Study Protocol

Funding Opportunity: PAR-16-020 Clinical Trial Readiness for Rare Neurological and Neuromuscular Diseases (READISCA)

Protocol Title: Clinical Trial Readiness for SCA1 and SCA3

Short Study Title: READISCA

Amendment 06

Date: May 23, 2021

Approved:

By: _____

Tetsuo Ashizawa, MD

Chair READISCA Steering Committee

Date: _____

By: _____

Henry L. Paulson, MD, PhD

Co-Chair READISCA Steering Committee

Date: _____

INVESTIGATOR AGREEMENT

I confirm that I have read and that I understand this protocol, and other study information provided by the READISCA Steering Committee. I agree to conduct this study in accordance with the requirements of this protocol and also protect the rights, safety, privacy, and well-being of study subjects in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.
- International Conference on Harmonization Harmonized Tripartite Guideline for Good Clinical Practice E6.
- All applicable laws and regulations, including, without limitation, data privacy laws and regulations.
- Regulatory requirements for reporting serious adverse events defined in this protocol.
- Terms outlined in the Consortium Agreement.

Signature of Investigator Date

Investigator Name (print or type)

Investigator's Title

Name of Facility

REASON FOR SUBSTANTIAL AMENDMENT 01, 02, 03, 04 and 05

This amended study protocol is prepared to remove some outcome measures in order to reduce the patient burden and focus on core clinical outcome assessments. Non-dominant hand 9HPT, non-dominant hand Click test, BARS, MOCA, CNRS, Physician Global Impression, Fall Questionnaire, UHDRS IV, Neuro-QOL, Beck Depression Inventory, Beck Anxiety Scale and Falls Diary were removed. Furthermore, the cut-off SARA score for premanifest and at-risk subjects was increased by 0.5 points to 2.5. Early stage subjects must now have a total SARA score of ≤ 9.5. SARA scoring was changed to accommodate potential decimal scores on items 5, 6, 7 and 8.

Additionally, some text passages were clarified, and errors were corrected in the study protocol.

- Blood samples for RNA will no longer be collected
- Blood samples for DNA genotyping will be collected at baseline or screening visit. Additional sample will be collected at the time of Lumbar Puncture if CSF is obtained. Additional sample will be collected for 50%-at-risk for confirmatory testing.

Detailed changes are summarized in the following table:

Section	Page	Old text	New text
Heading	All	Amendment 01, Version 1.3 Amendment 02, Version 1.4 Amendment 03 (removed version) Amendment 04 Amendment 05	Amendment 06
	All	Amendment 01: 23/Mar/2018 Amendment 02: 28/June/2018 Amendment 03: 22/May/2019 Date: March 17, 2020 Date: August 5, 2020	Date: May 23, 2021
	1	Amendment 01: Updated to include approval page	
Investigator agreement	2	Amendment 01: <<New section>>	
Reason for substantial amendment	3	Amendment 01: New section with details of amendment 01 Amendment 02: Details of amendment 02 Amendment 03: Revised age of inclusion criteria for premanifest and 50%-at-risk participants Added two additional US study sites Revised to include details of the telephone consenting process Added additional blood sample for confirmatory testing of 50%-at-risk participants Removed 18-month MR timepoint Added potential blood sample collection at screening visit Amendment 04: Added use of fluoroscopic guidance for lumbar puncture if needed Amendment 05: Added COVID-19 Screening and Questionnaire. Added potential remote evaluation Added plans for out-of-window study visits	
Summary	7	Amendment 01:	

		<<New section with protocol summary page>> Amendment 03: Subjects of either sex, aged 18 to 65 with presence of symptomatic ataxic disease with definite molecular diagnosis of SCA1 or SCA3 in the subject or first degree relative or Asymptomatic subjects of either sex aged 27 to 50 whose first-degree relative has a molecular diagnosis of SCA1 or SCA3 or Amendment 05: Appr. 22 sites globally	
Background and study rationale	8	Amendment 02: <<New section, changed to Advarra IRB>>	
US sites	9	Amendment 01: Added: Veronica Santini, MD and Trevor Hawkins, MD Removed: Drew Scott Kern, MD Amendment 02: Removed: Baylor College of Medicine – Paolo Moretti, MD, University of Texas Southwestern (UTSW) – Pravin Khemani, MD Added: Laura Scorr, MD Amendment 03: Added: Barrow Neurological Institute – Terry Fife, MD, Jacinda Sampson, MD and University of Pennsylvania – Pedro Gonzalez-Alegré, MD, PhD Amendment 04: Added: Ohio State University - Yasushi Kisanuki, MD, FAAN and Sandra Kostyk, MD, University of Iowa - Peggy C. Nopoulos, MD and Annie Killoran, MD, MSc Amendment 05: Removed: Barrow Neurological Institute – Terry Fife, MD Added: Peter Morrison, DO	Amendment 05: Removed Lauren Seeberger, MD
Study Population	10	Amendment 01: Early-stage patients (3-9.5), Premanifest carriers (0-2.5), 50%-at-risk subjects (0-2.5)	
Recruitment of prospective study subjects in the current study	10	Amendment 01: Subjects from remote locations will complete telephone consent process per GCP guidelines and HIPAA regulations. After which subject information will be forwarded to HMRICC to complete eligibility evaluations Amendment 02: Replaced Houston Methodist Institute Coordinating Center (HMRICC) Amendment 03: Added CoRDS and ClinicalTrials.gov	
Inclusion Criteria Table	10	Amendment 03: Premanifest carriers: Age (years) 27-65 50%-at-risk subjects: Age (years) 27-50	
Telephone consents	11	Amendment 01:	

		<<New section>> Amendment 03: Whenever possible, the informed consent process will be done in person. However, when the consent process cannot be reasonably conducted in person to support research related activities or preparatory work (as in the case of remote patients) a consent via telephone or comparable media may be deemed appropriate. The consent process will be documented, and accompanying signature pages will be sent via facsimile, email or certified mail as defined by ICH, GCP and applicable regulatory requirements. Further guidelines describing the phone consenting process will be clarified in the READISCA operations manual	
Study visits	11 - 12	Amendment 01: Updated section Amendment 02: New section for videotaping the SARA and storage on Box.com>> CSF with additional DNA and plasma collected by US sites will be sent to National Institute of Neurological Disorders and Stroke (NINDS) biomarker repository (BioSEND) at the University of Indiana. Lastly, whole blood will be collected at the baseline visit for the isolation of peripheral blood mononuclear cells. The collected blood will be shipped to RUCDR, the NINDS Human Cell and Data Repository at Rutgers University. Amendment 03: Additional blood sample will be obtained from 50%-at-risk participants for confirmatory molecular testing at the University of Utah. Blood samples for DNA will be obtained from all 200 enrolled subjects at the baseline visit, while Plasma will be obtained annually at each study visit. CSF will be obtained from those who are willing to have a lumbar puncture (LP), which will be performed according to the strict SOPs (see supplemental materials) at 2 time points from the baseline visit to the conclusion of the study. Amendment 04: The lumbar puncture procedure would be performed by experienced physician under fluoroscopic guidance if needed. Amendment 05: Added COVID-19 screening and questionnaire Added potential for completing some evaluations remotely	Amendment 06: Add SARA video access to researchers of neurodegenerative diseases
Study Visits Impacted By COVID-19 Related Site Closures	12	Amendment 05: <<New section>>	

Data	12 - 13	Amendment 02: <<New section>>	
Rater Qualification and Certification	13	Amendment 01: <<New section>> Amendment 02: Removed central raters	
MR data acquisition: Study population	13	Amendment 01: <<New section>> Amendment 03: Revised target age range for premanifest carriers and 50%-at-risk participants to 27-50 years to minimize the probability of including participants too far from disease onset and those who are highly unlikely to have an expansion of CAG repeats in SCA1 or SCA3.	
Study duration	13	Amendment 01: Added 3 time points for CSF collection Amendment 02: Blood samples for DNA will obtained from all 200 enrolled subjects at the baseline visits, while Plasma will be obtained annually at each study visit. CSF will be obtained from those who are willing to have a lumbar puncture (LP), which will be performed according to the strict SOPs (see supplemental materials) at 2 time points from the baseline visit to the conclusion of the study. All MR visits will include completion of SARA test prior to MR scan. Three additional time points may be included in the MR sub study to incorporate scans at 6 months, 18 months and 36 months. Amendment 03: Participants will be scanned (within ±2 weeks of the clinical study visit) at baseline, 6 month, 12 month, 24 month and 36 month time points during the READISCA study period. All MR visits will include completion of SARA exam prior to MR scan.	
MR Inclusion and exclusion criteria	13	Amendment 02: Participating sites will notify HMRICC as soon as potential MR candidate has been identified. HMRICC will subsequently pre-screen candidates using attached script. Safety and further screening evaluations will be remotely conducted by MR site where scanning will occur.	
Table 4	15	Amendment 01 and 02: Updated schedule of assessments Amendment 03: DNA sample may be collected at the screening or baseline visit Amendment 05: Added COVID-19 Questionnaire	
Adverse Events and Serious Adverse Events	16	Amendment 01: <<New section>>	

Data Safety and Monitoring Plan	18	Amendment 01: Robert Wilson, MD, PhD (University of Pennsylvania) and Anhar Hassan, MD (Mayo Clinic, Jacksonville) and John Caviness, MD (Mayo Clinic, Arizona) serve as members of the Data Safety Monitoring Committee. Removed Zbigniew Wszolek, MD (Mayo Clinic, Rochester)	
Protocol deviation	18	Amendment 01: <<New section>> Amendment 05: Allowance for delays caused by the COVID-19 pandemic	
Study Costs/ Compensation	19	Amendment 03: Added compensation for unsuccessful CSF collection, if LP needle penetrates the skin.	
Use of Data and/or Samples	20	Amendment 01: <<New section>>	
Ethical Conduct of the Study	21	Amendment 01: <<New section>>	
Clinical Trial Registration	21	Amendment 01: <<New section>>	
Appendix	22	Amendment 01: <<New section>>	

Summary

Funding: NIH PAR-16-020 Clinical Trial Readiness for Rare Neurological and Neuromuscular Diseases (U01)

Title of Protocol: Clinical Trial Readiness for SCA1 and SCA3 (READISCA)

Participant population:

Number of Participants:

170 for Year 1 and 200 for Years 2-5

- Early stage Subjects: 60
- Premanifest mutation carriers: 60
- 50%-at-Risk subjects: 60*
- Previously diagnosed early stage patients: 20

Number of sites:

Appr. 22 sites globally

Duration of Study: Maximum 60 months

Study AIMS:

AIM1: To establish world's largest cohort of premanifest and early-stage SCA1 and SCA3 individuals.

AIM2: To validate MR morphological, biochemical (MRS), and functional (resting-state fMRI) biomarkers in premanifest and early SCA1 and SCA3.

AIM3: To adapt recent developments on statistical design and analysis of small population trials to SCAs.

Main Criteria for Inclusion*:

1) Signed informed consent (no study-related procedures may be performed before the subject has signed the consent form)
2) Subjects of either sex, aged 18 to 65 with presence of symptomatic ataxic disease with definite molecular diagnosis of SCA1 or SCA3 in the subject or first degree relative or
3) Subjects with definite molecular diagnosis of SCA1 or SCA3 or
4) Asymptomatic subjects of either sex, aged 27 to 50 whose first-degree relative has a molecular diagnosis of SCA1 or SCA3 or

5) Subjects of age ≥18 with previous diagnosis of early stage SCA1 and SCA3 6) Subjects capable of understanding and complying with protocol requirements 7) No changes in physical/occupational therapy status within two months prior to enrolment
Main Criteria for Exclusion: 1. Subjects currently receiving or having received within 2 months prior to enrolment into this study, any investigational drug 2. Subjects who do not wish to or cannot comply with study procedures 3. Genotype consistent with other inherited ataxias 4. Changes in coordinative physical and occupational therapy for ataxia 2 months prior to study participation 5. Concomitant disorder(s) or condition(s) that affects assessment of ataxia or severity of ataxia during this study 6. AIM 2 exclusion criteria also includes the following: Inability to undergo MRI scanning, Weight over 300lbs, Presence of structural abnormalities such as subdural hematoma or primary or metastatic neoplasms, concurrent illnesses or treatment interfering with cognitive function such as stroke or normal pressure hydrocephalus

* We expect approximately 50% of these subjects will be tested negative for SCA1 and SCA3, and they will serve as controls. If we do not have 30 control subjects, we will recruit additional asymptomatic subjects who are of either sex aged 18 to 65 with neither SCA1 nor SCA3 mutations to serve as additional controls. Exclusion criteria described above also applies to control subjects.

Introduction

Background and study rationale

Spinocerebellar Ataxias (SCAs) are a group of rare, inherited diseases (affected population fewer than 200,000 people in the United States [US]) involving progressive cerebellar degeneration, leading to devastating disability and, eventually, death. SCA type 1 (SCA1) has shown the fastest progression rate among common SCAs, and SCA3 has the highest prevalence in most parts of the world, including the US and Europe. These characteristics make SCA1 and SCA3 attractive SCAs for future clinical trials of disease-modifying treatments. Furthermore, SCA1 and SCA3 are caused by an expansion of polyglutamine-coding CAG repeats in their respective disease-causing genes, similar to other SCAs and Huntington's disease, in which common, pathogenic mechanisms have been extensively studied. While there are no efficacious treatments at present, promising disease-modifying drugs are being developed, based on excellent scientific premise. These include: (1) MSK1 inhibitors intervene in a SCA1-specific pathogenic pathway; (2) citalopram, a widely used anti-depressant, for SCA3; and (3) inhibitory RNA reagents that are designed to decrease the levels of SCA1- and SCA3-disease causing proteins, ATXN1 or ATXN3, respectively. These drugs were all identified by either unbiased screenings or by focusing on upstream targets and reduce the levels of ATXN1 or ATXN3 in disease models. Envisioning clinical trials to test efficacy of these candidate drugs, we identified currently existing gaps in clinical trial readiness, which include a lack of power, based on the anticipated effect size of candidate drugs and the currently available small cohort size of SCA1 and SCA3 subjects. We also became aware of the lack of robust biomarkers of disease-modifying therapeutic efficacy. Furthermore, we recognized that early interventions are essential for increasing the probability of efficacy of disease-modifying drugs. Finally, due to limitations in the cohort size, drugs' effect size, and responsiveness of currently available clinical outcome assessment (COA) measures, we noted that we need to adapt emerging statistical designs and analyses for clinical trials with small sample sizes to conduct SCA1 and SCA3 trials. To fill these gaps, we propose three Aims in close collaborations between US and European SCA consortia:

Aim 1. To establish the world's largest cohorts of premanifest and early-stage SCA1 and SCA3 individuals by combining and expanding existing cohorts, COA data, and biofluid samples (blood and cerebrospinal fluid) from US and Europe.

Aim 2. To validate MR morphological (Volumetry/voxel based morphometry/diffusion), biochemical (MRS), and functional (resting-state fMRI) biomarkers in premanifest and early SCA1 and SCA3.

Aim 3. To adapt recent developments on statistical design and analysis of small population trials to SCAs.

This international, multi-site effort will enable trials of disease-modifying therapies for SCA1 and SCA3 within 5 years, by generating a well-characterized (demographic, genetic, COA, and biomarker) early-stage cohort and establishing multi-modal criteria for patient stratification to enable highly efficient and robust trial designs. For this study, we intend to use a **Central Institutional Review Board (IRB)** for which *Advarra IRB will be the IRB of Record*. The Houston Methodist Research Institute has an ongoing contractual agreement with Advarra IRB.

Study Design:

Aim 1. Organize the world's largest cohorts of patients with these SCAs by combining available cohort from both sides of the Atlantic, focusing on preclinical mutation carriers and early-stage patients who carry the SCA1 or SCA3 CAG repeat expansion.

Participating Study Consortia in Aim 1:

The US sites mostly consist of the Clinical Research Consortium for Study of Cerebellar Ataxias (CRC-SCA), which has collected longitudinal clinical data from SCA patients, using NIH funding (RC1 NS068897, PI: Ashizawa) from 2009 to 2012 and has been continuing the effort with recent reinvigoration by a National Ataxia Foundation (NAF) grant (PI: Paulson). The two European sites, headed by Dr. Thomas Klockgether (the German Center for Neurodegenerative Diseases [DZNE], Hospital University of Bonn, Bonn, Germany) and Dr. Alexandra Durr (Brain & Spine Institute [ICM], Hôpital Pitié Salpêtrière, Paris, France), are headquarters of European consortia for clinical studies of SCAs. Dr. Klockgether is a leader of the European Ataxia Study Group (EASG), which has conducted the EUROSCA natural history study of patients with SCA1, SCA2, SCA3 and SCA6, and the RISCA study to characterize carriers of gene mutations of these SCAs. Dr. Klockgether is currently conducting the European SCA3/Machado-Joseph Disease Initiative (ESMI), funded by the Joint Programme – Neurodegenerative Disease Research (JPND). Dr. Durr leads the Spastic Paraplegia and Ataxia Network (SPATAX), another major consortium primarily based in France. These European studies have utilized COA measures, most of which are identical to those used by the CRC-SCA and those used in the proposed study. Sites and investigators of these consortia are shown below. However, the data from previous studies have already been compiled within the CRC-SCA database at UCLA and within the DZNE database at the University Hospital Bonn. Drs. Klockgether and Perlman (UCLA) will exchange their datasets for joint analyses of disease progression data and other clinical characteristics. The clinical study data and the US and European imaging data will be archived in the NIMH Data Archive (NDA).

US sites:

- Columbia University – Sheng Han Kuo, MD
- Emory University – George Wilmot, MD, PhD and Laura Scorr, MD
- Houston Methodist Research Institute – Tetsuo Ashizawa, MD
- Johns Hopkins University – Liana Rosenthal, MD and Chiadi Onyike, MD
- Harvard University/Massachusetts General Hospital – Jeremy Schmahmann, MD, PhD
- Northwestern University – Puneet Opal, MD, PhD
- Ohio State University – Yasushi Kisanuki, MD, FAAN and Sandra Kostyk, MD
- Stanford University – Sharon Sha, MD and Veronica Santini, MD and Jacinda Sampson, MD
- University of Alabama Birmingham – Talene Yacoubian, MD, PhD and Marissa Dean, MD
- University of California Los Angeles – Susan Perlman, MD
- University of California San Francisco – Michael Geschwind, MD, PhD and Alexandra Nelson, MD, PhD
- University of Colorado –Trevor Hawkins, MD
- University of Chicago – Christopher Gomez, MD, PhD
- University of Florida – SH Subramony, MD
- University of Iowa – Peggy C. Nopoulos, MD and Annie Killoran, MD, MSc
- University of Michigan – Henry Paulson, MD, PhD and Vikram Shakkottai, MD, PhD
- University of Minnesota – Khalaf Bushara, MD
- University of Pennsylvania – Pedro Gonzalez-Alegré, MD, PhD
- University of Rochester Medical Center – Peter Morrison, DO and Alexander Paciorkowski, MD
- University of South Florida – Theresa Zesiewicz, MD

- University of Utah – Stefan Pulst, MD, PhD

European sites:

- German Center for Neurodegenerative Diseases (DZNE)/ University Hospital of Bonn, Bonn, Germany – Thomas Klockgether, MD
- ICM (Brain and Spine Institute) /Hôpital Pitié Salpêtrière, Paris, France – Alexandra Durr, MD, PhD

Comparing Available Datasets from US and Europe:
The exchange of datasets between the European consortia and the CRC-SCA will involve only de-identified data. The CRC-SCA data have been shared with outside investigators, under guidance by the NIH data sharing policy. The joint US-European team of statisticians will compare the de-identified datasets, including demographics, such as age at baseline, age at onset, mode of onset (the first symptom), duration of disease, gender of transmitting parent, race, ethnicity, nationality, and the region of residence. The team will also compare results of COA measures of ataxia at the baseline visit, including the Scale for Assessment and Rating of Ataxia (SARA) scores, timed motor performance measures, such as the Timed 8-Meter Walk (T25FW) and the 9-Hole Peg Test (9HPT), the number of non-ataxia movement disorders, the Unified Huntington Disease Rating Scale IV (UHDRS-IV; for activity of daily living), EQ-5D (patient-reported quality of life), the Montreal Cognitive Assessment (MOCA), Beck's Depression/Anxiety Index, and the Functional Staging for Ataxia. Genotypes will be also analyzed for correlations with the age at onset and other clinical data. The joint team will determine whether the US and European datasets can be combined as a single cohort. Data transfer and sharing will be done in compliance with the EU General Data Protection Regulation (GDPR) and NIH Data Sharing Policy. De-identified data from US and European participants will be combined in Paris, where Dr. Tezenas du Montcel will perform the primary statistical analysis and share the statistical outcome with US statisticians and then report to the Steering Committee.

Prospective Data Collection:

Study population:

We plan to enroll a total of 200 subjects, including 60 early-stage patients (SARA total score 3-9.5), 60 premanifest gene mutation carriers (SARA total score 0-2.5), 60 50%-at-risk subjects (SARA total score 0-2.5), and 20 patients, who had a SARA total score <10 during the previous (2009-2012) natural history study. Confirmatory genotyping for SCA1 and SCA3 will be performed on blood samples obtained at the baseline visit. For 50%-at-risk subjects, genotyping results will be released to the subject's designated physician or genetic counselor after a release of information form has been signed. We strongly recommend genetic counseling for the disclosure of DNA results. We expect that the SCA1-to-SCA3 enrollment ratio will be approximately 1 to 2 in the US and 1 to 1.2 in Europe, based on the previous studies. We anticipate <5%-attrition rate and plan to replace subjects, who drop out, with new subjects. The low attrition rate is expected because study subjects have mild or no disability and low mortality. During the study, there will be individuals who will phenoconvert and some subjects who will want to know the DNA testing results. Subjects who become clinically ataxic will be categorized in the phenoconversion group. Asymptomatic subjects, who receive positive DNA results, will be included in the premanifest carrier group. Prospective data will be categorized at the end of the study into five groups: (1) premanifest carriers whose SARA total score remain in the 0-2.5 range; (2) mildly affected early-stage patients who had an initial SARA total score 3-9.5, which may increase in subsequent years; (3) premanifest participants who phenoconvert during the study period with a SARA total score increase from the 0-2.5 range to ≥3; (4) SCA1 and SCA3 patients who had a SARA total score <10 during the 2009-2012 CRC-SCA natural history study (or during the ESMI, SPATAX or EUROSCA studies) who may have higher SARA scores during this study; and (5) gene mutation negative controls. Their annual disease progression will be characterized by statistical analyses of annual changes of COA measures, with the SARA total score as the primary outcome measure.

Study duration: Five years

Recruitment of prospective study subjects:
We will recruit subjects from clinics of the US and European institutions participating in this project. The NAF, Coordination of Rare Diseases (CoRDS), ClinicalTrials.gov and local ataxia patient support groups in the US and Europe will help the recruitment by referring potentially eligible subjects to these clinics. Participation in the

study will also be solicited from members of patient support networks and registries, including the National Ataxia Patient Registry, RISCA Registry, and SPATAX Registry.

Inclusion Criteria					
Study population category	**N**	**Genetic diagnosis of SCA1/SCA3**	**Age (years)**	**SARA total score**	**Gait**
Early-stage patients	60	(+) in the subject or 1st degree relative	18-65	3-9.5	Ambulate without an assisting device (SARA gait subscore <5)
Premanifest carriers	60	(+) in the subject	27-65	0-2.5	Within the normal range
50%-at-risk subjects	60	Unknown and (+) in 1st degree relative	27-50	0-2.5	Within the normal range
Previously early-stage patients	20	(+) in the subject	Any age	<10 in 2009-2012	Any state
Additional controls	If needed	(-) in the subject	18-65	0-2.5	Within the normal range

Exclusion criteria (at least one)

- Known genotype consistent with other inherited ataxias
- Concomitant disorder(s) that affects assessment of ataxia or severity of ataxia during this study
- Investigational drugs taken in 2 months prior to participation in this study
- Changes in coordinative physical and occupational therapy for ataxia in 2 months prior to study entry
- Unwillingness to provide a DNA sample at the enrollment in this study

At the participating clinics, these subjects will be screened with the inclusion and exclusion criteria (see above), and those who are eligible will be enrolled in the longitudinal study, where subjects will be evaluated annually according to the READISCA protocol (see supplemental materials). Controls will mainly consist of at-risk persons with no SCA gene mutations, and if necessary, we will recruit additional control subjects from spouses and other relatives, who are known to have no SCA mutations. Whenever possible, the informed consent process will be done in person. However, when the consent process cannot be reasonably conducted in person to support research related activities or preparatory work (as in the case of remote patients) a consent via telephone or comparable media may be deemed appropriate. The consent process will be documented, and accompanying signature pages will be sent via facsimile, email or certified mail as defined by ICH, GCP and applicable regulatory requirements. Further guidelines describing the phone consenting process will be clarified in the READISCA operations manual. If participant meets eligibility criteria for MR enrollment, travel arrangements will be made on behalf of remote participants to one of the recruiting sites in the US to complete baseline evaluations.

Any recruitment materials (such as radio/newspaper/television/internet advertisements, posters, flyers, telephone scripts and letters) used for this study must be approved by central IRB prior to use during this study.

Table 3. Estimated enrollments for clinical evaluation of study subjects.

	[1]Year 1	Year 2	Year 3	Year 4	Year 5
Enrolled in Year 1	170	162	153	146	138
Enrolled in Year 2		38	36	34	33
Enrolled in Year 3			11	10	10
Enrolled in Year 4				10	10
Enrolled in Year 5					9
[2]**Total**	**170**	**200**	**200**	**200**	**200**[3]

[1]We anticipate <5% annual attrition rate (shown are figures based on 5%).

[2]We intend to maintain the total number of visits at 200 every year.

[3]We intend to follow these subjects beyond Year 5 with a limited battery of COA measures.

Annual follow up-visits will occur within ±4 weeks of anniversary dates of the baseline visit. We anticipate an attrition rate of <5% per year and plan to replace subjects who drop out with new subjects as shown in the table. The low expected attrition rate is based on the fact that the study subjects are either in early stage or premanifest stage of the disease and unlikely to miss the scheduled visits due to disability, death, or financial reasons.

Study Visits:

All subjects will undergo the same baseline and annual follow-up visits, which include many components of the CRC-SCA protocol developed based on the battery of tests described in our earlier papers. Briefly, the protocol of clinical evaluation includes: (1) detailed history including demographics, the age of ataxia onset, concomitant medications, and procedures; (2) physical assessment including vital signs, anthropometry; (3) performance-based COA measures, including SARA (videotaped), T25FW (T8MW), Cerebellar Cognitive Affective Syndrome Scale (CCAS Scale); (4) Composite Cerebellar Functional Scale (CCFS) which includes the 9HPT and the click test; (5) COA measures based on physicians observations, such as Inventory of Non-Ataxia Signs (INAS), Functional Staging; and (6) other assessments including self-reported outcome measures, such as Patient Global Impression, EQ-5D, PHQ9, Friedreich Ataxia Activity of Daily Living (FA-ADL), and Fatigue Severity Scale. All SARA exams will be videotaped using specified camera equipment to be provided to each site. SARA videos recorded on site will be uploaded to a cloud storage website at Box.com. HMRICC has an existing business associate agreement with Box.com which will allow transfer of PHI from the covered entity to the business associate in compliance with HIPAA and HITECH regulations. Additionally, permissions are configured in such a way that links cannot be shared with persons outside the consortium. Once videos have been uploaded to Box.com downloading and editing capability will be restricted to the HMRICC. Furthermore, levels of access will be customized to individual site needs, such that viewing access is restricted to only videos uploaded by respective sites. Face recognizable video containing PHI will be restricted to local research site, HMRICC, central raters and researchers of neurodegenerative diseases.

Estimated time for these assessments is 2 hours.

Efforts must be made to complete in person evaluations whenever possible, however a subset of the clinical evaluations may be collected remotely as needed. Any remote evaluations must be completed within +/- 24 hours of the face-to-face evaluations. These evaluations must be completed with study personnel via a secure, HIPPA compliant video conference platform. Remote assessments completed alone, without accompanying in-person assessments will not meet the criteria for a study visit.

Evaluations Which Must Be Face-to-Face	**Evaluations Which May Be Remote**
Eligibility Criteria	Family History
General Physical Assessments	Demographics
SARA	Age of Ataxia Onset
Timed Tests (T25FW)	Concomitant Medications
CCFS	Therapy Review
CCAS	General Medical History
INAS	Patient Global Impression
Functional Staging	EQ-5D
Category of Participant at Current Visit	PHQ9
	Fatigue Severity Scale
	Friedreich's Ataxia Activities of Daily Living
	COVID-19 Questionnaire

Blood samples for DNA will be obtained from all 200 enrolled subjects while Plasma will be obtained annually at each study visit. Additional blood sample will be obtained from 50%-at-risk participants for confirmatory molecular testing at the University of Utah. Cerebrospinal fluid (CSF) will be obtained from those who are willing to have a lumbar puncture (LP), which will be performed according to the strict SOPs (see supplemental materials) at up to 2 time points from the baseline visit to the conclusion of the study. The lumbar puncture procedure would be performed by experienced physician under fluoroscopic guidance if needed. If participant declines CSF collection at baseline visit, the participant will be allowed to continue participating in the study. Please note that additional blood samples for DNA banking (DNA_{LP} samples) will be obtained from all who consent to have LPs. This collection can be done at either of the LP procedure timepoints.

Baseline DNA samples from the US will be sent to the University of Utah, while European samples will be stored by DZNE (Bonn) and SPATAX (Paris). To ensure assay uniformity, aliquots of European Baseline DNA samples will be tested in year 5 at UTAH. CSF, plasma and LP DNA samples collected by US sites will be sent to National Institute of Neurological Disorders and Stroke (NINDS) biomarker repository (BioSEND) at the University of Indiana. Lastly, whole blood will be collected at the baseline visit for the isolation of peripheral blood mononuclear cells. The collected blood will be shipped to RUCDR Infinite Biologics, the NINDS Human Cell and Data Repository at Rutgers University.

COVID-19 screening procedures will be implemented in advance of the study visit as well as on the day of the visit. Sites should follow their local guidelines regarding COVID-19 screening and testing requirements. Pre-visit screening may be done via telephone or similar HIPAA compliant communication methods. A review of literature showed a subset of COVID-19 patients had neurological consequences detected on brain imaging (Long, et al. 2020) and we anticipate that these consequences may be long term. It is not yet clear what impact such neurological symptoms would have on study objectives. While we do not expect exposure in many of our study participants, this survey will help us determine whether participants may have been exposed to the virus, and if so, whether they have ever tested positive.

Study Visits Impacted by COVID-19 Related Site Closures:

- Schedule the follow-up visits based on the previous visit, not the baseline visit schedule. For example: If the 12-month visit is delayed, the 24-month visit should occur 1 year after the 12-month visit instead of 2 years after the baseline visit.
- If the clinic visit was performed without the MR visit, schedule the missing MR visit as soon as possible. Schedule the next clinic and MR visits so that there is at least 6 months between the two MR visits.
- If the 6-month MR visit was not scheduled, skip this visit and continue with the original MR protocol timepoints.

Data:

Study sites will be provided access to an Electronic Data Capture (EDC) system that has been fully validated and conforms to 21 CFR Part 11 requirements. Designated study site staff will be trained on the EDC system. Study site staff will not be given access to the EDC system until they have been trained. Designated study site staff will enter the data required by the protocol into the eCRFs. Data entered by study site staff will be reviewed for completeness and accuracy. Authorized study site staff will respond to queries sent to their site and make any necessary changes to the data.

Rater Qualification and Certification:

In order to ensure satisfactory training of raters and quality execution with regards to data collection, SARA raters assigned to this study will be required to adhere to certain requirements prior to participation in the study. All raters will be required to watch a training video, then submit a sample SARA video following proposed videotaping guidelines to HMRICC for final approval prior to rating any subjects in this study. Whenever possible, each study participant should have the same rater throughout the duration of the study. Additionally, training on completion of other clinical outcome measures will be presented on scheduled web-based teleconferences as well as outlined in operations manual.

Telephone Contact:

Sites will maintain contact with subjects every 6 months for the duration of the study as reminders for the study logistics, including follow-up visits.

Aim 2. **MR morphological (Volumetry/voxel based morphometry/diffusion), biochemical (MRS) and functional (resting-state fMRI) biomarkers in premanifest and early SCA1 and SCA3.**
The imaging sites will include the four CRC-SCA sites involved in the ongoing Bioengineering Research Partnership (BRP) project, namely the University of Minnesota, Johns Hopkins University, Harvard University/Massachusetts General Hospital, and University of Florida, as well as two sites in Europe: Paris and Bonn. Dr. Öz has an ongoing collaboration with Drs. Alexandra Durr (MPI) and Sophie Tezenas du Montcel (Paris). In addition, Hôpital Pitié Salpêtrière in Paris has a large SCA1 cohort, including premanifest subjects. The DZNE in Bonn is included as the central site of the ESMI effort and the central imaging site of EASG, with broad access to SCA cohorts at all stages and extensive imaging expertise.

MR data acquisition:

Study population:

We will enroll a subset of the prospectively enrolled 200 subjects in Aim 1. These subjects will consist of 30 (20 premanifest and 10 early-phase) subjects with the SCA1 mutation, 40 (27 premanifest and 13 early-phase) subjects with the SCA3 mutation and 30 controls (drawn from at-risk subjects who test negative for SCA1 or

SCA3 gene mutations and from spouses and other relatives who do not have symptoms, signs or risks of ataxia). We will enrich the SCA1 and SCA3 cohorts by inviting participants with a predicted time to ataxia onset within 10 years, based on their CAG repeat length and current age. For premanifest carriers and participants who are 50%-at-risk, we will target an age range of 27-50 years to minimize the probability of including participants too far from disease onset and those who are highly unlikely to have an expansion of CAG repeats in SCA1 or SCA3.

Study duration:

Participants will be scanned (within ±2 weeks of the clinical study visit) at baseline, 6 month, 12 month, 24 month and 36 month time points during the READISCA study period. All MR visits will include completion of SARA exam prior to MR scan. Three additional time points may be included in the MR sub study to incorporate scans at 6 months, 18 months and 36 months.

Inclusion and exclusion criteria:

Inclusion criteria for Aim 2 will be the same as those in ***Aim 1*** and willingness to undergo MR procedures.

Exclusion criteria for Aim 2 will be the same as those in ***Aim 1*** and inability to undergo MRI scanning (claustrophobia, presence of paramagnetic substances or pacemakers in body, weight over 300 lbs.), pregnancy or lactation in women of childbearing potential not protected by an effective means of contraception, and other neurological diseases than those of interest. In addition, potential study participants will be excluded from the study if any of the following criteria are present at any point in the study: structural abnormalities such as subdural hematoma, primary or metastatic intracranial neoplasms, and concurrent illnesses or treatments interfering with cognitive function, such as a stroke or normal pressure hydrocephalus.
Participating sites will notify HMRICC as soon as potential MR candidate has been identified. HMRICC will subsequently pre-screen candidates using attached telephone script. Safety and further screening evaluations will be remotely conducted by the MR site where scanning will occur. After eligibility requirements have been met, travel arrangements will be made on behalf of participant to one of the four MR performing sites by HMRICC.

MR study:

Before the 3 Tesla field strength MR scanning, the participant will be asked to remove any metallic objects (watches, keys, chains, hair pins, glasses, jewelry, coins, etc.), items with magnetic data (credit cards), and other valuables, all of which will be safely stored during the procedure. During the MRI scan, the participant will be asked to lie still on a flat surface like a table, which slides into a large magnet bore of the scanner with coil placed around the participant's head. The MR protocol, including structural MRI, diffusion tensor imaging (DTI), MR spectroscopy (MRS), and resting state functional MRI (rs fMRI), will be completed in the estimated one-hour scan time. The participant will be able to communicate with the researcher and will be able to get out of the magnet if the participant feels sick, claustrophobic, or tired. The MR scan will take approximately one to one and a half hours.

For data consistency, we will use Siemens 3T Prisma scanners with multi-band capability and 32-channel receive-head coil, which are available at all four US (and additional two European) sites and have an excellent record of clinical applications. The state-of-the-art one-hour protocol allows for detection of premanifest abnormalities. Structural and functional acquisition parameters are based on the Human Connectome Lifespan imaging protocol, which provides additional normative data. Protocol harmonization and training assure the consistent data acquisition and cutting-edge data sharing, involving all six sites, has been planned with Flywheel, Inc. as the partner.

European MR data will be compiled with US counterparts by Dr. Öz and colleagues at the University of Minnesota, who will share the imaging outcomes with Dr. Tezenas du Montcel for statistical analysis. Drs. Durr and Tezenas du Montcel will use the MR data for Aim 3.

Schedule of events table:

Table 4. Study visits	Screening visit (Likely same day as baseline)	Baseline visit	Year 2 follow up visit	Year 3 follow up visit	Year 4 follow up visit	Year 5 follow up visit
COVID-19 Questionnaire	✓		✓	✓	✓	✓
Eligibility	✓					
Demographic data	✓					
Age at onset	✓					
Therapy review	✓		✓	✓	✓	✓
Complete History[1]	✓					
Interval History[2]			✓	✓	✓	✓
General physical assessment	✓					
Prior DNA result check (if available)	✓					
Baseline DNA result check[3]			✓			
Concomitant medications	✓		✓	✓	✓	✓
SARA[4]	✓		✓	✓	✓	✓
Plasma		✓	✓	✓	✓	✓
Whole blood (PBMCs)		✓				
9HPT, T25FW, Click Test		✓	✓	✓	✓	✓
CCAS		✓	✓	✓	✓	✓
INAS, Functional stage		✓	✓	✓	✓	✓
Patient Global Impression, EQ-5D, PHQ9, FA-ADL, Fatigue Severity Scale		✓	✓	✓	✓	✓
Category of participant			✓	✓	✓	✓
MRI/MRS/fMRI[5]		✓	✓	✓	✓	✓
Blood sampling[6]	✓	✓				
CSF sampling (optional)[7]		✓	✓	✓	✓	✓
AE/SAE review		✓	✓	✓	✓	✓
Conclusion of study participation						✓

[1]Complete history include general medical history. [2] Interval history will include general medical history, age at onset and therapy review. [3]The result of baseline DNA testing will be checked as soon as it becomes available before the Year 2 follow up visit. [4] SARA will be done for assessment of inclusion criteria at the screening visit, but it will not be repeated at the baseline visit if both visits occur on the same day. SARA may also be re-evaluated at imaging site prior to each scan. All SARA assessments will be videotaped following recommended guidelines. [5]MRI/MRS/fMRI will be performed at specified timepoints for three consecutive years. Therefore, the first MRI/MRS/fMRI study may be performed in the first, second, or third year. Additional scanning may be obtained at 6 months, 18 months and 36 months.[6] DNA samples will be collected at screening or baseline visit. An additional DNA sample may be collected to confirm results of genetic testing for 50%-at-risk participants. [7] CSF samples may be collected up to 2 times. Additional DNA sample will be collected during either the first or second LP procedure.

Aim 3. Adapt recent developments on statistical design and analysis of small population trials to SCAs.

We will confirm the disease progression rate for the clinical outcomes and MRI and MRS biomarkers in premanifest carriers and early-stage patients. Linearity of evolution of clinical outcomes will be confirmed in early-stage diseases. We will examine whether expected linearity of the disease progression on MRI and MRS measures correlates with disease progression on COA measures after the disease onset in premanifest participants. Mixed models and joint mixed models will be used in order to establish the joint evolution of clinical and MR biomarkers and to define a threshold for non-linear evolution. The effect sizes for each COA and MR biomarker will be calculated and the MR biomarker(s) most sensitive to premanifest disease will be identified. We will also confirm that the CAG repeat size is a genetic modifier that can be used to predict the age of ataxia onset, using mixed models incorporating the predictors via interactions. We will also define

subgroups with different evolution to optimize the statistical power of future trials by lowering all sources of variability. For this purpose, trajectory models such as Latent Class Mixed Model will be implemented both on baseline severity data and disease evolution. For simulations, we will test following strategies: (1) using external control data including historical controls, (2) using relaxed statistical error rates, (3) optimizing the design using "umbrella trial," group sequential design, crossover, n-of-one, and re-randomization designs, and (4) using repeated measures.

Adverse Events/Serious Adverse Events

Serious adverse events are defined as any untoward medical occurrence during the study period

- results in death,
- is life-threatening
- requires inpatient hospitalization or causes prolongation of existing hospitalization
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect, or
- Requires intervention to prevent permanent impairment or damage.

The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Adverse events are further defined as "Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product or exposed to a research related environment and which does not necessarily have to have a causal relationship with this treatment.

All adverse events, including those associated with MRI/MRS procedures, will be reported to the Steering Committee via the HMRICC, which will coordinate necessary measures to address medical needs that resulted from the adverse event. Serious adverse events will be reported within 24 hours to the HMRICC, officials at the NIH, and the IRB. HMRICC will immediately notify the Steering Committee members. The Steering Committee will act to address the problem and implement measures to prevent similar problems. The Steering Committee will also report all adverse events to the *Data and Safety Monitoring Committee.*

Potential Risks

An assessment of potential risks for this project includes the following:

- Risk of loss of confidentiality: Participants will be asked to provide medical information documenting their condition for this research project. Such information will be stored in a secured access database at the site where the participant is enrolled in the study. Only research personnel working on this READISCA project at that site will have access to the data. If results of the study are published, the identity of participants will not be disclosed. Data and biological materials obtained from participants will be de-identified before they are sent to other sites for analysis and storage. The newly assigned code to de-identify the data can be linked to the participant's PHI only by the site investigator and coordinator who enrolled or examined the participant. Any information, which link the de-identified data to PHI, will be kept in either a locked cabinet or a password-protected computer file, which only the site investigator and coordinator can access. All PHI will be protected by the Health Insurance Portability and Accountability Act (HIPAA) and the Genetic Information Nondiscrimination Act (GINA) in the US and equivalent laws in Europe.
- Risk of falls and resultant injuries during neurological evaluations: While the risk is minimal, participants may have balance problems and may fall during study visits.
- Risks and/or complications of *optional* spinal tap: Risks of a lumbar puncture (LP) procedure, include (maximal complications in parentheses) local pain and irritation at the site of needle injection, a 5% to 30% risk of post-LP headache, and less than 1% risk of infection (meningitis), avulsion of nerve root (focal neurological deficit), bleeding into spinal canal (cauda equina syndrome with paralysis of legs), and extremely rare brain herniation syndromes (death). If a participant develops a headache in upright posture that significantly improves by lying down, but does not improve after 12 hours of bed rest, fluids, or ibuprofen/Tylenol®, a blood patch may be necessary.

- Risks associated with venipuncture: Venipuncture may cause local subcutaneous bleeding or puncture site infection. Some individuals may have vagal syncope, which may result in fainting.
- Risk of claustrophobia: Some individuals may become claustrophobic during MR testing and if this occurs, the scan will be discontinued immediately.
- Risks associated with the 3T scanners: The FDA has classified these systems as devices of non-significant risk and serious risks are not expected. However, some participants may experience minor dizziness when moving in and out of the system, headache, flashing light sensation, or a metallic taste. Should participants experience any of these effects and wish to discontinue the study, they will be taken out of the scanners. These symptoms should resolve after the scan is discontinued. The only significant risk associated with utilizing high magnetic field strength magnets is that if highly magnetic materials are brought into the room containing the high magnetic field strength scanner, they will become projectiles and can cause injuries. To date, there have been no serious and/or permanent adverse effects associated with our 3T scanners. The participants are therefore at low risk during the study. At our centers, we have many IRB-approved protocols that involve 3T scans of various patient populations, including neurodegenerative diseases.

The alternative to participating in this project is to decline. Participants will be at no risk if they decline to participate.

2. PROTECTION AGAINST RISKS

- Risk of loss of confidentiality: To protect participant confidentiality, participant information will be kept in their private medical chart or in records maintained by the investigators. Such records will be kept in locked cabinets or password-protected computer files in locked rooms. Participants will not be identified by name in any data presentation.
- Risk of falls and resultant injuries during neurological evaluations. We will provide sufficient physical support to prevent falls during study visits.
- Risks associated with venipuncture: Pressure application and sterile techniques are effective ways to reduce hemorrhage and infection. If the participant feels lightheaded or dizzy, venipuncture will be halted.
- Risks for complications of spinal tap (optional): LP procedure will be performed using good standard technique, under the guidance of clinical site PIs. Study personnel will monitor the volunteer and assess vital signs before and after the LP. Risk of headache will be reduced by using a small diameter atraumatic needle, replacing the stylet before the needle is withdrawn, and inserting the spinal needle with proper orientation. Risk of infection will be minimized by proper sterile technique and never passing through overlying tissue which could be infected. Risk of avulsion of nerve root will be minimized by conscientious technique and listening to the volunteer's report of symptoms during the procedure. Risk of bleeding into the spinal canal will be minimized by taking a history concerning coagulopathies, performing blood coagulation studies when indicated, and not performing LP on patients, who have abnormal results. Risk of brain herniation syndromes will be prevented by evaluating for papilledema and CT scanning for mass lesions, when indicated. Moreover, no LPs will be performed on any participant for whom it is in any way deemed a high risk.
- Risk of claustrophobia: Participants will be screened for claustrophobia prior to the scan, and if claustrophobia occurs during the scan, it will be discontinued immediately.
- Risks associated with the 3T scanners: Participants will be closely screened for contraindications to MRI scanning, and to prevent injury, routine safety procedures will be followed closely, prior to the participant entering the scanner area. If a participant should become anxious during the scanning procedure, due to feelings of claustrophobia, the scanning will be immediately stopped. Investigators can communicate via intercom with participants, while they are being scanned. A number of steps are taken to protect against potential risk, in addition to keeping time-varying magnetic fields (dB/dt), radio frequency (RF) power deposition, and acoustic noise within FDA guidelines. During each study, the participants are continuously monitored visually and can communicate with the researcher at all times. They can immediately be removed from the scanner, if needed. RF power deposition is monitored with software and hardware protection systems and kept below the FDA-approved limit. Acoustic noise is reduced by 12 dB, using a shield placed inside the gradient, using acoustic foam padding, and using specialty ear plugs (Howard Leight Industries, San Diego, CA), which are able to reduce the acoustic noise by 33 dB. To minimize any

discomfort, the participant will be moved inside the scanner at a very slow speed. Furthermore, we will continue to monitor any discomfort, by administering exit questionnaires to all participants. While inside the scanner, participants will have access to a "panic" alarm, which notifies the investigators of a problem and the desire to immediately stop the study. If a participant employs this alarm, they will be immediately taken out of the scanner and any problems will be addressed.

Data and Safety Monitoring Plan

This is not an interventional treatment trial. However, as standard practice for clinical studies in our centers, a detailed data and safety monitoring plan will be developed. Site investigators or coordinators will report all adverse events associated with clinical evaluations, venipuncture, LP, and MRI/MRS procedures to the HMRICC (Dr. Ashizawa, Director) at HMRI. Serious adverse events will be reported to the HMRICC and IRB within 24 hours, and the HMRICC will immediately notify the Steering Committee members and NINDS officials. The HMRICC and the Steering Committee will ensure that all necessary medical measures are taken to circumvent the adverse event. The Steering Committee will implement measures to prevent the recurrence of similar problems. The Steering Committee will perform quality control on all incoming data reported by the Director of the HMRICC. The Committee will document protocol deviations and violations, and reconcile incomplete or inconsistent data and errors. The Committee will also decide on corrective actions to prevent or minimize further occurrences of such problems. All protocol deviations, adverse events and serious adverse events will be reported to the Data and Safety Monitoring Committee. Robert Wilson, MD, PhD (University of Pennsylvania), Anhar Hassan, MD (Mayo Clinic, Jacksonville) and John Caviness, MD (Mayo Clinic, Arizona) serve as members of the Data Safety Monitoring Committee.

Protocol Deviations

Any protocol deviation as relates to study procedures must be recorded and reported to the HMRICC at the earliest possible time. Some events that can be classified as protocol deviations include but are not limited to the following:

- When one or more of the clinical outcome measures was not completed
- Study visits completed outside of window (+/- 4 weeks from anniversary of baseline visit)*
- COA completed by rater without appropriate and documented training

Additionally the following events will be recorded as irregularities during quality checks by HMRICC:

- SARA rated by multiple raters for the same patient
- Imaging visit completed outside (+/-) 2 week window of clinic visit.*

*If delays were caused as a result of the COVID-19 pandemic, study visits may be completed outside of window following the guidelines described in the study visit section.

3. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE PARTICIPANTS AND OTHERS

There will be no financial or immediate health benefits for our participants. However, information obtained from this study is expected to improve patient care through better assessment of disease progression or treatment in the future. The risks of participation are clearly defined for our participants and every effort has been taken to minimize their risk. We believe that the risks of participation are greatly outweighed by the benefits to be gained for others.

4. IMPORTANCE OF KNOWLEDGE TO BE GAINED

We believe that the information gained in this investigation will be important in establishing sufficiently large cohorts for clinical trials, developing robust disease progression markers to monitor treatment effects, and identifying the feasible clinical trial designs for disease-modifying drugs to treat SCA1 and SCA3. We believe that the insights gained from this project will ultimately have a significant impact on reducing the morbidity and early mortality associated with these diseases, and such insights may well be applicable to other neurodegenerative disorders. Given the enormous personal and public health burden of neurodegenerative diseases, we believe the limited risks our participants will be asked to incur are significantly outweighed by the new knowledge gained.

Notification of Significant New Findings

Personnel in the MR center maintain a list of the names and contact information of all participants included in research at the MR facilities. This information is required and will be used to notify participants of significant new information about the effects of MR on human health that develop over the course of MR research. Participant's identifying information is stored securely, and it is maintained in a confidential manner by persons with oversight of research conducted at the MR center.

Study Costs/Compensation

The study participant will not be responsible for the cost of any study procedure.

Compensation will be provided to participants who undergo the spinal tap procedure. Participants will be compensated $100 after CSF is collected, or for unsuccessful CSF collection, if LP needle penetrates the skin.

$50 compensation will also be provided to participants for completed MR scan visits.

Research Related Injury

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment, and follow-up care as needed. Care for such injuries will be billed in the ordinary manner to the study participant or his/her insurance company. If the participant thinks that he/she has suffered a research related injury, the participant should immediately notify the study staff. The research staff should take timely actions to minimize and treat the injury.

Incidental Findings on MRI

The images or pictures created during this study are for research purposes only and are not intended to provide health care to the participant. However, if the study participant is a control participant, and if the results from the MR show aberrations in the images, a Radiologist, trained in analyzing MR images, will assess the images. The images will not contain any information that leads to the participant's identity. There will be no financial charge to the participant for having the Radiologist assess the images. The investigator for this study will contact the participant, if the recommendation is to further investigate the unusual results of the pictures. However, further medical follow up is not a part of this study, and the study does not have funds set aside for this purpose. Therefore, if the results do show something unusual, any medical follow up cost will be the participant's responsibility and/or the responsibility of the health insurance carrier.

Confidentiality

The records of this study will be kept private. In any publications or presentations, we will not include any information that will make it possible to identify study participants. The record for the study may, however, be reviewed by the research staff directly involved in the study, or by departments at individual sites, with appropriate regulatory oversight. The information from the study will not be entered into the participant's permanent medical record. However, confidentiality is not absolute. Study data will be encrypted, according to current individual site policies for protection of confidentiality. The risks to the participant and his/her family from genetic research are very low. DNA samples will be identified only with a study code. In the event of an unexpected breach of confidentiality, the Genetic Information Non-Discrimination Act (GINA) will help protect the participant from health insurance or employment discrimination based on genetic information. However, GINA will not regulate life or disability insurance.

Protected Health Information (PHI)

PHI created or received for the purposes of this study is protected under the federal regulation known as HIPAA.

Voluntary Nature of the Study

Participation in this study is entirely voluntary. The study participant may choose not to be a part of this study or may withdraw from the study at any time, without affecting the relationship with the participant's physician or other medical staff.

Use of Data and/or Samples

At any time during the study, the participant may revoke authorization to use data and/or samples. However, the sponsor and the study investigator can still keep and use any information that is already received to the extent necessary to preserve the integrity of the research study.

Sample Size Justification

For Aim 1 study of prospective data collection the sample size is guided by feasibility (regarding our realistic ability to enroll premanifest and very early stage subjects across US and Europe), rather than power. The sample size of 200 participants will ensure enrollment of >60 participants with SCA1 and >100 participants with SCA3, which should provide sufficient power at least equivalent to the previous US and EUROSCA studies.

For Aim 2 the sample size is determined based on the RISCA study, which included imaging data from 26 SCA1 mutation carriers and 9 SCA3 mutation carriers and reported significant VBM alterations in SCA1 and trends in SCA3. Based on these data, a Wilcoxon rank sum test will have 95% power to show a difference between controls (mean=0.018, SD=0.019) and SCA (mean= 0.016, SD=0.0019) with 30 subjects per group (α=0.025 to take into account the two comparisons of SCA1 and SCA3 versus controls, two-tailed test). Note that this power computation is conservative since the RISCA study included only at-risk subjects, whereas the current study will include both premanifest and early manifest participants. In addition, based on our MRS preliminary data, a Wilcoxon-Mann-Whitney test will have 94% power to show a difference in NAA at baseline between premanifest subjects who have disease onset within the 5 years of the READISCA (conservatively estimated at 1/3 of the participants i.e. n=7, mean = 1.2) and those participants who remain in the premanifest stage (n=14, mean = 2) for the SCA1 group (standard deviation of 0.4, α=0.05, two-tailed test). We will recruit a larger sample size for the SCA3 cohort because: 1) imaging alterations did not reach significance for SCA3 mutation carriers in the RISCA study; 2) the diffusion MRI abnormalities were less pronounced in SCA3 than those in SCA1; and 3) SCA3 is the most common SCA, enabling recruitment of a larger cohort.

Statistical Methods

For Aim 1 data from the participating centers will include demographics, repeat length (expanded and normal alleles), and scores from the SARA, timed measures, patient-reported outcomes (PRO), and health-related quality of life (QoL) for “core ataxia COA”. Range, scale, and outliers of the combined data will be evaluated. Sex will be considered as a biological variable in all statistical analyses. The cluster structure of combined cross-sectional data will be assessed using a variety of non-linear manifold learning methods to identify similarities and dissimilarities. Association and equality of means will be evaluated using parametric and non-parametric inferential hypothesis testing. Longitudinal analyses for repeated measures will be performed using mixed models and generalized estimating equations (GEE regression). As the drop out processes could differ between cohorts the models will take into account the process (pattern mixture models or joint model if necessary).

Propensity Matching: *Post hoc* group comparative analysis will include propensity matching of SCA1/SCA3 subjects from US and those from Europe which have similar covariate profiles, in order to minimize confounding from covariates. Separate logistic regression runs will be performed to obtain the logit for each subject, where the dependent variable is the group (US cohort vs. European cohort) and covariates are nuisance factors such as gender, age, medical history, etc. Histograms of the fitted logit will be constructed for each group, and subjects will be randomly drawn from the same bins within the two histograms. This approach will be bootstrapped so that repeated sampling of the two groups of subjects with similar confounder profiles can be employed during modeling.

For Aim 2 centralized data analysis will be performed. De-identified MRI and MRS data will be analyzed in a blind fashion at UMN, as described previously for volumetric, VBM and MRS analyses. Diffusion MRI data will be analyzed using tract-based spatial statistics (TBSS), which is part of the FMRIB’s Software Library (FSL), and tractography to delineate particular white matter (WM) pathways (e.g., cerebellar peduncles). Functional (rs fMRI) data will be pre-processed using FSL, followed by between-group analyses using seed-based connectivity analysis for particular regions of interest, and independent component analysis/dual regression (MELODIC/FIX)) for resting-state networks (RSNs). Normative data from the Human Connectome Lifespan project will supplement matched-control data acquired prospectively. Outcome measures will consist of cerebellar and brainstem volumes, gray matter (GM), and WM loss metrics from VBM, metabolite concentrations, fractional anisotropy, mean diffusivity, radial and axial diffusivity, as well as degree of co-activation within RSNs of interest (e.g., motor function). These multi-modal imaging data will then be incorporated in the predictive model to stratify future clinical trial cohorts in Aim 3.

To compare the imaging biomarkers between SCA1, SCA3 and control cohorts, globally and in a pairwise fashion, we will use Wilcoxon rank sum test. In addition, premanifest carriers and early-stage participants will be compared using Wilcoxon rank sum test. Within each SCA group, premanifest and manifest patients will be compared regarding the biomarkers at baseline and during follow-up. If this approach is not conclusive, cluster approaches will be carried out. Sex will be considered as a biological variable to assess potential sex-based differences in imaging results. Finally, the MR biomarkers will be included in the predictive model (Aim 3).

Ethical Conduct of the Study:

This study will be conducted with the highest respect for the individual participants (i.e., subjects) according to the protocol, the ethical principles that have their origin in the Declaration of Helsinki (18th World Medical Assembly, 1964) and its last revision (Fortaleza, October 2013) (48), the ICH Harmonized Tripartite Guideline for GCP and local laws and regulations of the country where the study is performed.

Clinical Trial Registration:

In order to ensure that information on READISCA reaches the public in a timely manner and to comply with applicable laws, regulations and guidance, HMRICC will be the record owner on ClinicalTrials.gov. HMRI contact information along with participating sites and recruiting status will be registered and available for public viewing.

Appendix – READISCA Telephone Screen

Have you ever been screened or completed any research studies for spinocerebellar ataxia? ☐Yes ☐No

If "YES" – Are you currently receiving treatment from the research study? ☐Yes ☐No

If "YES" schedule screen three months after completion of interventional study.

Are you looking to participate in READISCA Clinical Trial Readiness for SCA1 and SCA3? ☐Yes ☐No
If "YES" please explain the study to the potential participant verbally, providing all pertinent information (purpose, procedures, risks, benefits, alternatives to participation), and allow the potential participant ample opportunity to ask questions. If participant agrees, send ICF to participant.

Are you experiencing symptoms of spinocerebellar ataxia? ☐Yes ☐No
Do you or family member of yours have a genetic diagnosis of SCA1 or SCA3? ☐Yes ☐No
If necessary, are you willing to undergo video screening assessment?* ☐Yes ☐No
Are you willing to participate in MRI/MRS sub study? ☐Yes ☐No

If "YES" complete the MRI Questions checklist below

****Offered to potential participants from remote areas. Contact information should be provided to HMRICC as soon as signed Informed consent form is received from participant***

How did you hear about us? ☐NAF ☐CoRDS ☐Clincaltrials.gov ☐Flyer ☐Friend ☐Web ☐Other

Age:	Gender: ☐M ☐F	***If female:*** Pregnant or nursing? ☐Yes ☐No

Do you currently receive any of the following therapies? ***Or*** Have you ever used the following in the past?*	***If "YES" –*** How often do you currently use? ***Or*** *How* often did you use in the past?
☐ Physical Therapy	_____x per week/month/past*
☐ Speech Therapy	_____x per week/month/past
☐ Occupational Therapy	_____x per week/month/past
☐ Psychotherapy	_____x per week/month/past
☐ Inpatient Rehabilitation	_____x per week/month/past
	**Must have no changes in physical/occupational therapy status for 2 months prior to enrollment*

MRI/MRS Questions Prescreen Checklist:

Do you have any metal in your body? ☐Yes** ☐No ***If "YES", get more details and include comment below***

Are you claustrophobic or have difficulty in enclosed areas? ☐Yes** ☐No ***If "YES", exclude.***

Have you ever lost consciousness? ☐Yes** ☐No ***If "YES",*** for how long? ***If LOC >10 mins, exclude.***

Have you ever worked with metals (welder, electrician, etc.)? ☐Yes** ☐No ***If "YES",*** did you wear safety glasses? ***If no, exclude.***

Do you weigh over 300lbs ☐Yes** ☐No ***If "YES", exclude.***

Do you know anyone else who may qualify for the study? ☐Yes ☐No ***If "YES"*** Are you willing to contact the person(s) and ask them to contact us? ☐Yes ☐No ***If "YES" please provide site phone and email contact.***	**Meets AIM 2 eligibility criteria**? ☐YES ☐No** ***(why not?)*** **HMRICC contacted?** ☐**Yes**** **Date:** ☐No** ***(why not?)***	**List of Potentially Prohibitive Metals** Pacemaker ☐Yes ☐No Stimulator ☐Yes ☐No Infusion pump ☐Yes ☐No Insulin pump ☐Yes ☐No Prosthesis ☐Yes ☐No Metal joints ☐Yes ☐No Ear implants ☐Yes ☐No Vascular clips ☐Yes ☐No Tattoo ☐Yes ☐No Hearing aid ☐Yes ☐No Permanent make-up ☐Yes ☐No	_____ First name, Last initial _____ Phone # ***(and/or E-mail)*** Personal #? ☐Yes ☐No **Phone screen completed by:** **Date:**

Mailing Address: