain this preliminary data, multi-voxel studies (MRSI), will also be utilized to obtain data over larger regions of muscle.

In order to demonstrate the reproducibility of the MRI/MRS procedure over time, we will invite 5 subjects with FSHD and 5 control subjects to return for a second MRI/MRS scan. The physical examination, timed function testing, and dynamometry will be repeated to ensure that
these measurements are unchanged, and an identical imaging protocol will be performed and compared to the study obtained during the first visit.

As recent data has suggested that ultra-high field MRI and MRS can improve the accuracy of metabolite measurement in skeletal muscle, 20 selected study participants (15 affected subjects with FSHD, 5 unaffected controls) will also be scanned using the 7T MRI scanner at the F.M. Kirby Research Center for Functional Brain Imaging. These participants will be selected from the initial pool of 60 cases and controls based on their strength testing measurements. We will specifically select subjects with differing degrees of muscle strength in order to examine as wide a spectrum of diseases severity as possible. The 7T imaging protocol will include MRS and conventional T1 and T2 anatomic MRI sequences, but not imaging for muscle fat content measurement or T2 relaxation times. The 7T scan is also expected to take approximately 2 hours to allow extra time for positioning and adjustment of imaging parameters (such as shimming). The subjects who are selected for and agree to do the 7T imaging protocol will return on a separate date for this part of the protocol.

b. Study duration and number of study visits required of research participants.

Each research participant that meets the screening requirements will undergo at least 1 study visit. This will include the informed consent, history, physical examination, dynamometry measurements, timed function tests, and MRI scan. This visit is expected to take 3 hours (1 hour for consent, interview, examination, dynamometry, and timed function testing; 2 hours for the MRI scan). If requested by the participant, these elements can be divided over 2 visits.

Participation in the reproducibility study will require 1 additional outpatient visit which will take approximately 2.5 hours.

Participation in the 7T MRI scan will require 1 additional outpatient visit which will take approximately 2 hours.

The duration of the study is expected to be approximately 2 years.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

The research team members who conduct the screening examinations for enrollment will not be blinded to the subject’s diagnosis, as participation in the study requires that the research team members confirm the genetic diagnosis of FSHD. The post-processing and interpretation of the MR data will be performed by members of the Radiology Department at Johns Hopkins Hospital who will be blinded to the subject’s diagnosis.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

Participation in the study will have no effect on routine care and will not require any current therapy to be stopped.

e. Justification for inclusion of a placebo or non-treatment group.
f. Definition of treatment failure or participant removal criteria.

There is no treatment being offered in this study. Participants may be removed from the study if they are unable to cooperate with the physical examination, dynamometry measurements, or the MRI scan.

g. Description of what happens to participants receiving therapy when study ends or if a participant’s participation in the study ends prematurely.

Participants in this study will not receive therapy.

5. Inclusion/Exclusion Criteria

For subjects with FSHD:

Inclusion criteria:
1. Previous genetic testing that was positive for the mutation that causes FSHD
2. Ability to give informed consent
3. Ability to physically perform strength testing, timed function testing, and dynamometry in the upper and lower extremities.

Exclusion criteria:
1. Contraindication to magnetic resonance imaging
2. Inability to complete strength testing, timed function testing, and dynamometry due to contractures or limb amputation.

For healthy control subjects:

Inclusion criteria:
1. Previous genetic testing that was negative for the mutation that causes FSHD or willingness to undergo FSHD genetic testing
2. Ability to give informed consent
3. Ability to physically perform strength testing, timed function testing, and dynamometry in the upper and lower extremities

Exclusion criteria:
1. Contraindications to magnetic resonance imaging
2. Inability to complete strength testing, timed function testing, and dynamometry due to contractures or limb amputation.

For diseased control subjects:

Inclusion criteria:
1. Diagnosis of a disease that causes muscle wasting. This would include:
   a. Genetic diagnosis of Becker muscular dystrophy, myotonic muscular dystrophy, or limb-girdle muscular dystrophy.
   b. Muscle biopsy diagnosis of polymyositis, dermatomyositis, or inclusion body myositis
2. Previous genetic testing that was negative for the mutation that causes FSHD or willingness to undergo FSHD genetic testing
3. Ability to give informed consent
4. Ability to physically perform strength testing, timed function testing, and dynamometry in the upper and lower extremities

Exclusion criteria:
1. Contraindications to magnetic resonance imaging
2. Inability to complete strength testing, timed function testing, and dynamometry due to contractures or limb amputation.

6. **Drugs/Substances/Devices**

Not applicable to this proposal.

7. **Study Statistics**

a. Primary outcome variable.

The major metabolites that are typically seen in proton spectra of muscle include creatine, intramyocellular lipids, extramyocellular lipids, and choline-containing molecules, and these will be the focus of our initial analysis. Metabolite levels will be normalized to water and calculated using an area-under-the-curve analysis. These metabolite levels will be correlated to clinical strength measurements in the respective muscles.

b. Secondary outcome variables.

None

c. Statistical plan including sample size justification and interim data analysis.

Our initial goal will be to include 30 subjects with FSHD and 30 control subjects. As there are no prior studies utilizing this protocol in the FSHD population, estimates of the required sample size were based on 3 data sources: in vitro proton spectroscopy data for choline in skeletal muscle of patients with limb-girdle muscular dystrophy, nuclear magnetic resonance spectroscopy data obtained from mouse models of muscular dystrophy (mdx), and $^{31}$P spectroscopy data for skeletal muscle in patients with FSHD. Correlation analyses between quantitative imaging/spectroscopy parameters and muscle strength testing (MMT and QMT) will be performed to determine their potential efficacy as biomarkers.

d. Early stopping rules.

Study participants can end their participation in the study protocol at any time and members of the study team may terminate a participant’s involvement if they find that there are contraindications to MRI or if the subject experiences discomfort from the MRI scan, such as severe claustrophobia or excessive heating of tattoos.

8. **Risks**

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

Magnetic resonance spectroscopy and magnetic resonance imaging without contrast are considered to be a minimal risk procedures at the magnetic fields described in this protocol. Subjects that are
susceptible to injury from high-power magnetic fields will be identified using the Johns Hopkins Radiographic Sciences Department screening form prior to imaging. Study team members who will be in the control room or scanning room of the research MRI suite are required to undergo MRI safety training provided by the Radiographic Sciences Department. There are no known effects of MR on pregnancy, but there may be unknown effects. It is also commonly observed that women with FSHD can experience a decline in strength during pregnancy which cannot be accounted for by our current understanding of the pathophysiologic mechanisms of disease. Therefore, pregnant subjects will not be included in the study.

Magnetic resonance spectroscopy and imaging at 7 Tesla is also considered to be a minimal risk procedure. However, if subjects move rapidly within or around a magnet with a field of 7 Tesla, they may feel dizzy. We therefore will advise them to move slowly around the magnet. Once they are positioned on the table, we will move the person into the magnet slowly.

The physical examination, dynamometry, and timed function testing are both commonly done during routine neurology clinic visits. The risks of the physical examination are minimal, but may include some muscle fatigue, particularly in subjects who have FSHD.

The genetic testing for FSHD will require a single 10ml blood draw that will take place at the time of screening. The risks of a routine peripheral venous blood draw are minimal, but may include pain, bruising, bleeding, fainting, and infection.

While the subjects who have FSHD will know their diagnosis before their enrollment, there is a small chance that a participant that is enrolled as a normal control will have a positive gene test for FSHD. The prevalence of FSHD in the general population is estimated to be 7 per 100,000, and in an asymptomatic participant, the chance of a positive genetic test is likely lower. A positive test result may result in psychosocial stress for the participant and his or her family.

b. Steps taken to minimize the risks.

Subjects may experience discomfort from noise in the scanner. This will be minimized with the use of headphones or earplugs. Subjects may also experience physical discomfort from positioning in the scanner, which will be minimized with cushioning and repositioning maneuvers. Subjects may also develop claustrophobia in the scanner. The study can be aborted at any time if this develops.

When performing MRI and MRS studies using the 7 Tesla MRI scanner, we will advise participants to move slowly to minimize the chance of developing dizziness. We will also move the participant slowly into the magnet once they are positioned on the MRI table.

Blood draws for the genetic testing will be performed by trained nurses or phlebotomists at the Kennedy Krieger Outpatient Clinic in order to minimize the risks associated with blood draws.

If a control subject’s genetic test is positive for the mutation that causes FSHD, the subject will be directly and confidentially informed of this result by members of the research team and genetic counseling will be made available to them.

c. Plan for reporting unanticipated problems or study deviations.
Any complications from MRI/MRS scanning will be reported to the IRB.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

Risks associated with breach of confidentiality will be minimized by de-identifying personal identifiers when imaging data is analyzed. The imaging data itself will be stored in secure data-storage facilities in the radiologic department at Johns Hopkins Hospital. Codes will be kept in a password protected computer at the Kennedy Krieger Institute that will only be accessible to the PI.

e. Financial risks to the participants.

None. All costs of participation will be covered by the study.

9. Benefits
a. Description of the probable benefits for the participant and for society.

There are no direct benefits to the participants of this study. This study is designed to develop research biomarkers which will be used in future clinical trials for the benefit of the muscular dystrophy community.

10. Payment and Remuneration
a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

None

11. Costs
a. Detail costs of study procedure(s) or drug(s) or substance(s) to participants and identify who will pay for them.

The costs of the study include personnel costs and the costs of scanner use. These costs will be covered by grants and donations to the Center for Genetic Muscle Disorders at the Kennedy Krieger Institute. Imaging procedures that are done at Johns Hopkins Hospital will be covered by a subcontract with the Kennedy Krieger Institute. As the majority of participants in this study are expected to be local to the Baltimore area, travel expenses will not be covered.