Date: _July 1, 2013_____
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Johns Hopkins Medicine - eForm A

1. Abstract

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

X-linked adrenoleukodystrophy (X-ALD), a progressive neurodegenerative disease, is caused by a defect in the ABCD1 gene. The disease has multiple phenotypes, but the most common adult form is adrenomyeloneuropathy (AMN), which presents as a slowly progressing spastic paraparesis with sensory and autonomic dysfunction. In a previous study we linked corticospinal tract (CST) and Dorsal Column (DC) abnormalities to lower extremity sensory loss and weakness in men with AMN; however, there have been no studies evaluating these relationships in woman heterozygote carriers (i.e., women with AMN). It is unknown, in women with AMN, how the pattern of damage in the brain and spinal cord relates to disability and if these patterns predict responsiveness to treatment. While conventional magnetic resonance imaging (MRI) techniques have been used to evaluate progression of X-ALD disease burden, attempts at correlating clinical disability with axonal injury have had limited success due to clinical rating scales that do not adequately measure all aspects of the disease, coupled with an inability for routine imaging techniques to quantify relevant fiber tracts in the brain and spinal cord. We propose to comprehensively evaluate the CST and DC across the brain and cervical spinal cord with the purpose of understanding mechanisms of disability in women with AMN. We hypothesize that: 1) tract-specific magnetization transfer imaging (MTI) and diffusion tensor imaging (DTI) will predict specific sensory and motor impairments (i.e., sensory loss and weakness) and disability; and 2) tract-specific MTI and DTI will predict who is likely to respond best to progressive resistive training (PRT). For this project we are using a rigorous biologic approach that combines clinical evaluation, neuroimaging, and measures of impairment and disability to provide a more precise prognosis in terms of physical disability and to predict who is likely to respond to a physical intervention. We predict that many of the women will improve their strength and function following the PRT program. Specifically, we predict that axonal loss will be associated with irreversible disability. By contrast, demyelination may have potentially reversible injuries that would benefit from PRT in improving disability. The linking of this information will not only be important for better defining disability in women heterozygote carriers of AMN but it will also help to guide physicians and rehabilitation therapists in predicting who is likely to respond to rehabilitative interventions, as well as for optimizing outcomes for future neuroprotective pharmacological interventions.

2. Objectives (include all primary and secondary objectives)

Primary objectives:

1) To determine to what extent pathological abnormalities (i.e., MT measures of demyelination, and DTI measures of axonal integrity) are associated with sensory and motor impairments and locomotor patterns (i.e., kinematic walking measures and quantitative motor impairments) in women with AMN.
2) To determine the extent to which tract specific anatomy (demyelination, axonal loss) predict who will respond best to three months of progressive resistance training (PRT) in female carriers.

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)

Adrenomyeloneuropathy (AMN) is the adult phenotype of X-linked adrenoleukodystrophy (X-ALD), a progressive disorder that affects the nervous system, adrenal cortex and testes due to a defect in the gene ABCD1, which has been mapped to Xq28. Adult onset of symptoms of disease not only occurs in 60% of males with the X-ALD mutation in their most productive years of life but also 50% of female carriers, though often a decade later than that of the men. Diagnosis and treatment studies for AMN have principally focused on men despite the prevalence of symptoms in female carriers. AMN is characterized by progressive non-inflammatory axonopathy in the corticospinal (CST) and Dorsal Column-Medial Lemniscal (DC-ML) tracts of the brain and spinal cord. There is additional evidence of demyelination caused by secondary inflammatory mechanisms. AMN including women carriers have been characterized as having primarily lower extremity spasticity, with abnormalities in vibration sensation, strength, walking, and balance that progress during 5 to 20 years in a variable pattern. Our preliminary data show that women carriers can have symptoms that are as severe as the men. There are currently no medical treatment options available for women carriers; these patients have dismal hopes for improvement, except with rehabilitation. There is a glaring need for more knowledge and more effective rehabilitative treatment approaches for women ALD carriers.

Conventional magnetic resonance imaging techniques along with the Loes scale (i.e., categorical rating scale) have been used to evaluate progression of X-ALD disease burden in children with moderate success. Pathologic studies show that dying back of axonal loss is a primary pathogenic mechanism of AMN and much of the permanent disability results from axonal damage. However, attempts at correlating clinical disability with axonal injury have had limited success due to clinical rating scales that do not adequately measure all aspects of the disease, coupled with an inability for routine imaging techniques to depict specific clinically involved fiber tracts in the brain and spinal cord. Our preliminary data show that we can distinguish female carriers from control subjects using novel techniques such as magnetization transfer (MT) imaging. In addition, we have previously shown that damage measured using tract specific MRI, specifically magnetization transfer and diffusion tensor imaging (DTI) correlates with physical impairments in individuals with the degenerative disease of multiple sclerosis.

In conjunction with advanced DTI and MT metrics developed at the Kirby Imaging Center of the Kennedy Krieger Institute, we plan to capitalize on the tools within our motion laboratory, having thorough experience with them in assessing spasticity, ataxia, sensation, and muscle strength in individuals with AMN and in individuals with multiple sclerosis. We predict that MT imaging and DTI, which measure the integrity of white matter and fiber tracts in the brain and spinal cord, will aid in understanding the extent to which axonal/myelin anatomy are associated with sensory and motor impairments as well as locomotor patterns with the goal of understanding how to treat physical disability more effectively.

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).

We will test female heterozygote carriers of X-ALD and healthy age- and gender-matched controls.

Women who are presently patients of the Neurogenetics Center of the Kennedy Krieger will be informed of the study at their routine visits. This center cares for over 100 individuals who are symptomatic carriers for this condition and it is not expected that recruitment will be an issue. However, we will also post announcements on the Kennedy Krieger Neurogenetics and the United Leukodystrophy Foundation (ULF) websites.

Women who contact us will undergo a phone screening to determine eligibility. During the telephone screening they will be asked, "Are you or do you think you might be pregnant?" If they are pregnant they are not eligible for the study. They will also be asked if there are any reasons that would prevent them from participating in an exercise program. If yes, they will be determined ineligible to participate. If they are eligible we will schedule them for their initial testing session. If they are not interested in participating or found to be ineligible we will destroy any information we have collected from them.

For healthy control subjects.
We will use posted advertisements posted in the Kennedy Krieger and in the planned fitness facilities(i.e., Curves). We will also post an announcement on the webpages of the Kennedy Krieger and ULF. Interested subjects will be told to contact the research coordinator for a telephone screening. If they are found to be eligible we will schedule them for their initial testing session. If they are found to be ineligible we will destroy any information we have collected from them.

Women will be screened during a phone interview using a standardized script. Information on the woman will be collected at that time, but if found to be ineligible or she chooses not to participate this information will be destroyed.

Women who are eligible will be scheduled for a baseline assessment. Baseline assessment will consist of a standardized sensorimotor examination including a gait assessment, MRI of the brain and cervical spinal cord, and exercise instruction. Screening medical information will be reviewed at the visit, including a question about whether the subject might be pregnant. If there are questions or concerns about eligibility or the institution of an exercise program, the PI or physician examiner will make a determination of participation.

We will examine subjects at the level of functional disability (i.e. clinical rating scales), at the level of impairment (i.e., strength and sensation), and at the level of anatomy (i.e., MTI and DTI). The general paradigm includes four parts, requiring 1.5-3.5 hours for testing, depending on the number of activities done. Part 1 is the clinical neurological evaluation which includes clinical rating scales along with measures of lower extremity impairments and quality of life, requiring one hour. Part 2 is the laboratory testing of global walking requiring 30 minutes. Part 3 is evaluation and instruction for a custom exercise program, requiring one hour. Part 4 involves conventional imaging and measures of DTI and MTI in the brain and spinal cord to be completed in the F.M. Kirby Center in KKI, across the hall from the Motion Analysis

Laboratory (requiring one hour). We will allow rest breaks as needed by each individual. Parts 1, 2, and 3 will be conducted at the Motion Analysis Laboratory located in KKI. All subjects will participate in 3 testing visits, one at baseline, one at 12 weeks and one at 18 weeks from the baseline visit. All subjects will be asked to participate in parts 1-4 at the baseline visit, and only parts 1 and 2 during visits 2 and 3.

Thus, for testing the breakdown for the time is as follows:
Baseline visit: 1 hr. clinical exam, 30 min. gait test, 1 hr. exercise instruction,1 hr. MRI scan
12th week visit: 1 hr. clinical exam, 30 min. gait test
18th week visit: 1 hr. clinical exam, 30 min. gait test

All subjects will undergo a functional sensorimotor assessment at every testing session with the purpose of systematically documenting clinical status. The primary investigator or physical therapist and research coordinator will conduct this exam. The exam will include standard clinical measures of sensation (vibration, proprioception), strength, tone (Modified Ashworth), and passive range of motion. In addition, we will use standardized quantitative scales to evaluate walking, ataxia, and overall disease impairment in these individuals . Lastly, self report measures of quality of life and pain will be used.

After the functional assessment, locomotion will be evaluated. Each subject will be asked to walk several times across a 25-foot walkway to evaluate locomotion, and to stand still on a force plate to evaluate balance; all movements will be done in the Motion Analysis Laboratory at the Kennedy Krieger Institute. To track walking we will use a GaitRite System. This is a walkway that has pressure activated sensors that measure the temporal (timing) and spatial (two dimension geometric position) parameters of the foot as it lands on its pressure activated sensors. For balance, a vital component of locomotion, we will assess the extent of postural sway while standing on a static force plate. The force plate is embedded in the floor of the laboratory and is very sensitive to small amounts of movement that are often missed by observation. Force plate data will be collected at 1000HZ using a Kistler 9281 force plate (Kistler Instrument Corp., Switzerland). During these tasks we may also evaluate muscle activity using electrodes to record surface electromyography (EMG) signals. Markers will be pasted on the surface of the skin over relevant muscle groups. Data from the EMG markers will be collected at 1000Hz and time locked using Optotrak software. During all movements an examiner will guard each subject and rest breaks will be given as needed.

To begin the exercise intervention a physical or occupational therapist will evaluate their strength, flexibility, and level of activity. Then subjects will be given an exercise program to follow at a designated fitness facility. The physical or occupational therapist will teach the subject how to do each exercise and tell them where they will be done. The progressive resistance training program will consist of 12 weeks of guided resistance training 3 times weekly. Subjects will be asked to do one day of resistance training at their home weekly. A member of the research team (i.e., a physical or occupational therapist or research coordinator) will contact each subject by phone or email several times over the first 12 weeks of the study to answer any questions and encourage subjects to continue with their exercise program. Compliance will be tracked by the facility (i.e., Curves). They will send the PI monthly reports stating the number of times each participant has used the facility. Subjects will also be given an

exercise log to complete. If participants miss a training session a substitute session will be arranged on an alternate day.

Subjects will also go through Magnetic Resonance Imaging (MRI) in the F.M. Kirby Center at the Kennedy Krieger Institute. Prior to the start of imaging each subject will be asked to complete a standard questionnaire, this will ensure that the subject is able to safely enter the area where the MRI scan will take place. Subjects will undergo imaging without contrast and without sedation. For this study we will use a 3T Philips GyroscanNT (Best, The Netherlands) system to obtain high-resolution anatomical brain and spinal cord images. In the brain and spinal cord, diffusion tensor imaging (DTI) will be performed as well as magnetization transfer (MT). Conventional brain and spinal cord (STIR, T1w, T2w) sagittal and axial images may also be acquired. The total scanning time will be approximately 1 hour.

Analysis of the DTI images will be done using custom written software (DTI-Studio) or custom Matlab programs to calculate the diffusion tensor variables at each pixel and create 2D color maps of white matter pathways. Analysis of MT will be calculated on a voxel by voxel basis by the following equation: $MTR=1-S/S_0$ where S and S_0 are the images acquired with and without MT prepulse, respectively.

The measurements made with these activities are for research purposes only and at this time will have limited direct clinical utility, but results can be given to the subject or their physician. If the subject would like us to provide results they will be instructed to let us know during their testing, or they can ask us over the telephone after their visit and we will send the information to them.

Incidental findings on MRI will be provided to the patient by Dr. Raymond by either phone or if necessary in a clinical visit. The individual will be assisted in obtaining follow up care.

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

Each testing session will require about 1.5 hours of time, with one session requiring 3.5 hours of time due to a 1-hour MRI scan and an hour for exercise instruction. All subjects will be tested a total of three times: baseline, 12 weeks, and 18 weeks after baseline. Based on the results of this study we will ask subjects if they would be willing to come back for future visits, this is not mandatory for participation.

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

All subjects in the study will take part in the exercise intervention. We are unable to blind the subjects or testers to the intervention which has been discussed in other similar studies as a limitation. However, we have specified guidelines that the PT will follow for progression of the exercise program to alleviate bias as much as possible.

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

N/A

e. Justification for inclusion of a placebo or non-treatment group.

N/A

f. Definition of treatment failure or participant removal criteria.

Patients selected for the study will be removed if they: 1) inform us that they become pregnant during the study, 2) are unable to follow the requirements of the study, or 3) request to be removed.

We are not aware of a particular pregnancy risk, but the inclusion of a subset who are pregnant would be difficult to interpret compared to non-pregnant participants.

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

The subject's medical care will continue unaltered when the study ends or if a subject is removed from the study.

5. Inclusion/Exclusion Criteria

Inclusion criteria for women with X-ALD

- confirmed diagnosis, X-ALD heterozygote carrier
- no medical contraindication to participating in a strength training program
- able to follow complex directions as determined by a score of ≤1 on a subset of questions taken from the NIH Stroke scale (Brott et al. 1989)
- hip flexion strength: 6.6-15.8kg or hip extension strength: ≤18.3kg
- normal passive range of motion at hips/knees/ankles
- scale of 1-6 on the EDSS
- able to walk ≥50m
- age 21-65

Exclusion criteria

- Evidence of other neurological deficit that could interfere, such as previous stroke or muscle disease
- congestive heart failure
- cancer
- orthopedic conditions
- severe pain that precludes study participation
- seizures
- pregnancy
- other medical condition that precludes participation in an exercise program, e.g., unstable angina, uncontrolled diabetes, uncontrolled hypertension

Healthy controls have the same exclusion criteria as women with AMN with 2 exceptions, (1) they can be age 21-70 years, and (2) they will not be carriers for X-ALD. They must have normal neurological function.

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

N/A

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

N/A

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

N/A

Study Statistics

a. Primary outcome variable.

Primary outcome variables for sensorimotor impairments include: vibration sensation, strength (average values from the hip and ankle muscles, both flexion and extension, will be used), and spasticity from the ankle flexor/extensor muscles bilaterally.

The primary variables of interest for white matter integrity in this analysis will be fractional anisotropy (FA) and magnetization transfer ratio (MTR).

Other primary outcome variables include correlations to determine relationships between sensorimotor impairments and between white matter integrity variables in the tracts of interest. Multiple regression analysis we will also be used determine to what extent sensorimotor impairments are related to white matter integrity variables.

The primary outcome variable for the exercise intervention we will calculate a functional composite score (FS) for each person that will incorporate 4 tests that functionally challenge the lower extremity: 1) sit to stand, 2) Up and Go test, 3) 25-foot timed walk, and 4) 6-minute walk test.

b. Secondary outcome variables.

To determine response to three months of the exercise intervention:
1. We will calculate pre- to post- trial differences between groups using an unpaired t test. Changes between pre- and post-trial within groups will also be compared with paired t tests.

2. To determine change in hip flexion, FS from post- to follow up session within groups will be compared with paired t tests. [($Exercise_{followup}$-$Exercise_{post}$) vs ($Exercise_{post}$-$Exercise_{pre}$)], will be evaluated.
3. To evaluate possible time and group interactions during the intervention within groups we will use multivariate ANOVA's. Significant interactions will be evaluated with a mixed effects linear regression.
4. To determine change in EDSS scores over each session we will use with Wilcoxon signed rank test.
5. To determine whether specific changes in the primary outcome measures are predicted by the changes in tract specific demyelination and axonal loss (i.e., MTR and FA) we will determine how much each impairment changed from the pre- to post- session.
6. We will conduct separate correlations to determine the relationship between the primary outcomes, and extent of demyelination and axonal loss pre- and post-intervention.
7. Multiple regression analyses will also be used to determine which pattern of white matter damage (i.e., demyelination, axonal loss) best predicts hip flexion and FS.

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

Preliminary studies of heterozygote data from our laboratory suggest that a minimum of 12 subjects are needed to obtain adequate statistical power. Hip flexion strength data from female carriers are: 11.2 ± 4.6 kg. Using these values, differences between groups on the order of one SD will be statistically significant with a power of 0.8 given a sample size of 12 subjects. To account for attrition, we have added 25% to this total. Therefore, our initial plan is to have 15 female carriers for this project and 15 healthy controls. All power calculations assume two-tailed tests with $\alpha = 0.05$. Analysis will be done using Statistica software (StatSoft Inc., Tulsa OK) or Stata (StataCorp).

d. Early stopping rules.

None.

7. Risks

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

The potential risks in this study are very low. The study is entirely non-invasive. Subjects may become fatigued or lose their balance during walking or standing and they may have slight muscle soreness, this has occurred very rarely in past studies. There is a potential that subjects may have an allergy/sensitivity to the tape used for marker placement, in this situation we will use a stocking net that we have in the lab to hold the marker in place instead of tape. Subjects may become uncomfortable while in the MRI scanner. Any incidental findings on MRI will be provided to the patient by Dr. Raymond by phone or if necessary in a clinical visit. If there are new findings the individual will be assisted in obtaining follow up care. There is a potential risk of time commitment (missing work or school) and therefore we will be flexible and practical in scheduling convenient appointments.

b. Steps taken to minimize the risks.

All subjects will complete the exercise intervention with supervision. A licensed physical or Occupational therapist will progress the exercise intervention for each individual based on their capabilities. Subjects will be given rests whenever needed or requested.

During the walking and balance tests we will place a gait belt on all subjects and 1-2 investigators will walk behind them in case they lose their balance. We will also give a rest break when subjects feel fatigued, short of breath, or simply want a break. They will be allowed to stop any test at any time or rest between tests. In previous studies there has been no incident that would suggest any significant potential risk.

The effects of magnetic fields in an MR scanner have been extensively studied, and there are no known significant risks with an MR exam. The subject may be bothered by feelings of confinement (claustrophobia), and by the noise made by the magnet during the procedure. To address the discomfort due to the loud noises we will provide earplugs for the subject to wear while he/she is in the magnet. The subject may not participate in this study if he/she has a pacemaker, an implanted defibrillator or certain other implanted electronic or metallic devices. To assure that subjects are safe to enter the MR area we will ask a series of standard questions to determine whether any of the subjects in this study have had brain surgery for a cerebral aneurysm or have implanted medical or metallic devices, shrapnel, or other metal.

c. Plan for reporting unanticipated problems or study deviations.

In the case of an adverse event the PI will assure first and foremost that the subject is safe. The PI will also report the event to the JHM IRB, the KKI Office of Research Compliance, and to the European Leukodystrophy Foundation.

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

There are minimal legal risks associated with this study as it is a minimal-risk study and all appropriate procedures will be followed to protect confidentiality.

e. Financial risks to the participants.

None.

8. Benefits

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

There are no individual medical benefits for participating in this study. Participants may improve their strength and functional status through exercise training. We will verbally review the MRI scan and results from the clinical evaluation with each participant. This information may be helpful in guiding subjects toward further rehabilitation steps.

In a previous study we linked corticospinal tract abnormalities to lower extremity weakness in men with AMN; there have been no studies evaluating these relationships in female carriers (i.e., women with AMN). It is unknown, in women with AMN, how the pattern of damage in the brain and spinal cord relates to disability and if these patterns predict responsiveness to

treatment. Our comprehensive methodology and careful quantification of sensory and motor impairments and walking may be a more sensitive way to quantify subtle changes in disability, and may be more closely related to the function of the corticospinal tract. Our results, therefore, have the potential to support new clinical markers that more closely follow changes in physical function in AMN. We are also directly testing a treatment intervention that will provide scientific rationale for predicting the best responders to a progressive resistance training program. We will evaluate not only the improvement in physical function but also the extent that myelin and axon structure and integrity can predict who best responds to the intervention. Overall, the linking of clinical impairments and disabilities with anatomic MRI measures will not only be important for better defining disability in female carriers but it will also help to guide physicians and rehabilitation therapists in predicting who is likely to respond to rehabilitative interventions, as well as for optimizing the effects of future neuroprotective pharmacological interventions.

9. 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.

All subjects will be compensated $25 for participating in the laboratory sessions. Subjects will be paid in cash upon completion of the study. If subjects leave the study before it is completed, they will not receive payment.

10. Costs

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

The subjects will not be charged for any of the procedures in the study. They will only be responsible for the cost of any travel to and from the Institute as well as any meals they eat while participating. The costs of parking at the Institute's garage will be covered. All subjects will be provided $150 for the costs of their participation in the exercise program. MRI scan fees for women carriers will be covered by a research grant from the European Leukodystrophy Association. Requests for further financial support are underway through the NBRU, and NIH mechanisms. Funding for up to four individuals is also available to help offset the financial burdens of travel costs.